BIOGEOTECHNOLOGY OF METALS

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General problems of microbiological hydrometallurgy are discussed, such as microorganisms and their role in the oxidation of sulfide minerals, geochemical activity, theory of growth and development of bacteria with good prospects for use in hydrometallurgy. Modern aspects of technologies for dump, underground, and tank techniques of bacterio-chemical leaching of metals from ores and concentrates are highlighted. New trends in the development of biohydrometallurgy as well as its environmental aspects are reviewed.

This volume is intended for microbiologists, geochemists, hydrometallurgists, and biogeotechnologists.

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"Non, mille fois non, il n'existe pas une categorie de sciences aux quelles on puisse donner le nom de sciences appliquées.

Il y a la science et les applications de la science, liées entre elles comme le fruit à l'arbre qui l'apporte."

(Pasteur, 1871)

No, thousand times no, there is no category of science which could be named applied science.

There are science and applications of science related to it as the fruit to the fruit-bearing tree.

(Pasteur, 1871)

PREFACE

This volume is published in the framework of the International Project on Microbiological Leaching of Metals from Ores initiated by the UNEP/UNESCO/ICRO Panel on Microbiology and implemented under an official agreement between the United Nations Environment Programme, the USSR State Committee for Science and Technology, and the Environment Protection Committee of the People's Republic of Bulgaria, in cooperation with the Council for Mutual Economic Assistance.

The Institute of Microbiology of the USSR Academy of Sciences, Moscow, acts as the focal point for the Project.

The objective of the Project is to contribute to the training of specialists from developing countries in various fields, such as microbiological hydrometallurgy, biogeotechnology, and development and application of low-waste technologies promoting rational use of natural resources.

Under the Project's programme, an International Training Course on Microbiological Leaching of Metals from Ores was held on May 24 to June 20, 1982, for trainees from developing countries. Theoretical principles of bacterial leaching were taught at the Institute of Microbiology of the USSR Academy of Sciences, Moscow, while classes on theoretical and practical aspects of dump leaching were held in Bulgaria at the Higher Institute of Mining and Geology, Sophia, and at the NIPRORUDA Institute, Sophia, as well as at the Vlaikov Vryh mine. Tank leaching technique was covered at the Department of Experimental Research of the Ministry of Geology of the USSR, Tula.

The International Seminar on Modern Aspects of Microbiological Hydrometallurgy held in Moscow on June 21—26, 1982 was attended by the Course trainees and internationally reputed scientists in this field who presented papers on recent research advances in biogeotechnology.

This volume comprises both papers presented at the International Seminar and lectures, in a revised form, delivered at the International Training Course.

Presentations included in this volume cover a wide spectrum of microbiological and technological problems related to the use of bacteria in biogeotechnology as well as their role in geochemical processes. It is shown that microorganisms can efficiently be used for dressing ores and other types of mineral raw materials, for extracting metals from solutions and for oxidation of methane in coal mines.

INTRODUCTION

The importance of microorganisms in the turnover of matter in nature was first established by Louis Pasteur over a hundred years ago. His findings provided the foundation for a new branch of science: general microbiology. The concepts originally introduced by Pasteur were later brilliantly developed by S. Winogradsky, M. Beijerinck, W. Bavendamm, P. Dorff, R. Lieske, H. Molish, E. Newman, C. van Niel, S. Waksman, C. ZoBell, V. Vernadsky, V. Omeliansky, G. Nadson, B. Isatchenko, V. Tauson, N. Kholodny, V. Butkewich and other researchers. It is largely due to their efforts that, in 1930s, geological microbiology, studying the involvement of microorganisms in the turnover of chemical elements in the biosphere, has been established as a science in its own right.

For a long time geological microbiology was regarded as a purely theoretical science due to the enormous time span of geological processes.

The mining of mineral deposits by man, however, has been destroying the natural environment that kept minerals intact for thousands of years, thus providing conditions for a markedly greater rate of oxidation and reduction processes. Gradually, it has become obvious that many microbiological processes in mineral deposits are so intensive that they can be of practical importance. This resulted in the emergence of a new branch of geological microbiology in the last two decades: biogeotechnology. **Biogeotechnology is concerned with applications of microorganisms for geotechnological mining and processing of minerals; for metal extraction from solutions; and for industrial effluent treatment with a view to removing metals and protecting the environment.**

Today, the following principal domains of biogeotechnology can be listed:

1. Biogeotechnology of metals:

a) biohydrometallurgy, i.e. biological leaching of metals from mineral raw materials (ores, concentrates as well as wastes and intermediate products of various industries);

b) biodressing, i.e. biological treatment of mineral raw materials for the purpose of making them suitable for further processing or utilization, e.g. low-grade bauxite ore treatment, removal of iron from sands, kaolins and clays as well as of arsenic from sulphide concentrates and of phosphorus from manganese ores, use of microorganisms or microbial metabolites in flotation;

c) biological accumulation and concentration of metals from pregnant solutions;

d) biological treatment of industrial effluents containing ions of heavy metals;

e) biological desulphurization of coal and oil, production of sulphur from sulphates;

f) studies on the role of microorganisms in element concentration in nature and in formation of mineral deposits.

2. Use of microorganisms for enhanced oil recovery.

3. Oxidation of methane in coal layers utilizing microorganisms.

4. Studies on the role of microorganisms in the corrosion of various materials.

Any domain of biogeotechnology includes the following research objectives:

(1) Physiological characteristics and growth and development patterns of microorganisms with regard to particular techniques of metal production;

(2) Ecology and geochemical activity of microorganisms in mineral deposits and in rocks;

(3) Simulation of microbiological processes and development of flow charts for processing various minerals;

(4) Commercial testing of mineral processing technologies utilizing microorganisms, their economic assessment and introduction;

(5) Study of the role of microorganisms in the solution of the problem of the environment protection.

This volume consists of 7 parts containing lectures where all the above mentioned problems of biogeotechnology of metals are treated in varying degree of detail. The introductory paper highlights the history of this science, its objectives and major trends in its development both in the present and in the future.

This volume is intended for various categories of readers including microbiologists, biotechnologists, geochemists, geotechnologists, and other specialists dealing with the problems of minerals extraction and processing.

BIOGEOTECHNOLOGY OF METALS, ITS HISTORY, TASKS AND TRENDS OF DEVELOPMENT

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INTRODUCTION

Biogeotechnology of metals is the science of extracting metals from ores, concentrates, rocks, and solutions under the impact of microorganisms or their metabolites at normal pressure and at temperature of 5 to 90°C. These processes are largely connected with the direct effect exerted by the bacteria on the minerals (oxidation or reduction), and also with the effect exerted by their metabolites such as mineral and organic acids, proteins, peptides, polysaccharides, peroxides and others. At the present time, at least three types of microbiological processes important for metal recovery are known.

1. Oxidation of sulphide minerals, elemental sulphur, ferrous iron and a number of other metals in reduced form, by lithotrophic microorganisms.

2. Formation by organotrophic microorganisms of organic and inorganic compounds, peroxides, etc., capable of disintegrating the minerals in rocks through dissolution, formation of complexes and chelates, and oxidation of individual chemical elements.

3. Formation by organotrophic microorganisms of a large amount of biomass and also of organic compounds capable of accumulating or precipitating non-ferrous metals and rare elements from solutions.

Biogeotechnology of metals as a science concentrates on the study of biological, chemical, physico-chemical and other processes aimed at providing a technology for dressing ores and processing industrial products.

The main tasks facing this science are the following:

1. The study of the geochemical activity of microorganisms during the hypergenic processes occurring in mineral deposits and weathering crusts, the isolation of new microorganisms active in the leaching and enrichment of minerals as well as the study of microbial communities and their role in these processes.

2. The selection of highly active microbial mutants, the study of the physiology of their growth and development in extreme technological conditions of metal extraction.

3. The study of the mechanism and kinetics of biological leaching of metals from ores and concentrates, and also of the ore dressing and extraction of metals from solutions.

4. The study of the complex of technological measures involved in extracting metals by the biological methods as well as of the economics of technological processes.

THE HISTORY OF BIOGEOTECHNOLOGY OF METALS

Leaching of metals. Methods of leaching copper from ores have been known since the 15th century. Thus, back in 1497 in Northern Hungary copper was obtained by the cementation method [1]. In 1566, in the same place a complete cycle of leaching was carried out using an irrigation system. Around 1750 two hundred tonnes of cement copper were obtained annually by the hydrometallurgical method. In Germany the leaching of copper from dumps was also practised in the 16th century [2]. Forced dump leaching of copper ores began to be used in Spain at the Rio Tinto mine in 1725. However, copper was extracted from the mine waters here as early as 1670 [3]. In the territory of the USSR dump leaching was carried out in Kedabek (Azerbaijan) at the end of the last century [4]. In America leaching of copper from ores was begun in the 1920s at Bisbee (Arizona) and Tyrone (New Mexico) [3, 5].

In the USSR the extraction of copper from solutions obtained from mines of copper-pyrite deposits was widely practised in the Urals from the mid-1940s to the fifties. In 1949, 5,730 tonnes of copper were mined. An occasional practice was to wet the ore either by water or by mine drainage solutions.

Later on, dump leaching of copper was begun by a number of companies in the south-western part of the USA, in Peru, Africa, Australia, Yugoslavia, Bulgaria, and other countries.

In the sixties, the industrial underground leaching of uranium was begun in Canada. Later, some other countries began the leaching of uranium (Portugal, the USA, South Africa, and France). The essence of this technology consisted in the ores, primarily oxide ores or pyrite-containing sulphide ores, being wetted with weak solutions of sulphuric acid (pH 1.5—2.0) containing ions of iron. It was considered that the leaching process was carried out chemically.

DISCOVERY OF THE MICROORGANISMS IMPORTANT IN HYDROMETALLURGY

At the end of the last century for the first time the Russian scientist Winogradsky formulated the concept of lithotrophy. There can be no doubt that his research stimulated the study of the role of microorganisms in the cycle of sulphur and other elements in nature. Soon, in 1902, the eminent Dutch microbiologist Beijerinck isolated a new autotrophic microorganism *Thiobacillus thioparus*, which was able to oxidize sulphur and a number of its reduced compounds at high pH values of the medium.

Rudolfs and Helbronner were the first to conduct research (in 1921—1922) which showed that some unidentified sulphur oxidizing

microorganisms were capable of oxidizing pyrite and sulphides of zinc [6, 7].

At the same time, Waksman and Joffe [8] isolated the autotrophic acidophilic microorganism *Thiobacillus thiooxidans* which was able to oxidize sulphur and a number of its reduced compounds to sulphuric acid.

The first suggestions concerning the role likely to be played by bacteria in the formation of sulphates in mine waters at coal deposits were made by Carpentor and Herndon in 1933 [9], but they did not succeed in establishing the biological nature of this process experimentally.

The first evidence of the biological oxidation of ferrous iron at low pH were obtained by Colmer and Hinkle in 1947 [10]. They isolated a pure culture of bacteria *Thiobacillus ferrooxidans* which were responsible for the oxidation of Fe^{2+} in acid mine waters.

These pioneering works by American microbiologists undoubtedly laid the foundation for the development of biogeotechnology of metals. A quantitative evaluation of the role of *T. ferrooxidans* in the oxidation of Fe^{2+} and of sulphide minerals was made later [11—14].

In the same period the occurrence of *T. ferrooxidans* in nature was studied in the main, and it was shown that the chief habitation of these organisms was the acid drainage waters in coal mines and deposits of sulphide ores. It became increasingly obvious that *T. ferrooxidans* are important for the development of the zone of oxidation and natural leaching of metals from ores. These processes gained special strength in ore mining when ore was crushed and access was provided for the surface water and oxygen.

Thus, in the period from 1947 to 1960 the main physiological features of *T. ferrooxidans* were essentially revealed and also its occurrence in nature, which allowed the direct solution of the practical tasks of leaching non-ferrous metals and uranium from ores, using microorganisms.

THE FIRST PRACTICAL STEPS IN BIOGEOTECHNOLOGY OF METALS

The first patent for the utilization of *T. ferrooxidans* for formation of $Fe_2(SO_4)_3$ in the technology of leaching metals from ores and for processing of sulphide concentrates was granted in the USA in 1958 [15] and was used commercially in dump leaching of copper in the Bingham Canyon [16].

In the USSR, the first pilot industrial tests on underground biological leaching of copper were conducted in 1964 by Golomzik, Nagirnyak, and Karavaiko at the Degtyar mine [4]. These tests allowed a quantitative evaluation to be made of the activity of *T. ferrooxidans* in natural conditions and to prove the possibility of intensifying their activity in industrial conditions at a lower temperature (11—15°C).

In this period and later on, the presence of *T. ferrooxidans* was proved in ore dumps and in solutions of the installations at most deposits where dump and underground leaching of copper or uranium had already been started [4, 5, 17, 18]. It became obvious that *T. ferrooxidans* was found everywhere in ores and solutions where there was dump and underground leaching of copper, uranium and other metals and was important for intensification of such an ancient natural process of great industrial significance. At the present time, considerable quantities of copper and uranium are obtained by the dump and underground methods throughout the world with the help of bacteria.

The science of tank biological leaching of metals from ores and concentrates is considerably younger. Ten to fifteen years were needed to cover the way from laboratory experiments with diluted pulp (S : L = 1 : 50, 1 : 100) at the beginning of the sixties to semi-industrial tests on thick pulps (S : L = 1 : 5) in the mid-seventies as well as from an extremely low rate of oxidation of sulphide minerals (several mg per litre per hour) to technologically acceptable rates (up to 1 g per litre per hour and even more) [19]. The first thing which was done in that period was the solution of a technological problem of paramount importance, namely a scheme was worked out for the process and equipment which allowed continuous leaching in thick pulps. In Canada for the first time the semi-continuous leaching of uranium was achieved by the Bureau of Mines by the method of counterflow decantation [20].

Somewhat later in the USSR a technological scheme was worked out for direct flow leaching of metals in Pachuka tanks with an airlift system for agitating and aerating the pulp [21, 22]. This scheme provided optimal conditions for the life activity of bacteria during the processing of sulphide concentrates in a thick pulp.

Other branches of biogeotechnology of metals such as the use of microorganisms in the selective separation of sulphides at the flotation stage as well as for the sorption of metals from solutions are in the initial stages of development.

Thus, in spite of the fact that the process of dump and underground leaching of metals has been consciously used by man for some 500 years now, it only became known in the 1950s that bacteria played an important role in this technology. Nevertheless, it may be said that the processes of microbiological oxidation of sulphide minerals in nature occur ever since oxygen and microorganisms have appeared.

THE MODERN ASPECTS OF BIOGEOTECHNOLOGY OF METALS

One of the most important tasks facing this science is the search for and study of various groups of microorganisms which are promising for use in hydrometallurgy.

At the present time, microorganisms which are promising for the extraction of metals are known both among lithotrophs and organotrophs. A brief description of them is given below.

Lithotrophic bacteria. When evaluating the role of microorganisms in the leaching of metals and concentrates, usually only the activity of *T. ferrooxidans* is taken into account. Nevertheless, as can be seen from the data in Table 1, during the leaching of metals from ores at low pH values various lithotrophic bacteria are found which are capable of oxidizing sulphur, Fe^{2+} or sulphide minerals, and also a number of other elements. At the same time, the rate of Fe^{2+} oxidation, according to Keenan, may increase 500,000 times [in ref. 23] and that of sulphide minerals by dozens, hundreds and thousands of times depending on the specific conditions [4].

T. thioparus is capable of oxidizing some sulphide minerals (PbS, Bi_2S_3, Sb_2S_3, FeS) at high pH values [43—45]. Later, a new trend emerged in experimental work on the leaching of metals from carbonate ores with the help of this group of bacteria [46]. The fact that the bacteria important for hydrometallurgy belong to essentially different systematic groups (including archaebacteria, for example, *Sulfolobus*) attracts attention. They are widespread in nature although frequently in quantities insufficient to ensure a high rate of oxidation processes, at least at low temperatures (Table 2).

Some bacteria form cenoses not infrequently providing both for the oxidation process and for their own existence. For example, *Leptospirillum ferrooxidans*, *T. thiooxidans*, and *T. organoparus* are found in nature together with *T. ferrooxidans* and are in the final count trophically dependent on it. A number of species of bacteria, which form cenoses consisting of *L. ferrooxidans* (which oxidize

Table 1

Lithotrophic bacteria and their communities important for hydrometallurgy

Microorganisms and their associations	Reference	Microorganisms and their associations	Reference
Thiobacillus ferrooxidans (+ *T. acidophilus*)	4, 10 24—29	Same + *T. ferrooxidans*	37
Leptospirillum ferrooxidans	30—32	*Sulfolobus brierley*	38—40
L. ferrooxidans + *Thiobacillus organoparus* (*T. acidophilus*), *T. thiooxidans*	33, 34	*Sulfobacillus thermosulfidooxidans* Same + *L. ferrooxidans*	41 42
Thiobacillus TH 1, 2, 3	35, 36	*T. thioparus* *T. neapolitanus*	43—45 Our unpublished data

Table 2

Occurrence of lithotrophic microorganisms in ores

Microorganisms	Number of cells in 1 ml or 1 g	Reference
T. ferrooxidans	$10^1 - 10^9$	Our data
T. thiooxidans	up to 10^6	—"—
S. thermosulfidooxidans at 22–25°C	up to 10^6	—"—
L. ferrooxidans	up to 10^6	Our data
Thiobacillus TH 1, 2, 3	up to 10^6	35

Fe^{2+}), and *T. organoparus* or *T. thiooxidans* (which oxidize S^{2-}, S^0), are capable of oxidizing sulphide minerals as well [33, 34]. Some heterotrophic bacteria, for example, *Beijerinckia lacticogenes*, can provide *T. ferrooxidans* with nitrogen compounds, which leads to the intensification of the oxidizing processes and leaching of metals from ores in media poor in this element [47, 48].

Therefore one of the main tasks for the future is to study the role of the complex of microorganisms found in ores in the oxidation processes during leaching of metals, including tank leaching, and to investigate the principles governing the functioning of microbial associations during changes in the factors of the medium.

Thermophilic bacteria have been discovered in considerable amounts in the hot spots in ores and in the therms (Table 2). In their oxidizing activity at high temperatures, they are no less active than *T. ferrooxidans* [49], or even much more so [5, 38, 42, 50]. Thus, thermophilic bacteria are more stable to Mo (up to 2 g per litre) and oxidize MoS_2 more actively. The thermophiles also exhibit great activity in leaching nickel from ores [50]. However, thermophilic bacteria require the presence of organic substances in the medium. In particular, their development activity grows in the presence of yeast extract, glucose, and glutamin.

Experimental research has shown that the thermophilic bacteria can utilize organic substances from other mesophilic and thermophilic bacteria; e.g. thermophilic bacteria related to thiobacilli — from *T. ferrooxidans* [37]; *S. thermosulfidooxidans* — from the thermotolerant bacterium *L. ferrooxidans* [42]. There are grounds to believe that in nature thermophilic bacteria obtain the necessary organic substrates both from other bacteria and from solutions and rocks.

Thus, an important task is that of making an in-depth study of thermophilic bacteria and their associations, and of the kinetics of the oxidizing processes caused by them for the purpose of using them in hydrometallurgy.

Organotrophic bacteria. Until recently little attention was paid to the microbiological processes of the leaching of metals at high pH values of the medium. This concerns the processing of all types of ores and rocks for which acid leaching processes cannot be employed. They include rocks, carbonate ores, etc. The best known technology for extracting metals at high pH values is the soda leaching of uranium. Microbiological studies during the soda leaching of uranium have been conducted by Hungarian researchers [51—54]. It has been shown that leaching of uranium at pH 9.5—10 results from chemical and biological processes, the mixed microflora being more active than the pure cultures during leaching of uranium under such conditions. Soda leaching showed the presence of microscopic fungi, microscopic algae, and thionic bacteria. The number of these bacteria may be as high as 10^6—10^7 cells per gramme. The most active of them in leaching of uranium is *T. thioparus*, while *T. denitrificans* and *T. novellus* are somewhat less active. It may be suggested that these bacteria facilitate leaching of uranium due to the oxidation of sulphur compounds. The heterotrophic microflora facilitate leaching of uranium owing to the formation of organic acids, and the algae owing to the release of oxygen.

During the study of microflora from other ore deposits most researchers have tried to find specific microorganisms responsible for the transformation of various elements. For example, nitrifying bacteria, forming nitrous and nitric acids, facilitate leaching of the more mobile elements and concentration of the less mobile ones e.g. nickel which is accumulated in the crust of weathered rocks, resulting in the formation of residual deposits. However, heterotrophic bacteria facilitate leaching of Ni since the latter partially migrates in the form of complex with organic substances [55, 56]. The specific microorganisms must include those reducing chromium and oxidizing Sb^{3+}, Mo^{4+}, and As^{3+} [57—59]. In auriferous deposits microorganisms which possess a great ability to dissolve gold owing to the formation of amino acids are widespread [60].

A greater part in the degradation of ores and rocks is evidently played by the entire cenosis of microorganisms. The latter ensures not only release of elements, but also their migration at high pH values owing to the formation of chelates and organic complexes. The study of such cenoses is one of the most important tasks facing microbiologists.

There are numerous data available on the utilization of organotrophs for leaching metals from rocks and ores. In many cases impurities are leached while the ore is enriched and its qualities are improved (bauxites, manganese ores). It may be considered that the microorganisms grown on sugars exhibit good activity in leaching metals. However, it is difficult to say whether this will be the case when other sources of carbon and energy are employed.

Therefore, it is necessary to study the role of microorganisms in leaching of metals from rocks during the utilization of cheap organic substrates.

Finally, the technological aspects of employing organotrophic microorganisms or their metabolites in hydrometallurgy have not yet been solved. Thus, Wenberg *et al.* [61] showed that copper can be best leached using solutions from the cultivation of microscopic fungi. In this case, a large amount of biomass and organic acids are accumulated because the toxic effect of copper is precluded.

During leaching of Li by "silicate" bacteria it was shown that this element leaches more effectively at pH 3.0 and below, whereas the microorganism itself grows in a neutral medium [62].

Leaching of Au proceeds more intensively in an alkaline medium while the microorganisms forming amino acids develop at pH 7.0. Therefore, this metal is best leached by bacterial metabolites under conditions (pH 9—10, high temperature, etc.) precluding the development of ordinary microflora, which causes decomposition of the gold-dissolving substances [63].

A systematic study of the role of thionic bacteria in leaching of copper at high pH values is conducted in Poland [46]. Thionic bacteria isolated from flotation wastes and developing at pH ~9.0 are used. The bacteria oxidize CuS. However, the success of leaching also depends on the availability of cheap complexing agents.

As complexing agents the authors used Na_2EDTA and humic acids isolated from brown coal, and 93.3 and 73 per cent of copper were extracted respectively. It should, however, be noted that many metals including undesirable ones, are capable of forming complexes with both EDTA and other complexing agents. Therefore, to achieve selective extraction of desired elements, the conditions of leaching are to be controlled. Baner and Lindstrom [64] showed that at pH 9.5 addition of CO_2 increased solubilization of copper. At the same time, Zn and Ca would not leach, i.e. selective extraction of copper was achieved.

The above also shows that the organotrophic microflora greatly contributes to disintegration of ores and rocks and to leaching of metals. However, the mechanism of this process and the technological conditions of its implementation are still insufficiently clear. Their study is one of the important tasks facing microbiologists and technologists.

PROBLEMS OF INTENSIFICATION OF THE MICROBIOLOGICAL PROCESSES OF METAL LEACHING

The rate of leaching of metals depends on both microbiological and physico-chemical and technological factors, the main ones being the number and activity of bacteria.

The activity of the strains of *T. ferrooxidans* isolated from natural substrates and their resistance to heavy metals vary, [65—67] a fact determined by the pre-history of their existence [14]. Consequently,

when studying the role of bacteria in leaching of metals from ores, one should first isolate active strains from natural substrates. These cultures are also used to obtain highly active mutants which are adapted to the entirety of extreme factors.

For tank leaching of metals it has been shown that only a culture of bacteria adapted to the entirety of factors occurring in the commercial metal leaching is highly effective and stable [68].

In experiments mutants of *T. ferrooxidans* were obtained by a number of authors [69, 70]. Recently the important role of plasmids in the resistance of various bacteria to heavy metals has been noted [71]. Plasmids have also been discovered in *T. ferrooxidans* [72]. Complications arise in the use of laboratory-bred mutans in leaching of metals from ores and concentrates. The introduction of both "wild" strains of *T. ferrooxidans* and mutants into dumps did not make the oxidative processes more intensive [73]. Moreover, the mutant forms were displaced by the more active "wild" strains of bacteria which had adapted to the specific leaching conditions. Therefore, one of the tasks of intensifying the dump and underground leaching of metals from ores is the optimization of the physico-chemical factors as well as the utilization of large amounts of biomass of "wild" strains of bacteria. It is more realistic to use the laboratory-bred mutants of *T. ferrooxidans* or other bacteria in tank leaching.

As is known, the extreme factors of the medium such as low or high temperature, high metal concentrations, pH, hyperbaric pressure, etc., alone or in combination with other factors, suppress the growth of bacteria rather than their oxidizing activity [74—79]. Therefore, in the presence of such limiting factors the increment in the biomass is prolonged in time and the rate of the oxidation processes slows down. This makes the use of bacteria in the technology of metal leaching economically unacceptable. The problem can be solved by using a large amount of bacterial biomass adapted to the entirety of factors typical of different technological schemes.

The data showing the dependence of Fe^{2+} oxidation activity on the density of the bacterial population are shown in Table 3 [80].

It can be seen that the rate of Fe^{2+} oxidation grows sharply as the concentration of the biomass increases.

Table 3

The dependence of the Fe^{2+} oxidation rate on the density of the bacterial population ($t = 28^{o}C$)

Bacterial concentration, g/l	Oxidized Fe^{2+}, g/l per hour
0.005	0.6
1.2	15

As shown by us, at a temperature of 8°C an increase in the number of cells from 2.5×10^7 to 2.5×10^8 per ml tripled the Fe^{2+} oxidation rate.

The utilization of the concentrated biomass also allowed the rate of leaching of arsenic from gold-bearing concentrate to be increased 18-fold [81], and that of pyrite to be approximately doubled after the number of cells increased from 10^6 to 10^8 per ml [82].

Thus, the most effective means of considerably intensifying the oxidation processes both in the dump, underground and tank leaching is the utilization of a large amount of biomass of bacteria maintained in active state and adapted to the specific technological conditions.

Another task is to work out an effective method for obtaining a large amount of biomass of active bacteria for the purpose of using it in hydrometallurgy. In laboratory conditions and in pilot installations, cells were separated from pregnant solutions by means of centrifugation, filtration or flotation. The method of obtaining the biomass in a cultivator with electrochemical reduction of Fe^{3+} is being developed [83]. The BACFOX (BACterial Film OXidation) process [84, 85] has been suggested for Fe^{3+} regeneration and accumulation of the biomass in solution intended for dump and underground leaching of metals.

ROLE OF PHYSICO-CHEMICAL AND CHEMICAL FACTORS

The rate of bacterial oxidation processes also depends on chemical and physico-chemical factors (pH, Eh, O_2 and CO_2 content, electrode potentials of the sulphide minerals, temperature, mineral composition of ores and concentrates, etc.). However, special attention should be paid to those factors which not only allow the process to be accelerated but possibly change its nature and direction. For example, in a number of works it has been shown that silver ions accelerate bacterial oxidation of sulphide minerals to a considerable extent [87, 88]. The essence of the catalytic action of Ag^+, according to Miller and Portillo [89], consists in the following. When Ag^+ is added, the following reaction occurs: $CuFeS_2 + 4Ag^+ \rightarrow Ag_2S + Cu^{2+} + Fe^{2+}$.

On the surface of chalcopyrite a thin layer of silver sulphide is formed which consists of spherical particles, Fe^{2+} is oxidized by bacteria to Fe^{3+}. Silver sulphide rapidly reacts with the Fe^{3+} in the following reaction: $Ag_2S + 2Fe^{3+} \rightarrow 2Ag^+ + S^O + 2Fe^{2+}$.

Further, the cycle is repeated. Attention is attracted by the fact that the sulphide oxidation by this mechanism results in formation of elemental sulphur rather than of sulphuric acid.

The advantages of this process may consist in the following:

1) instead of weak sulphuric acid one can obtain elemental sulphur in the metallurgical process;

2) greater selectivity of the oxidation process with regard to different sulphide minerals may be expected;

3) creation of a simpler scheme for processing tailing solutions is possible.

This means that attention should be paid to the study of the joint effect of chemical and biological catalysts on the process of metal leaching.

Methods for intensification of leaching of metals at high pH values have still not been studied sufficiently. The heterotrophic bacteria need organic substrates. The rate of leaching of zinc at pH 8.5 from sulphide ores by means of *T. thioparus* increases with the addition of $Na_2S_2O_3$ (2.5—5.0 g per l) [86]. Possibly, the thiosulphate will play the same role in the leaching of metals from carbonate ores as do Fe^{2+}/Fe^{3+} during their sulphuric acid leaching with the participation of *T. ferrooxidans.*

ROLE OF MICROORGANISMS IN ORE ENRICHMENT

One of the most important tasks to be tackled in dressing polymetallic ores is the selective separation of different sulphide minerals at the stage of dressing, i.e. at flotation stage. The role of microorganisms in this process has still been poorly studied. It has been shown that sulphate-reducing bacteria *Desulfovibrio desulfuricans* and the products of their life activity can act as effective sulphidizers for oxide minerals. This process is based on the interaction of hydrosulphide ions which are formed during the development of bacteria, with the surface of oxide minerals and the formation of sulphides. Flotation of oxide lead and copper-molybdenum ores with culture of *D. desulfuricans* as a sulphidizer, has shown that the use of this culture in the flotation process is technologically and economically feasible [90, 91]. Furthermore, separation of artificially mixed galenite (PbS) and sphalerite (ZnS), and artificially mixed molybdenite (MoS_2) and chalcopyrite ($CuFeS_2$) has shown in principle that sulphate-reducing bacteria can be used as a desorbent of xanthate from the surface of minerals [92, 93]. Those minerals from the surface of which xanthate is removed, cannot be floated. This allows the minerals to be separated selectively at the flotation stage. Lipids formed by *Candida tropicalis* during its development on paraffin-containing substrates, are used as a substitute for expensive fatty acid collectors during flotation of oxide minerals [93]. Finally, it has been shown that during a short treatment by *T. ferrooxidans* of sulphide minerals (PbS, Cu_2S, $CuFeS_2$) their flotation activity is lowered. Both *T. ferrooxidans* and $Fe_2(SO_4)_3$ deactivated sphalerite which had been activated by ions of copper. At the same time, adsorption of xanthate by sphalerite decreases by 17 and 2.0—3.6 times respectively [94].

Thus, there are grounds for stating that the flotation properties of sulphide minerals can be changed with the help of bacteria and that this phenomenon may be used for the selective separation of sulphides in ore enrichment.

MICROORGANISMS AS BIOSORBENTS OF METALS

The presence of metal ions in solutions poses two important problems: their extraction from pregnant solutions and treatment of industrial wastewaters. It has long been known that living organisms are capable of accumulating non-ferrous, rare, and precious metals. A tendency has recently been noted for a systematic study to be made of the biosorption of metals from solutions and for the utilization of this phenomenon for practical purposes. Some data on the sorption of metals by microorganisms are cited in Table 4. It has been shown that the uranium content of yeasts or bacteria reaches 10—15 per cent of the dry weight, that of cobalt 9.9 per cent, and of cadmium 8—15 per cent. The rate of sorption depends on the organism (mainly on the activity of reactive groups on its surface), temperature, pH, modes of preparing the biomass, composition of the nutrient medium, and presence of organic substances and other metals [98, 101]. Selectivity in the incorporation of individual metals into the cells is reported [98]. The mycelium of microscopic fungi (waste of the microbiologi-

Table 4

Sorption of metals by microorganisms

Microorganisms	The process of sedimentation of the metals	Reference
Citrobacter sp., *P. putida*, *B. subtilis*, *Saccharomyces cerevisiae*, *P. aeruginosa*, *Rhizopus arrhizus*, a mixed culture of denitrifying bacteria, *S. lipolytica*, *Candida utilis*, *Streptomyces*, *Mucor rouxii*, *Phycomyces blakesleanus*, *Ghoanephora cucurbitarum*, algae	Biosorption of radioactive elements (U) and other metals: Al, Mo, Ag, Cu, Cd, Cr, Mn, Co, Ni, Zn, Hg, Pb	95—109
Microscopic fungi (wastes of the microbiological industry)	Biosorption of Au, Ag, Pt, Pd	109—110
Zoogloea ramigera	Sedimentation of U and other metals	111
Sulphate-reducing bacteria	Sedimentation of metals in the form of sulphides	112, 113
Chromium-reducing bacteria	Reduction of Cr, sedimentation of Cr^{4+}	114

cal industry) is capable of accumulating many metals. Depending on the treatment of mycelium and temperature as much as 100 per cent of Rb, Hg, Zn, Cu, Ni, Co, Mn, and Cr were extracted from solutions whose volume was 50 ml and the concentration of the relevant metals was 0.5 mM [102]. The chelating ability of the mycelium of fungi is connected with the presence of chitin and chitosan. With the help of the mycelium of microscopic fungi, up to 96–98 per cent Ag and Au, up to 84 per cent Pt, and 92 per cent Pd may be extracted from gold and silver affinage solution [109, 110]. After ashing the precipitates contain up to 20 per cent of the total metals.

A method for extracting metals from solutions with the help of polysaccharides, synthesized by *Zoogloea ramigera*, has been developed by Swedish scientists [111]. Depending on the pH selective extraction of the metals U, Cd, Zn is achieved.

Finally, the existing method of sedimentation of the metals with the help of sulphate-reducing or chromium-reducing bacteria is employed in removal of metals from waste waters [112–114]. However, both the mechanisms of biosorption of metals and the technological basis for utilizing microorganisms in extracting metals from solutions have not yet been studied to a sufficient extent. This is one of the urgent problems to be tackled in the near future.

It is known that the biological accumulation and concentration of metal ions occurs both inside the cell and on its surface. The accumulation of metals on the surface of the cell is preferable to its accumulation inside the cell. In the former instance, the metals can be easily washed away, and the biomass can be used again. In the latter case the cells have to be disintegrated to extract the metals.

ECONOMICS

The profitability of dump and underground leaching of copper from ore has long been known. Thus, Shoemaker and Darrah [115] note that dump leaching of copper is characterized by low capital inputs. In leaching of copper from oxide ore (Cu – 0.5—1.0%) where 60 per cent of the copper is extracted and 5—9 kg of H_2SO_4 is expended per kilogramme of copper, the main outlays are those for ore mining and the consumption of sulphuric acid and scrap iron. The operational expenses are estimated at 0.497–0.905 dollars per kilogramme of copper. They can be lowered to 0.382—0.748 dollars per kilogramme of copper when cementation of copper is replaced by electrolysis or when extractants are used. The cost of one tonne of copper in the early years of operation of the dump leaching installations at the Nikolaevsky deposit was 152 roubles and in the next few years 218–353 roubles [116]. The cost of underground leaching of copper was also comparatively low, namely 260 300 dollars per tonne in Miami [117], 75—85 dollars per tonne in Bor [118], and at the Bingham mine approximately 80 dollars per tonne [in ref. 119].

Thus, the cost of copper obtained by the dump and underground methods varies depending on many factors (mining and geological conditions, type of ore, methods of metal extraction, and so forth). The profitability of bacterial leaching of uranium from ore has also been shown [17].

The economics of various schemes of the tank leaching of metals have been described in a number of works [120—122]. Thus, the operational costs of the bacterial method for leaching arsenic calculated according to the data of large-scale tests are 16—17 roubles per tonne and almost two times lower than the expenditure on the autoclave leaching of gold-arsenic concentrates [120]. The arsenic content falls from 5.8 per cent to 0.5—0.6 per cent.

Torma [121] gives the following estimate with regard to the economics of microbiological leaching of a lead sulphide concentrate. The minimum extraction of Zn, Cu, and Cd was assumed to be 95 per cent. The annual profits are 630,000 dollars. McElroy and Bruynesteyn [122] have shown that in processing 200 tonnes of chalcopyrite concentrate per day the cost of one pound of copper is approximately 13 cents, i.e. about the same as that with the pyrometallurgical process.

All the authors note a number of advantages of the bacterial method of processing, at least, of complex concentrates, avoiding pollution of the environment or cutting down the capital and operational costs.

CONCLUSION

The recent achievements of microbiology and hydrometallurgy allow the existing practice of processing many types of mineral raw materials to be significantly changed. First and foremost they allow huge low-grade ore reserves to be processed as well as the wastes of dressing plants and complex sulphide concentrates. Deposits at great depth are also made available.

Until now, only dump and, to a lesser extent, underground methods of biological leaching have been used for industrial recovery of copper and uranium from low-grade ores.

A technology of the tank biological processing of complex copper, copper-zinc, lead-zinc and arsenic tin- and gold-bearing concentrates has been tested in semi-industrial conditions.

Laboratory experiments have shown that use of microorganisms may be promising for extraction of gold, manganese and other elements from ores, rocks and wastes of metallurgical works. However, the technological problems of many of the processes which have already been studied, remain to be solved.

New methods of metal extraction are economically advantageous and ensure comprehensive utilization of mineral raw materials. The problem of environmental pollution is also considerably alleviated.

REFERENCES

1. Podanyi, T., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi et al. (Eds.), Hungarian Academy of Sciences; Pécs, Hungary, 109 (1980).
2. Power, R.L., In: Unit Processes in Hydrometallurgy, Warden and Breach Science Publishers, New York-London, 806 (1964).
3. Trussell, P.C., Duncan, D.W., and Walden, C.C., *Can. Mining J., 85,* 3, 46 (1964).
4. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.I., Role of Microorganisms in Leaching of Metals from Ores, Nauka, Moscow (1972) (in Russian).
5. Brierley, C.L., *Crit. Rev. Microbiol., 6,* 207 (1978).
6. Rudolfs, W., *Soil Sci., 14,* 1, 135 (1922).
7. Rudolfs, W., and Helbronner, A., *Soil Sci., 14,* 1, 459 (1922).
8. Waksman, S.A., and Joffe, I.S., *J. Bacteriol., 7,* 239 (1922).
9. Carpentor, L., and Herndon, J., *W. Va. Univ. Eng. Exploit. Stat. Res. Bull.,* 10, (1933).
10. Colmer, A.R., and Hinkle, M.E., *Science, 106,* 253 (1947).
11. Leathen, W.W., Braley, S.A., and McIntyre, L.D., *Appl. Microbiol., 1,* 61 (1953).
12. Temple, K.L., and Delchamps, E.W., *Appl. Microbiol., I,* 255 (1953).
13. Bryner, L.C., Beck, J.F., Davis, D.B., and Wilson, D.G., *Ind. Eng. Chem., 46,* 2587 (1954).
14. Kuznetsov, S.I., Ivanov, M.V., and Lyalikova, N.N., Introduction to Geological Microbiology, USSR Academy of Sciences, Moscow (1962) (in Russian).
15. Zimmerley, S.R., Wilson, D.G., and Prater, J.D., US Patent No. 28229964 (1958).
16. Zimmerley, S.R., Malouf, E.E., Prater, J.D., and Schellinger, A.K., US Patent No. 3330650 (1964).
17. Kuznetsova, E.G., *Uspekhi mikrobiologii, 6,* 153 (1970).
18. Groudev, S., and Genchev, F., In: Proceedings of the 4th Congress of Microbiology, Sofia, v. III, 237 (1977).
19. Duncan, D.W., and Bruynesteyn, A., *Can. Min. Met. Bull., 74,* 116 (1971).
20. McGready, H.H., Harrison, V.F., and Gow, W.A., *Can. Min. Met. Bull., 62,* 135 (1969).
21. Pol'kin, S.I., Karavaiko, G.I., Tauzhnianskaya, Z.A., and Panin, V.V., In: Proceedings of the 9th International Mineral Processing Congress, Prague (1970).
22. Pol'kin, S.I., Panin, V.V., Adamov, E.V., Karavaiko, G.I., and Chernyak, A.S., In: Proceedings of the 11th International Mineral Processing Congress, Cagliari, Paper 33 (1975).
23. Lacey, D.T., and Lawson, F., *Biotechnol. Bioeng., 12,* 29 (1970).
24. Torma, A.E., and Gabra, G.G., *Antonie van Leeuwenhoek, 43,* 1 (1977).
25. Lewis, A.J., and Miller, J.D.D., *Can. J. Microbiol., 23,* 319 (1977).
26. Soljanto, P., and Tuovinen, O.H., In: Biogeochemistry of Ancient and Modern Environment, P.A. Trudinger, M.R. Walter, and B.J. Ralph (Eds.), Australian Academy of Sciences and Springer Verlag, Canberra-New York, 469 (1980).
27. Torma, A.E., and Habashi, F., *Can. J. Microbiol., 18,* 1780 (1972).

28. Imai, K., Sakaguchi, H., Sugio, T., and Tano, T., *J. Ferment. Technol., 51,* 865 (1973).
29. Silver, M., and Torma, A.E., *Can. J. Microbiol., 20,* 14 (1974).
30. Markosyan, G.E., *Biologicheskii zhurnal Armenii, 35,* 26 (1972).
31. Markosyan, G.E., *Reports of the USSR Academy of Sciences, 211,* 1205 (1973).
32. Guay, R., and Silver, M., *Can. J. Microbiol., 21,* 281 (1975).
33. Markosyan, G.E., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (Ed.), Pushchino, 160 (1976) (in Russian).
34. Norris, P.R., and Kelly, D.P., *FEMS Microbiol. Letters, 4,* 143 (1978).
35. Brierley, J.A., and Le Roux, N.W., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York, 55 (1977).
36. Brierley, J.A., Norris, P.R., Kelly, D.P., and Le Roux, N.W., *Eur. J. Appl. Microbiol. Biotechnol., 5,* 291 (1978).
37. Brierley, J.A., In: Biogeochemistry of Ancient and Modern Environment, P.A. Trudinger, M.R. Walter, and B.J. Ralph (Eds.), Australian Academy of Sciences and Springer Verlag, Canberra-New York, 70 (1980).
38. Brierley, C.L., and Brierley, J.A., *Can. J. Microbiol., 29,* 183 (1974).
39. Brierley, C.L., *J. Less Common Met., 36,* 237 (1974).
40. Zillig, W., Stetter, K.O., Wunderl, S., Schutz, W., Priess, H., and Scholz, J., *Arch. Microbiol., 125,* 259 (1980).
41. Golovacheva, R.S., and Karavaiko, G.I., *Mikrobiologiya, 47,* 815 (1978).
42. Karavaiko, G.I., Kovalenko, T.V., and Golovacheva, R.S., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi *et al.* (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 95 (1980).
43. Sokolova, G.A., and Karavaiko, G.I., Physiology and Geochemical Activity of Thionic Bacteria, S.I. Kuznetsov (Ed.), Nauka, Moscow (1964) (in Russian).
44. Khalid, A.M., and Ralph, B.J., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York, 165 (1977).
45. Lyalikova, N.N., *Reports of the USSR Academy of Sciences, 176,* 1432 (1967).
46. Kunicki-Goldfinger, W., Leiczak, A., and Ostrowski, M., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi *et al.* (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 127 (1980).
47. Tsuchiya, H.M., Trivedi, H.C., and Schuler, M.L., *Biotechnol. Bioeng., 16,* 991 (1974).
48. Tsuchiya, H.M., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York, 101 (1977).
49. Chakrabarty, A.M., and Murr, L.E., *Biotechnol. Bioeng., 21,* 1685 (1979).
50. Le Roux, N.W., and Wakerley, D.S., In: Biogeochemistry of Ancient and Modern Environment, P.A. Trudinger, M.R. Walter, and B.J. Ralph (Eds.), Australian Academy of Sciences and Springer Verlag, Canberra-New York (1980).
51. Czako-Ver, K., Czegledi, B., Fekete, L., and Kecskes, M., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi *et al.* (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 95 (1980).

52. Fekete, L., Czegledi, B., Czako-Ver, K., and Kecskes, M., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi *et al.* (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 43 (1980).
53. Kecskes, M., Dobolyi, Cs., Czegledi, B., Fekete, L., and Czako-Ver, K., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi *et al.* (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 49 (1980).
54. Komaromy, Z.P., Czegledi, B., Fekete, L., Czako-Ver, K., and Kecskes, M., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi *et al.* (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 53 (1980).
55. Lebedeva, E.V., Lyalikova, N.N., and Bugel'sky, Yu.Yu., *Mikrobiologiya, 47*, 1101 (1978).
56. Lebedeva, E.V., and Lyalikova, N.N., *Mikrobiologiya, 48*, 517 (1979).
57. Lebedeva, E.V., and Lyalikova, N.N., In: 5th Congress of the All-Union Microb. Soc., Ecology of Microorg. Sec., Abstracts of Reports, 34, Yerevan (1975).
58. Abdrashitova, S.A., Mynbayeva, B.N., and Ilialetdinov, A.N., *Mikrobiologiya, 50*, 41 (1981).
59. Mynbayeva, B.N., *Bull. of Kaz. SSR Academy of Sciences*, Biology Series, *2*, 42 (1981).
60. Korobushkina, E.D., Cherniak, A.S., and Mineev, G.G., *Mikrobiologiya, 43*, 49 (1974).
61. Wenberg, F.H., Erbisch, F.H., and Volin, M.E., *Soc. Min. Eng. AIME*, 207, (1971).
62. Karavaiko, G.I., Krutsko, V.S., Melnikova, E.O., Avakian, Z.A., and Ostroushko, Yu.I., *Mikrobiologiya, 49*, 547 (1980).
63. Mineev, G.G., Syrtlanova, T.S., and Skobeev, I.K., *Ore Enrichment*, Irkutsk, *4*, 171 (1976) (in Russian).
64. Baner, D.J., and Lindstrom, R.E., *J. Met., 23*, 31 (1971).
65. Groùdev, S.N., *Compt. rend. Acad. Bulg. Sci., 33*, 1119 (1980).
66. Groudev, S.N., *Compt. rend. Acad. Bulg. Sci., 33*, 659 (1980).
67. Vanchi, G.A., Bodiu, A.P., and Sinko, I.I., In: Proceedings of the 8th International Mineral Processing Congress, Leningrad, 2, E-6 (1968).
68. Pol'kin, S.I., Panin, V.V., Karavaiko, G.I., and Adamov, E.V., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (Ed.), Pushchino, 115 (1976) (in Russian).
69. Tuovinen, O.H., and Kelly, D.P., *Arch. Microbiol., 95*, 153 (1974).
70. Groudeva, V.I., Groudev, S.N., and Markov, K.I., *Compt. rend. Acad. Bulg. Sci., 33*, 1401 (1980).
71. Chakrabarty, A.M., In:Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, and J.A. Brierley (Eds.), Academic Press, New York-San Francisco-London, 137 (1978).
72. Martin, A.W., Dugan, P.R., and Tuovinen, O.H., *Can. J. Microbiol., 27*, 850 (1981).
73. Groudev, S.N., Genchev, F.N., and Gaidarjiev, S.S., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, and J.A. Brierley (Eds.), Academic Press, New York-San Francisco-London, 253 (1978).
74. Roy, P., and Mishra, A.K., *J. Appl. Bacteriol., 51*, 387 (1981)

75. Kovalenko, T.V., and Karavaiko, G.I., *Mikrobiologiya, 50,* 326 (1981).
76. Kelly, D.P., and Jones, C.A., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, and J.A. Brierley (Eds.), New York-San Francisco-London, 19 (1978).
77. Kovalenko, T.V., and Karavaiko, G.I., *Mikrobiologiya, 50,* 913 (1981).
78. Norris, P.R., and Kelly, D.P., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, and J.A. Brierley (Eds.), New York-San Francisco-London, 83 (1978).
79. Davidson, M.S., Torma, A.E., Brierley, J.A., and Brierley, C.L., *Biotechnol. Bioeng. Symp., 11,* 603 (1981).
80. Grishin, S.I., Adamov, E.V., Panin, V.V., and Karavaiko, G.I., *Mikrobiologiya, 52,* 2, 322 (1983).
81. Grishin, S.I., Adamov, E.V., and Panin, V.V., Moscow Inst. of Steel and Alloys, Moscow, Paper deposited with TsNIIEI tsvetmet., May 24, 1982, No. 928 tsm. D82 (1982).
82. Chrostowska, D., Goss, M., and Buszman, E., In: Physicochemical Problems of Mineral Processing, Wroclaw, 215 (1981) (in Polish).
83. Kovrov, B.G., Denisov, G.V., Sekacheva, L.G., and Belyi, A.V., In: Chemosynthesis in Continuous Culture, Nauka, Moscow, 34 (1978) (in Russian).
84. Livesey-Goldblatt, E., *Processing, 1,* 5 (1980).
85. Groudev, S.N., *Compt. rend. Acad. Bulg. Sci ., 34,* 1437 (1981).
86. Rozek, B., Konopka, E., Rozek, S., and Tumidajski, T., In: Physicochemical Problems of Mineral Processing, Wroclaw, 149 (1981) (in Polish).
87. McElroy, R.O., Douglas, W., and Duncan, W., US Patent No. 3856913 (1974).
88. Snell, G.J., and Morgan, S.C., *Eng. Min. J., 178,* 100 (1977).
89. Miller, J.D., and Portillo, H.Q., In: Proceedings of the 13th International Mineral Processing Congress, Warszawa, *1,* 691 (1979).
90. Solozhenkin, P.M., Lyubavina, L.L., Yemelyanov, A.F., Pulatov, G.Yu., Samokhvalova, L.F., and Akhnazarova, T.N., In: Substance Composition and Enrichment Properties of Mineral Raw Materials, Nauka, Moscow (1978) (in Russian).
91. Solozhenkin, P.M., Lyubavina, L.L., Samokhvalova, L.F., and Pupkov, V.S., *Izvestia vuzov Tsvetnaya metallurgiya,* No. 3, 13 (1979).
92. Kupeeva, O.D., Solozhenkin, P.M., and Khan, G.A., *Ore Enrichment,* Polytechnical Inst. Publishing House, Irkutsk, 5, 158 (1977).
93. Solozhenkin, P.M., Lyubavina, L.L., Samokhvalova, L.F., and Fedorovskikh, L.F., *Tsvetnye metally,* No. 7, 8 (1977).
94. Kim, D.Kh., Klimenko, N.G., Karavaiko, G.I., and Kliueva, N.D., *Prikladnaya biokhimiya i mikrobiologiya, 17,* 2, 284 (1951).
95. Madgwick, J.C., and Ralph, B.J., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York, 85 (1977).
96. Beveridge, T.J., and Murray, R.G.E., *J. Bacteriol., 141,* 876 (1980).
97. Norris, P.R., and Kelly, D.P., *Dev. Ind. Microbiol., 30,* 299 (1979).
98. Sakaguchi, T., Horikoshi, T., and Nakajima, A., *J. Ferment. Technol., 56,* 561 (1978).
99. Strandberg, G.W., Shumate, S.E., and Parrott, J.R., *Appl. Environm. Microbiol., 41,* 237 (1981).
100. Danch, A., Kasprzak, A., and Chmielowski, J., In: Physicochemical Problems of Mineral Processing, Wroclaw, 245 (1981) (in Polish).

101. Muzzarelli, R.A.A., Tanfani, F., and Emanuelli, M., *Appl. Biochem.*, *3*, 322 (1981).
102. Chopra, I., *J. Gen. Microbiol.*, *63*, 265 (1971).
103. McLean, O.J., Lucis, Shanh, Z.A., and Zansz, E.R., *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, *31*, 699 (1972).
104. Doyle, J.J., Marshall, R.J., and Pfander, W.H., *Appl. Microbiol.*, *29*, 562 (1971).
105. Macaskie, L.E., and Dean, A.C.R., *Environm. Technol. Lett.*, *3*, 49 (1982).
106. Norris, P.R., and Kelly, D.P., *J. Gen. Microbiol.*, *99*, 317 (1977).
107. Hassett, J.M., Jennet, J.C., and Smith, J.E., In: Contaminants and Sediments, R.A. Baker (Ed.), Ann Arbor Sci. Publ. Inc., *2*, 409 (1980).
108. Olafson, R.W., Apel, K., and Sim, R.G., *Biochem. Biophys. Res. Common.*, *89*, 36 (1979).
109. Mineev, G.G., Cherniak, A.S., and Semenova, L.P., *Prikladnaya biokhimiya i mikrobiologiya*, *8*, 358 (1972).
110. Mineev, G.G., Semenova, L.P., Cherniak, A.S., and Abakumov, D.N., *Trudy Irgiredmet*, *27*, 122 (1972).
111. Norberg, A., In: 5th International Symposium of Environmental Biogeochemistry (Abstracts), Stockholm, 89 (1981).
112. Ilialetdinov, A.N., Enker, P.B., and Loginova, L.V., *Mikrobiologiya*, *46*, 113 (1977).
113. Brierley, C.L., and Brierley, J.A., In: Biogeochemistry of Ancient and Modern Environment, P.A. Trudinger, M.R. Walter, and B.J. Ralph (Eds.), Australian Academy of Sciences and Springer Verlag, Canberra-New York (1980).
114. Romanenko, V.I., Kuznetsov, S.I., and Koren'kov, V.N., British Patent No. 1475369 (1977).
115. Shoemaker, R.S., and Darrah, R.M., *Min. Eng.*, *20*, 68 (1968).
116. Shalamov, V.M., Khalezov, B.D., and Novomlyntsev, A.A., *Bull. Tsvetnaya metallurgiya*, No. 23, 17 (1974).
117. Adamov, E.V., and Panin, V.V., In: Ore enrichment, Results of Science, Moscow, VINITI, USSR Academy of Sciences, *8*, 5 (1974) (in Russian).
118. Hovanec, G.M., In: Symposium pracovniků banského prúmyslu Příbram, Sekce U, 243 (1972).
119. Tauzhnianskaya, Z.A., State of the Art of Bacterial Leaching Abroad, Tsvetmetinformatsiya, Moscow (1973) (in Russian).
120. Ilialetdinov, A.N., Kamalov, M.R., and Bozhko, N.A., *Bull. of Kaz. SSR Academy of Sciences*, Biol. Series, No. 2, 2 (1978).
121. Torma, A.E., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, and J.A. Brierley (Eds.), Academic Press, New York-San Francisco-London, 375 (1978).
122. McElroy, R.O., and Bruynesteyn, A., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, and J.A. Brierley (Eds.), Academic Press, New York-San Francisco-London, 441 (1978).

PART I

PHYSIOLOGY AND BIOCHEMISTRY OF MICROORGANISMS IMPORTANT IN BIOGEOTECHNOLOGY OF METALS

MICROORGANISMS IMPORTANT FOR HYDROMETALLURGY: CYTOLOGY, PHYSIOLOGY, AND BIOCHEMISTRY

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INTRODUCTION

Bacterial leaching of non-ferrous and rare metals results from the oxidation of sulfide minerals and ferrous iron. Under mesophilic conditions acidophilic *thiobacilli* play a leading role in these processes. To date they are most extensively used in hydrometallurgy. At the same time there are favourable prospects for the future application in bacterial leaching of thermoacidophilic bacteria of the genera *Sulfolobus* and *Sulfobacillus* as well as thermophilic forms of the genus *Thiobacillus* [1, 3]. Studies on the cytology, physiology, and biochemistry of these microorganisms are clearly needed if concrete problems of controlling oxidation processes are to be solved with a view to intensify them.

The organisms of the above genera are characterized by the ability to oxidize sulfur, divalent iron, and sulfides as sources of energy for growth at low pH. Taxonomically the group of microorganisms under consideration is very diverse. The genera *Thiobacillus*, *Sulfobacillus*, and *Leptospirillum* are incorporated into Eubacteria [4] while *Sulfolobus* belongs to Archaebacteria [4, 5]. With the G + C content varying greatly in *thiobacilli* species representing the extremities of the spectrum (about 20 per cent) [4], they are likely to represent different genera.

The following is the discussion of the main cytological, physiological, and biochemical properties of these microorganisms.

CYTOLOGY

The morphology of the bacteria to be examined has a great diversity (Figs 1, 2). *Thiobacilli* are typical pseudomonads, i.e. rod-shaped cells (0.5 to 0.8) x (0.9 to 1.5) μm in size with rounded ends and a

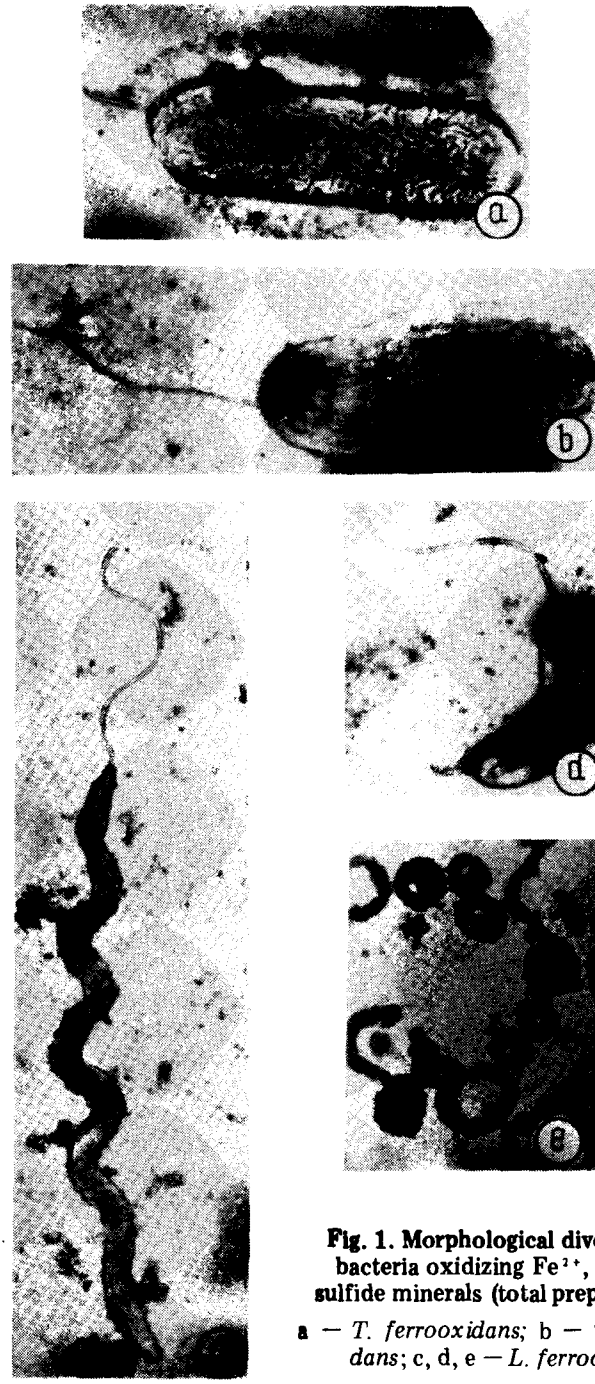


Fig. 1. Morphological diversity of bacteria oxidizing Fe^{2+}, S^0, and sulfide minerals (total preparations):

a — *T. ferrooxidans;* b — *T. thiooxidans*; c, d, e — *L. ferrooxidans*

polar flagellum (single as a rule) which enables them to move (Fig. 1a, b). The exception is a nonmotile thermophilic variant [6, 7]. Some *thiobacilli*, e.g. *T. ferrooxidans* form chains in a stationary phase [8]. Cell replication occurs by uniform division. A characteristic feature of *Leptospirillum ferrooxidans* belonging to the family Spirillaceae is pronounced polymorphism (Fig. 1c, d, e) [9—11]. Vibrios 0.9—1.1 μm long and 0.2—0.4 μm wide can be observed along with spiral forms having as many as two to five spires. Each cell has a polar flagellum 18—22 nm in diameter. Pseudococci are known to form under unfavourable growth conditions, i.e. aggregations of vibrios and cocci tightly twisted together, with sizes varying over a wide range. They reproduce either by uniform division or by separation of one of the spires.

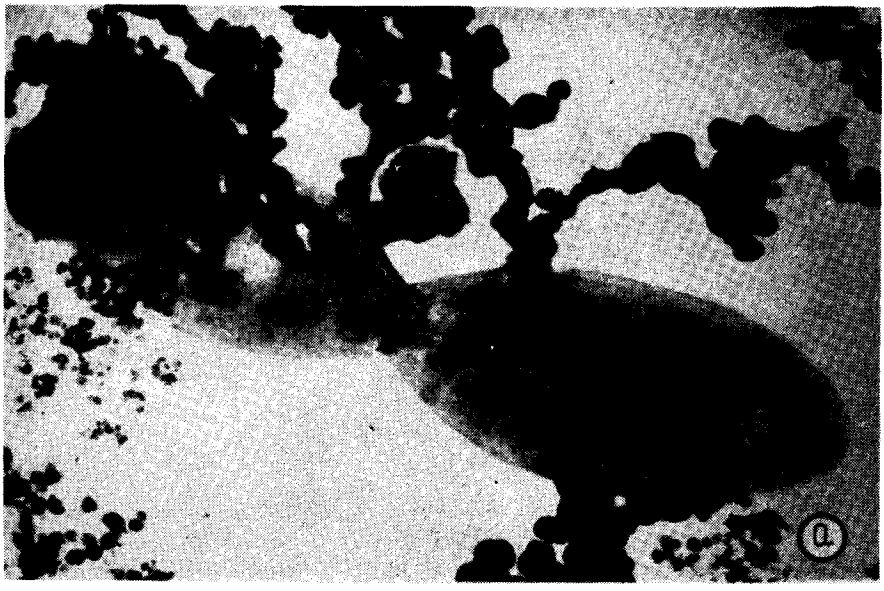
S. thermosulfidooxidans, the only known member of the genus *Sulfobacillus*, also exhibits polymorphism [12—15]. Growing cultures are dominated by rod-like cells (0.6 to 0.8) x (1.0 to 3.0) μm in size with rounded or pointed ends, isolated, coupled or forming short chains (Fig. 2a). Coccoid, wedge- or knob-shaped cells have also been observed. Rudimentary ramification is inherent in this organism. Its division is effected by septum formation. "Snapping" division or breakage is not infrequent, bringing about angular, circular or palisade distribution of cells. The flagellum with related structures is absent. The organism is able to produce endospores [13—15].

Microorganisms of the genus *Sulfolobus* are spherical or lobular in shape (Fig. 2b) and have a diameter 0.8—1.5 μm [16—22]. The cells are nonmotile and lack both the flagellum and endospores. The mode of multiplication has not been elucidated in detail. They are believed to replicate by septum formation [16, 22] or binary fission through cell constriction [20].

All these bacteria with the exception of *L. ferrooxidans* have been found to have pili [23, 24]. Besides, unique rhizoidal projections with a diameter of about 160 nm have been described in *S. thermosulfidooxidans* [13].

Various groups of microorganisms growing on ferrous iron, elemental sulfur, and sulfide minerals are apt to form mucous capsules (Fig. 1a) [12, 13, 25]. Mucus production undoubtedly contributes to the fast attachment or adhesion of the cells to a solid substrate.

The structure of the cell wall varies greatly in acidophilic bacteria oxidizing iron, sulfur, and sulfide minerals, for they belong to different taxonomic groups and some of them are differently Gram-stained. The rippled appearance of the cell wall is a prominent feature of *thiobacilli* [8, 26], its structure being typical of Gram-negative bacteria in general. It consists of a lipopolysaccharide membrane, an underlying electron-transparent layer of varying thickness, an electron-dense murein layer, and an internal electron-light layer (Fig. 3a). The structure of the cell envelope in *L. ferrooxidans* is also similar to that found in other Gram-negative bacteria [11]. A rigid murein layer ob-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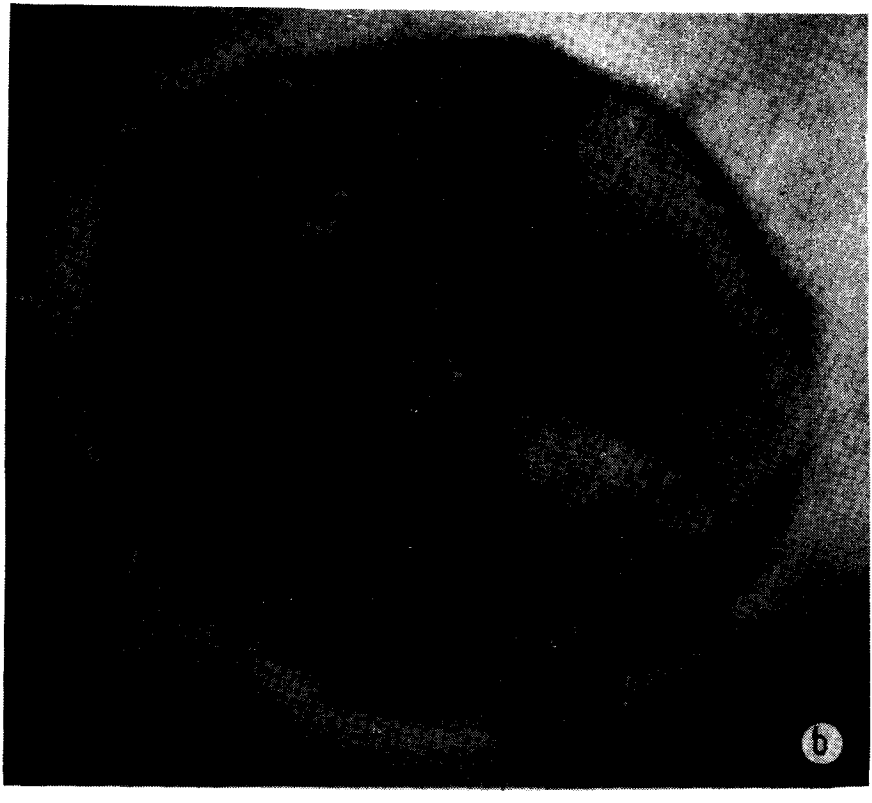

b

servable only in some portions of the section (Fig. 3b) is characteristic of the family Spirillaceae [27—29]. Gram-positive *S. thermosulfidooxidans* has a unicomponent cell envelope [14]. The structured S-layer is approximately 20—25 nm thick; immediately below is a rigid murein layer of the same thickness which is linked by bridges with the cytoplasmic membrane (Fig. 3c). The *Sulfolobus* spp. cells are Gram-negative [16]. The cell envelope of these Archaebacteria is composed of glycoprotein subunits about 15.5 nm in diameter arranged in a regular hexagonal array; a murein layer is absent [14, 16, 20, 21, 30].

Acidophilic *thiobacilli* are characterized by [26, 31] considerable morphological diversity of intracellular membrane structures. In ultrathin sections of the *T. thiooxidans* and *T. ferrooxidans* cells four-contoured as well as lamellar structures can be seen side by side with simple loop-shaped membranes (Fig. 4a, b, c). Younger cultures of *S. thermosulfidooxidans* contain metabolically active cells with a complicated cytomembrane system, vesicular invaginations dominating the periphery of the cells. Structures of complex appearance, including lamellar bodies are sometimes present [14]. Intracytoplasmic structures were shown to be absent in *L. ferrooxidans* [11].

The central part of the cell appears to be occupied by the nucleid electron-transparent areas with DNA strands (Fig. 5). The cytoplasm of young cells contains a large number of ribosomes and polyribosomes (Fig. 5). Large electron-dense inclusions, polyphosphates, are consistently associated with the nuclear region as a reserve material [32, 33]. The periphery of the cell appears to be occupied by polysaccharide granules (Fig. 5) [28, 34]. Acidophilic *thiobacilli* have been described to contain carboxysomes, hexagonal inclusion bodies comprising ribuloso-1,5-diphosphate carboxylase — a key enzyme of autotrophic carbon dioxide fixation [3].

It should be noted that *L. ferrooxidans* is capable of depositing Fe^{3+} on the outer surface of the cell and in this respect resembles iron bacteria which are known to form thick ferriferous extracellular sheaths [11]. It can be accounted for by the peculiarities of the chemical composition of the mucous capsule that synthesizes complex compounds containing Fe^{3+}. The extracellular iron deposition is likely to cause intracellular oxygen deficiency and contribute to the formation of coccoid cells (Fig. 6a). Meanwhile the murein layer undergoes lysis, and the outer cell membrane becomes separated from the cell forming a sphere around the organism (Fig. 6b).

Fig. 2. Morphological diversity of bacteria oxidizing Fe^{2+}, S^0, and sulfide minerals (total preparations):

a — *S. thermosulfidooxidans*; b — *Sulfolobus* sp.

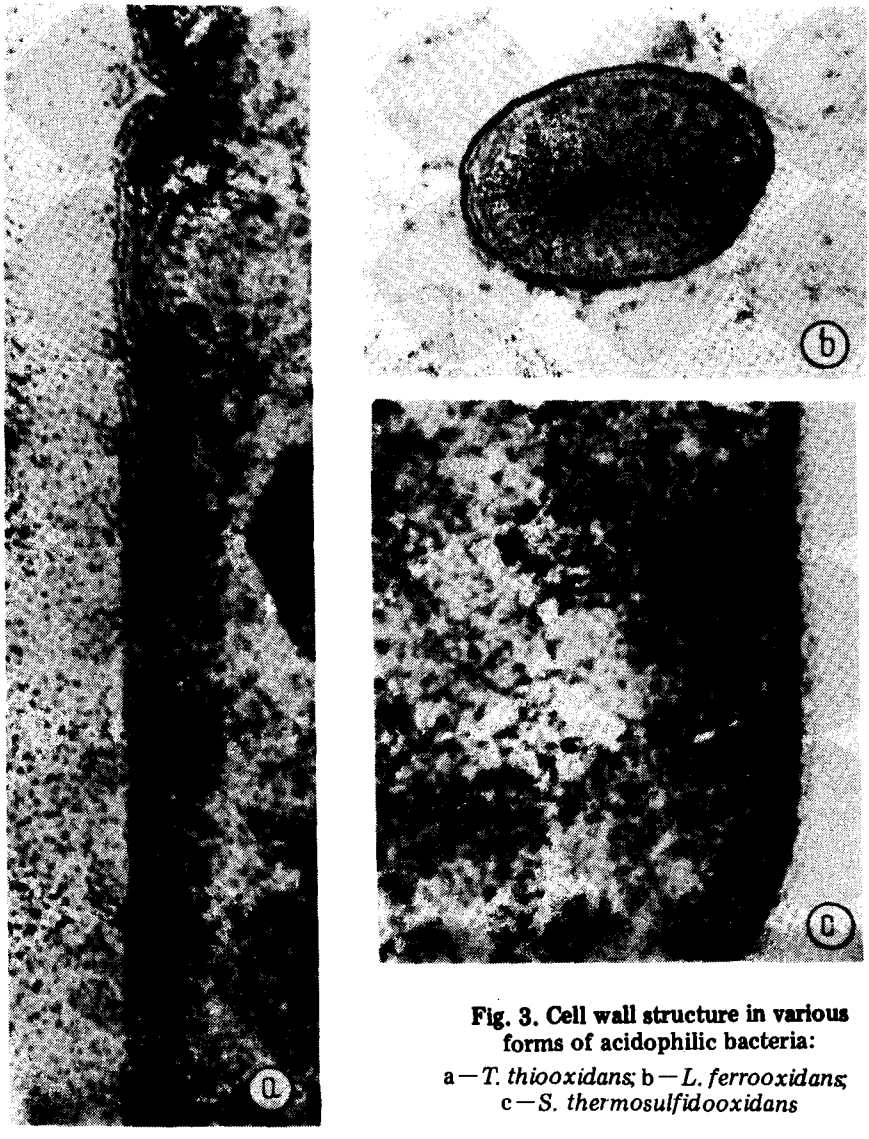


Fig. 3. Cell wall structure in various forms of acidophilic bacteria:
a — *T. thiooxidans*; b — *L. ferrooxidans*; c — *S. thermosulfidooxidans*

A prominent feature of *S. thermosulfidooxidans* is endospore production. Endospores are 0.7 x 0.7—0.8 μm in size, spherical in shape, and located in terminal or subterminal portions of the cell slightly or considerably dilating sporangia (Fig. 7) [12, 13, 15]. The process of sporification is similar to that in *Bacillus* [36] (Fig. 8). Both cortex and exosporium of the mature spore of *S. thermosul-*

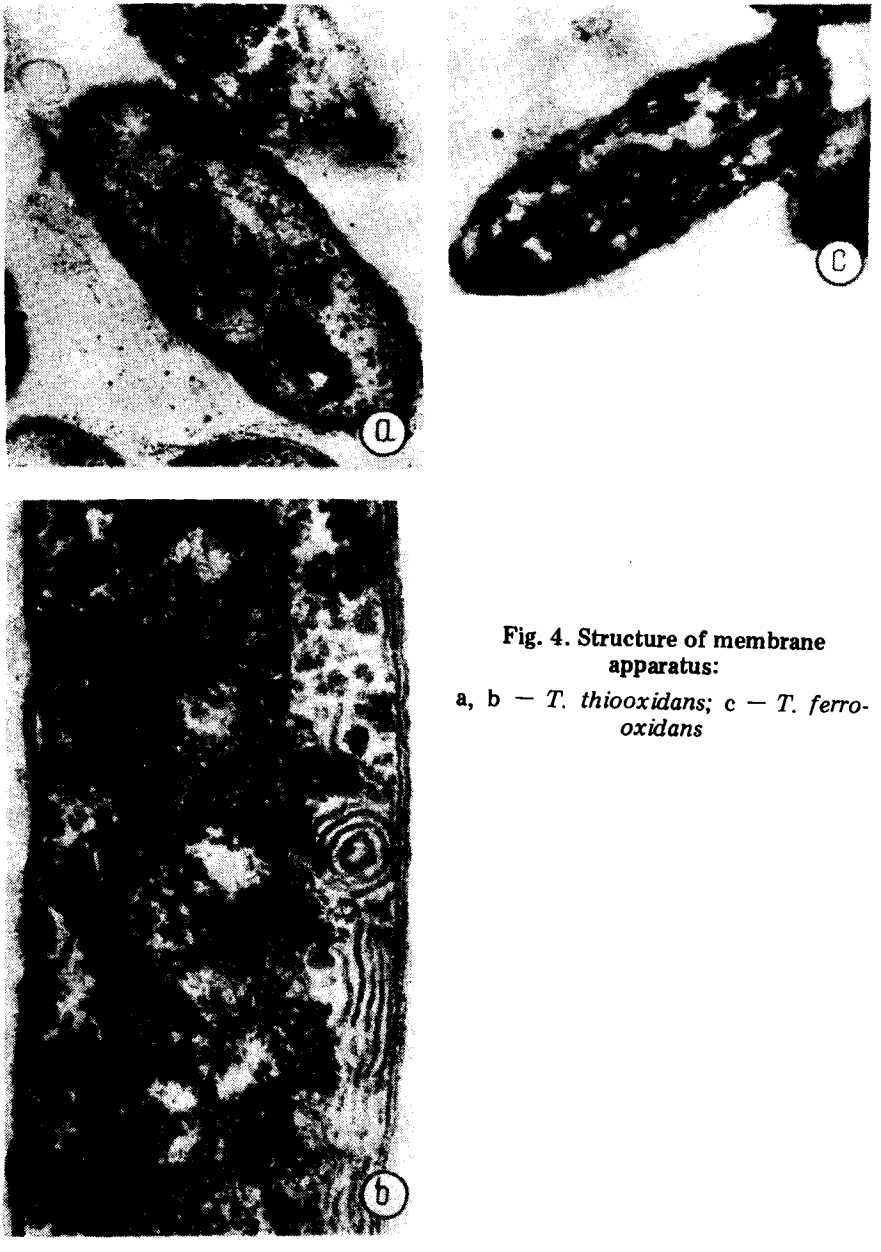


Fig. 4. Structure of membrane apparatus:

a, b — *T. thiooxidans*; c — *T. ferrooxidans*

fidooxidans are enveloped in a multilayer coat reminiscent of that in mesophilic [36] and thermophilic [37, 38] bacilli. Mature spores of this thermophile have considerable thermoresistance [12].

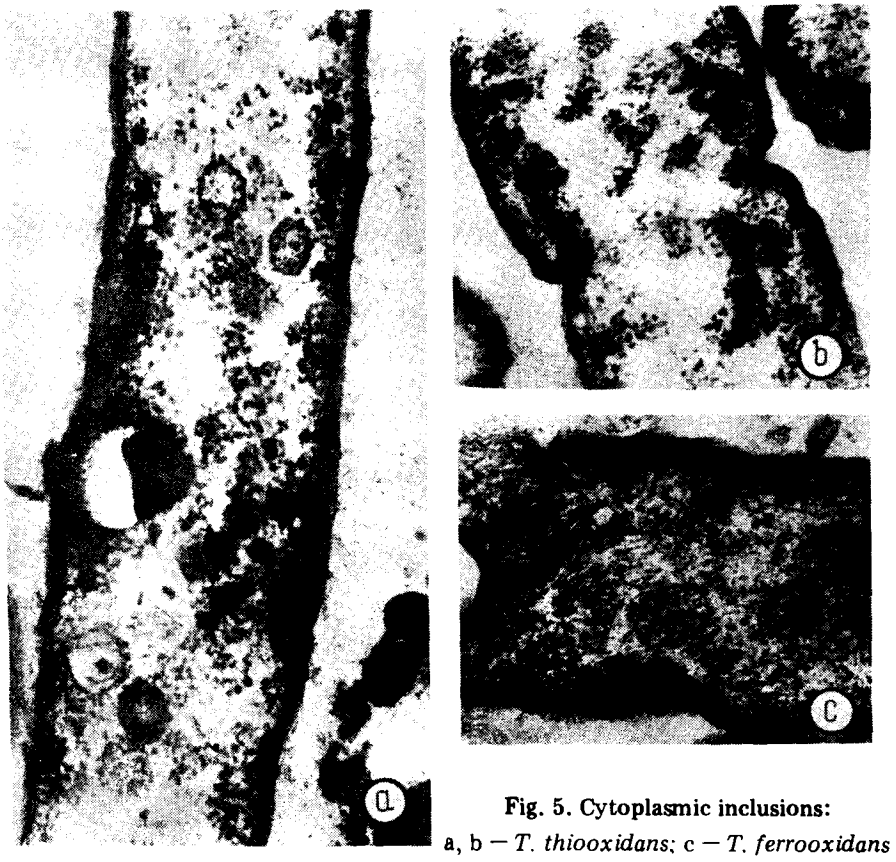


Fig. 5. Cytoplasmic inclusions:
a, b — *T. thiooxidans*; c — *T. ferrooxidans*

PHYSIOLOGICAL PROPERTIES

Table represents data on physiological properties of acidophilic bacteria oxidizing elemental sulfur, ferrous iron, and sulfide minerals. The data show that bacteria of this group comprise quite a number of microorganisms differing in physiological properties (temperature, sources of energy, and constructive metabolism).

Optimal temperatures vary from 28°C for mesophilic microorganisms to 50—80°C for thermophilic ones. The pH optimum for these bacteria falls within a narrow range between approximately 2.0 and 2.5. Except for certain differences, they utilize the same substrates as energy sources. Species of some genera, e.g. *T. ferrooxidans*, *S. thermosulfidooxidans*, and *S. brierleyi* obtain energy by the oxidation of both Fe^{2+} and S^{O} as well as sulfide minerals, while others can oxidize either ferrous iron or elemental sulfur. These organisms are classified as obligate lithoautotrophs or facultative autotrophs utili-

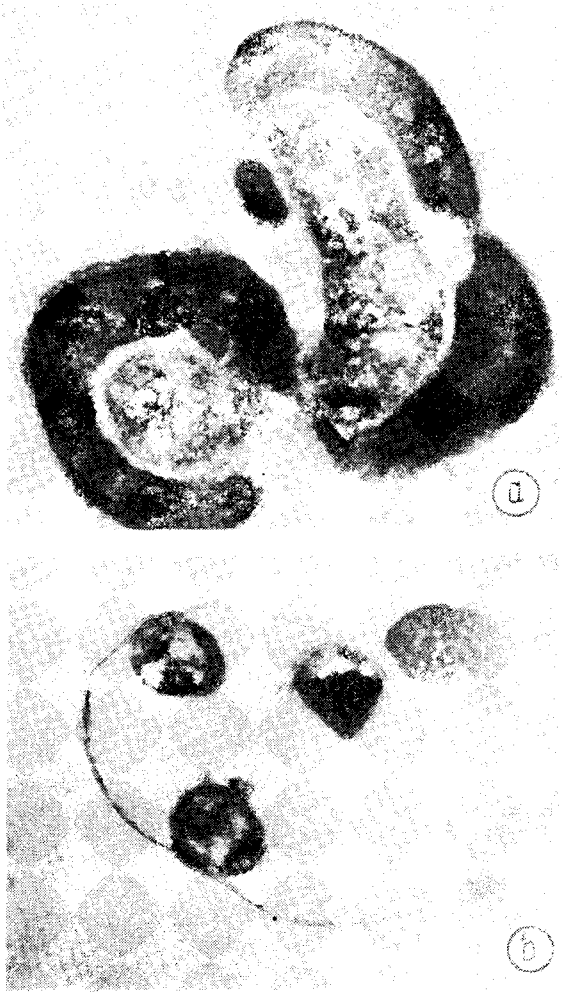


Fig. 6. Deposition of Fe^{3+} on the outer surface of *L. ferrooxidans* cells

zing CO_2 for constructive metabolism. However, thermophilic bacteria require yeast extract for growth. Representatives of *Sulfolobus* spp. and, to some extent, *S. thermosulfidooxidans* are able to shift completely to heterotrophic metabolism. *S. acidocaldarius* grows on media supplemented with yeast extract, tripton, peptone, casamino acids, casein hydrolyzate, ribose, and other sugars [16, 19, 55]. Restricted utilization of either glucose or sucrose has been shown to occur in *S. thermosulfidooxidans* [12].

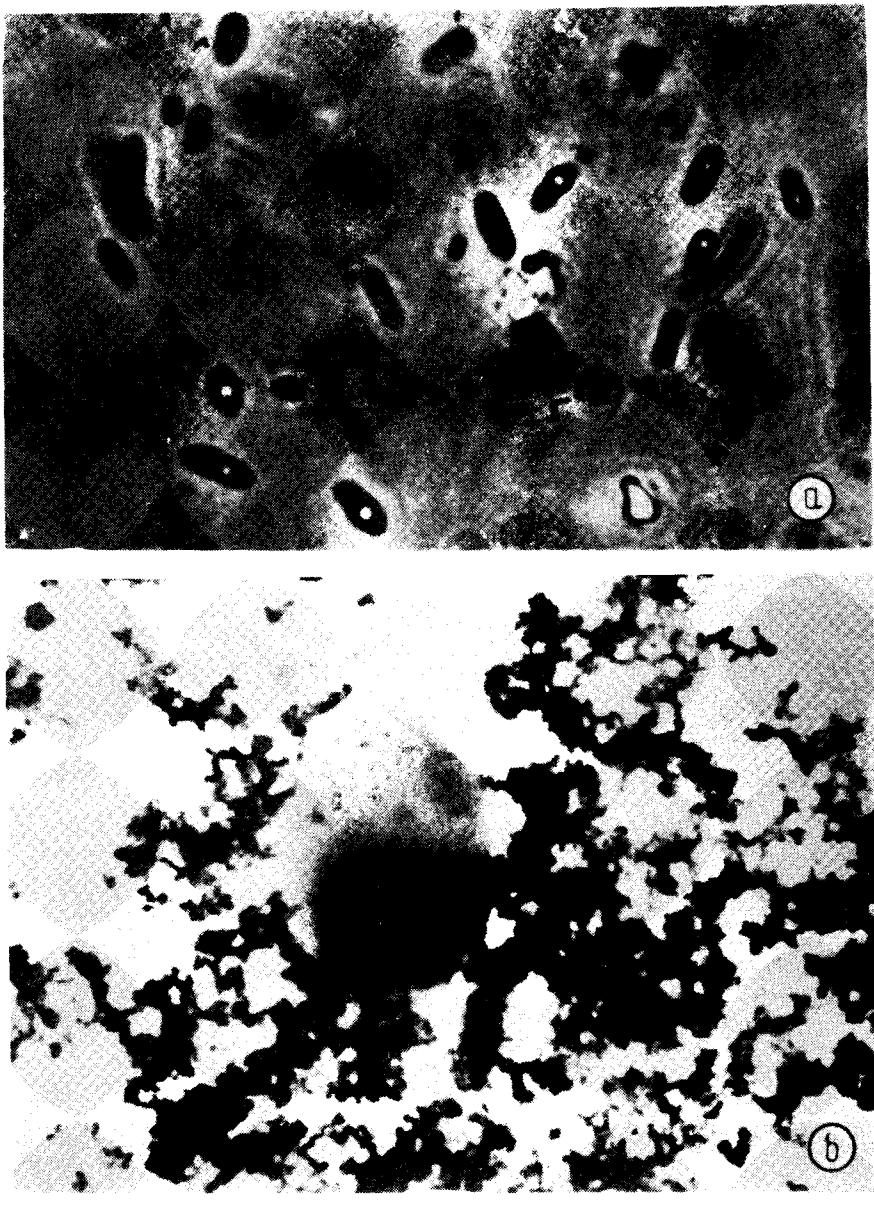


Fig. 7. Sporangia and spores of *S. thermosulfidooxidans*

The growth and development of autotrophic bacteria are commonly inhibited by organic compound [56, 57]. Yet they are also capable of syntrophic relationship with heterotrophic organisms. For example,

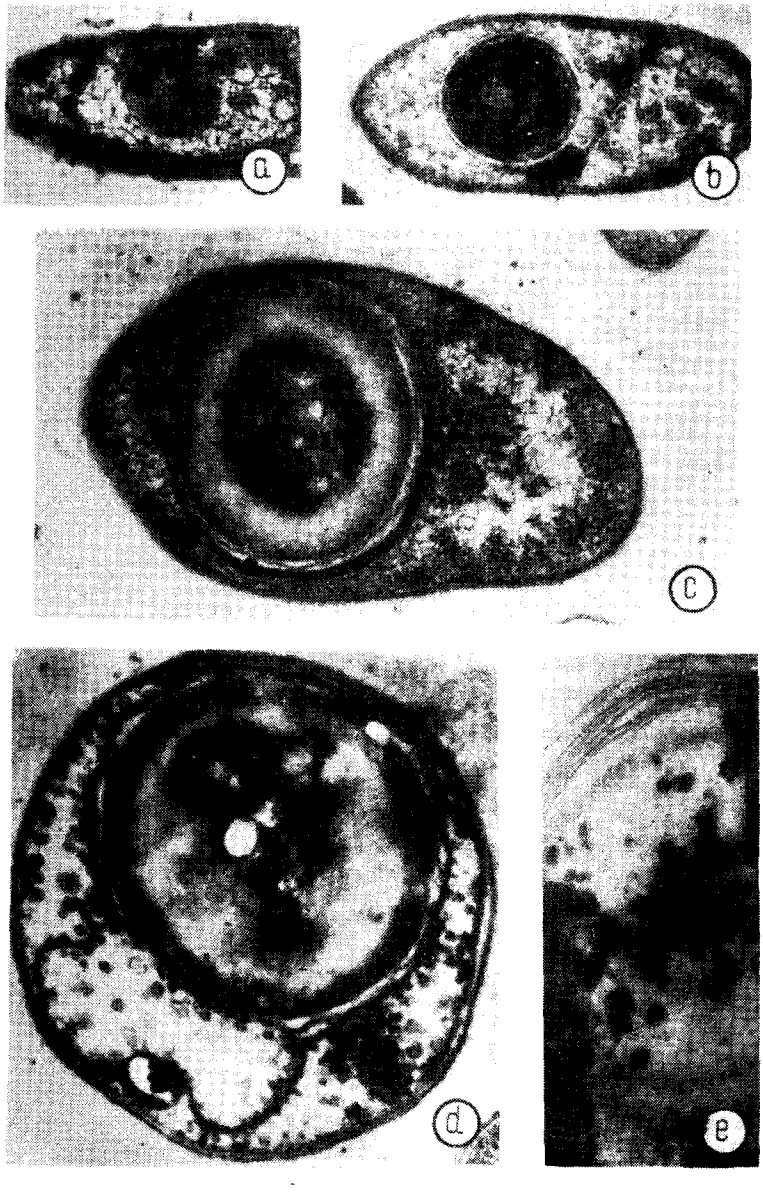


Fig. 8. Formation of spores in *S. thermosulfidooxidans*:

a — approximately stage IV; b — transition to stage VII; c — transition to stage VII; d — stage VII; e — a fragment of a mature spore with stratified covers

the presence of *Beijerinckia lacticogenes*, an acidophilic heterotrophic nitrogen fixer, enhances the oxidation of sulfide minerals by *T. ferrooxidans* in a medium lacking nitrogen sources [58]. The authors

Table

Physiological properties of acidophilic bacteria oxidizing Fe^{2+}, S^0, and sulfide minerals

Microorganisms	Habitats	Nutrients		Temperature		pH		References
		energy sources	carbon sources	range	optimum	range	optimum	
Thiobacillus thiooxidans	Sulfur and sulfide ore deposits, acid waters and springs	S, SO_3^{2-}, Sb_2S_3	CO_2	2.0–40	28	1.5–5.0	2.0–3.0	39–42
Thiobacillus ferrooxidans	Sulfur and sulfide ore deposits, coal fields, gold-bearing ore bodies	sulfide minerals, S^0, Fe^{2+}, S^{2-}, $S_2O_3^{2-}$, S_3^{2-}	CO_2	2.0–40	28	1.0–4.8	2.0–3.0	43–45
Thiobacillus organoparus (T. acidofillus)	Sulfur and sulfide ore deposits, acid springs	S^0	CO_2, glucose, galactose, fructose, xylose, ribose, arabinose, sodium citrate	2.0–40	28	1.5–6.0	3.0	9,46

Thiobacillus TH 1, 2, 3	Hydrothermal soils rich in sulfur, hot spots of experimental leaching facilities	Fe^{2+}, S^0, $S_2O_3^{2-}$, pyrite, pentlandite, chalcopyrite	CO_2 yeast extract, glutathione, cysteine, cystine	X-60	50	1.1—3.5	2.6	2, 3, 6, 7, 47, 51
Leptospirillum ferrooxidans	Sulfide ore deposits	Fe^{2+}	CO_2	2.0—40	30	1.5—5.0	3.0	9,52
Sulfobacillus thermosulfidooxidans	Sulfide ore deposits, hydrotherms	S^0, Fe^{2+}, sulfide minerals	CO_2, glucose, sucrose, glutamine	30—58	50—55	1.7—4.5	2.1—2.5	12,53
Sulfolobus acidocaldarius	Acid thermal soils, hydrotherms	S^0, yeast extract	CO_2, yeast extract, tripton, peptone, casamino acids, caseine hydrolyzate, glutamate, glutamine, aspartate, alanine, ribose, xylose	55—85	70—75	0.9—5.9	2.0—3.0	16,54
Sulfolobus brierleyi		S^0, Fe^{2+}, sulfide minerals	CO_2, yeast extract, sucrose,	45—75	70	2.0—7.0	1.5—2.0	17, 55, 21

Table , continued

Microorganisms	Habitats	Nutrients		Temperature		pH		References
		energy sources	carbon sources	range	optimum	range	optimum	
Sulfolobus brierleyi			lactose, α-keto-glutaric acid					
Sulfolobus solfataricus		S^0	CO_2, yeast extract, triptonic acids, glucose, sucrose, xylose, lactose, maltose, rhamnose	50—89	75—87	1.0—5.5	3.0—4.5	19, 21

believe *T. ferrooxidans* to produce organic acids as carbon sources for *B. lacticogenes* and to consume fixed nitrogen. In addition, the acceleration of *T. ferrooxidans* growth in the presence of a heterotrophic organism could be promoted by the consumption by the latter of the organic acids excreted by the autotroph into the medium.

A number of authors [59, 60] have demonstrated that some *T. ferrooxidans* strains can utilize organic substrates and shift from an autotrophic to a heterotrophic pathway. When incubated for 60 hours in various organic media, including complex ones, these strains displayed nothing but organotrophic growth. After 7 passages on a glucose-enriched medium the cells completely lost the capacity to oxidize ferrous iron still demanding low pH values. Harrison et al. [61] have shown that *T. ferrooxidans* is capable of oxidizing elemental sulfur, ferrous iron, and sulfide minerals and has a different genotype from heterotrophic microorganisms. For the oxidation of organic substances to proceed there must be organotrophic organisms present, such as *T. organoparus* or *Acetobacter acidophillum* [9, 46].

Bacteria oxidizing Fe^{2+}, S^{O}, and sulfide minerals at low pH are aerobic. If oxygen is deficient, *Thiobacillus* and *Sulfolobus* can utilize ferric iron as an electron acceptor, catalyzing its reduction [62] and using elemental sulfur as a donor of electrons. According to Brierley [55] *Sulfolobus* oxidizes elemental sulfur under unaerobic conditions while reducing hexavalent molybdenum $(MoO_4)^{2-}$ to pentavalent Mo_2O_5.

A distinctive physiological feature of all these microorganisms is their resistance to high concentrations of metal ions, which is of practical importance in hydrometallurgy. For example, *T. ferrooxidans* remains active in the presence of copper or uranium (U_3O_8) at concentrations of 55 and 12 g/l respectively [63]. Notably some metals (lead, mercury, and cadmium in particular) inhibit *T. ferrooxidans* growth even at low concentrations [64]. From the data of Kelly and Jones [65] it follows that Fe^{2+} and Fe^{3+} are likely to have a toxic effect on *T. ferrooxidans.* Carbon dioxide fixation is inhibited if Fe^{2+} concentration exceeds 10 g/l while Fe^{3+} in a concentration of 5.6 g/l suppresses the oxidation of ferrous iron. Extensive studies of the effect of Fe^{3+} on the ferrous iron oxidation by *T. ferrooxidans* have revealed the dependence of its inhibition on the concentration of Fe^{3+} as well as on the ambient temperature and physiological state of the culture [66, 67]. The extent of inhibition of bacterial activity by Fe^{3+} ions drops with the temperature. *T. ferrooxidans* has been observed to respond to unfavourable concentrations of ferric iron at incubation temperatures up to 26°C [67]. Fe^{3+} and Cu^{+} serve as competitive inhibitors of *T. ferrooxidans* activity, under unfavourable conditions it is bacterial growth that is inhibited first, not oxidizing power [66–68].

The mechanism of resistance to metal ions in *thiobacilli* remains to be elucidated. Imai et al. suggested that it involves a system which

blocks the transfer of ions into the cell [69]. Investigations of intracellular compartmentation of silver ions ($AgNO_3$) in *T. ferrooxidans* have shown that they accumulate mainly within the cell wall and cell membrane. The addition of reduced glutathione into the culture decreased the amount of Ag^+ associated with the membrane. This led the authors to suggest that GSH plays a significant role in the mechanism of resistance. Other authors believe that resistance to metal ions can be accounted for by the presence of specific plasmids [63, 70].

Physiological requirements of the microorganisms under consideration determine their distribution over ecological niches (Table). Acidophilic thermophilic bacteria are commonly found in hot spots of sulfide ore deposits, volcanic hot springs, and thermal soils. Mesophilic forms are reported to occur in acid waters and springs, sulfur and sulfide ore deposits where temperature does not exceed maximum values limiting growth of these species.

BIOCHEMICAL PROPERTIES OF ACIDOPHILIC BACTERIA, OXIDIZING FERROUS IRON, ELEMENTAL SULFUR, AND SULFIDE MINERALS

Metabolism in microorganisms is known to involve energy consumption and assimilation. The former furnishes bacteria with the energy for their physiological activity, and the latter results in the synthesis of organic matter to be used in cell construction.

Thiobacilli have most extensively been studied in terms of biochemical characteristics of the cell. For this reason they seem suitable for the discussion of the patterns of energy and constructive metabolism in chemolithotrophs of hydrometallurgical importance.

Energy metabolism. Adenosine triphosphoric acid (ATP) and proton potential are known to be involved in the energy transfer in living organisms. Hydrophilic properties preclude ATP from being a donor of energy for the processes which occur within membranes. In contrast to ATP, proton potential serves as a universal form of intracellular energy utilized in all kinds of chemical and osmotic processes. It is used in particular to secure the transport of a large number of substances, reverse electron flow, flagellum rotation, ATP and pyrophosphate synthesis and can be a source of heat generation [71].

An important property of proton potential is its ability to be transferred from one part of the membrane to another. Since bacteria possess a developed system of internal membranes permeating through the cytoplasm, the energy of proton potential is obviously available to any portion of the cell.

The mechanism of the formation of proton potential is explained by the chemiosmotic theory of Mitchell who postulated that the substrate oxidation occurs on the outer surface of the membrane

while molecular oxygen undergoes reduction on its inner surface. The resulting osmotic energy in the form of potential difference can be utilized in chemical reactions, e.g. ATP synthesis [72].

As proton potential is generated by respiratory enzymes, electrons in aerobic bacteria are transferred from a substrate to be oxidized to oxygen via specific pathways (redox chains) with increasing potential.

Participation of subcellular structures in the processes concerned with the oxidation of ferrous iron, elemental sulfur, and sulfide minerals. Bioenergy reactions of Fe^{2+}, S^{O}, and sulfide minerals oxidation are preceded by the substrate attachment to the cell wall surface [73—75]. Association of the microorganism with oxidized substrates is facilitated by various cell surface structures, viz. mucous microcapsules, pili, and peculiar projections [12, 13, 25]. The microcapsules envelope mineral particles forming a single bacterium-substrate system. *Sulfolobus* pili were shown to be involved in the attachment of cells to elemental sulfur [77]. The authors' evidence shows that the number of pili increases greatly when sulfur is utilized as a source of energy. Unique projections facilitating "anchorage" of cells on sulfide minerals have been described in *S. thermosulfidooxidans* [13].

The participation of the cell wall in the earlier stages of oxidation of ferrous iron, sulfur, and sulfide minerals was unambiguously demonstrated in *thiobacilli.* Chemical analysis together with cytochemical methods revealed the presence of Fe^{2+} and S^{O} ions in the cell wall during oxidation [78, 79]. The function of the outer membrane of the cell envelope (a lipopolysaccharide component) is believed to be of primary importance — its composition changes depending on the source of energy [80]. Studies on the ability of various cell structures of *T. ferrooxidans* to oxidize ferrous iron, elemental sulfur, and sulfide minerals showed the necessity of two membrane systems to be present, viz. the outer membrane of the cell wall and the cytoplasmic membrane [81].

The cell wall seems to participate both in the transformation of water-insoluble S^{O} and sulfide minerals and in the transport of substrates to the cytoplasmic membrane. However, the problem needs further investigation since an outer membrane is lacking in a number of sulfur-oxidizing bacteria, e.g. Archaebacteria and Gram-positive bacteria.

Bioenergy processes are thought to occur in the cytoplasmic membrane containing cytochromes which constitute the most important components of the electron transfer chain.

Oxidation of Fe^{2+}. To date the oxidation of Fe^{2+} has only been studied in *T. ferrooxidans.* The reaction of ferrous iron oxidation may be represented in the following general form:

$$2Fe^{2+} + 1/2\ O_2 + 2H^{+} \rightarrow 2Fe^{3+} + H_2O$$

According to the calculations of Tuovinen and Kelly, the oxidation of two Fe^{2+} ions results in the synthesis of one ATP molecule [82].

The electron transfer chain involves cytochromes *a*, *c*, and *b*, ubiquinone, and copper-containing proteins: rusticyanine and ferredoxin [83—91]. The composition of the chain is quite common. The only difference is the high levels of cytochromes *a* and *c* which amount to 50—140 times the average content in heterotrophic organisms [92]. Rusticyanine and cytochrome *c* amount to 5–10% of the total cellular protein [88—90]. Large percentages of these components are the result of the oxidation of substrates with high potentials [93].

Spectrophotometric studies have revealed the following sequence of the electron transfer [84]:

$$\text{rusticyanine} \rightarrow \text{cyt. } c \rightarrow \text{cyt. } c_1 \rightarrow \text{cyt. } (a_1 a_1) \rightarrow O_2$$

Rusticyanine, a protein composed of a single polypeptide chain with a copper atom, serves as the initial acceptor of electrons [84, 88, 89]. Addition of this component to the electron transfer chain causes a 4-fold stimulation of Fe^{2+} oxidation [94]. It has been shown with the help of nuclear magnetic resonance spectrometry that the earlier stages of oxidation occur on the outer surface of the cytoplasmic membrane while the terminal one (cytochrome a_1) is located within the membrane [95, 96]. A putative arrangement of the components of the electron transfer chain in *T. ferrooxidans* during Fe^{2+} oxidation is presented in Fig. 9 [97].

Such an array of the components in the electron transfer chain produces a membrane potential favourable for ATP synthesis.

Reverse electron flow. Fe^{2+} has a high potential and is incapable of NAD reduction necessary for autotrophic fixation of carbon dioxide. *T. ferrooxidans* therefore has another electron transfer chain responsible for the flow of no more than 10% of the overall amount of electrons. This is an energy requiring process [83]. In view of the potentials of quinone, cytochromes (c_1 and *b*), and ferredoxin, it seems reasonable to suggest that they are involved in this process. A possible pathway of the reverse electron transfer may be the Q-cycle (Fig. 10) [98, 99]. In this case only quinone can serve as an electron and proton carrier because of the great potential difference between its oxidized and reduced forms. Owing to its high potential, reduced ubiquinone is likely to accept electrons probably transferred from cytochrome *c*. The sites of ubiquinone oxidation and reduction are the inner and outer surfaces of the cytoplasmic membrane respectively. Electrons from oxidized ubiquinone are transferred to NAD by a carrier, e.g. ferredoxin. Q-cycle is sustained by the chemical potential, i.e. the difference between hydrogen ion concentrations on both sides of the membrane.

Oxidation of Cu^+, Sn^{2+}, and U^{4+}. No reliable evidence has been obtained to support the view that Cu^+ and Sn^{2+} oxidation is coupled with a phosphorylating system since both ions are readily subjected to chemical oxidation at low pH [100, 101]. U^{4+} oxidation by *T. ferro-*

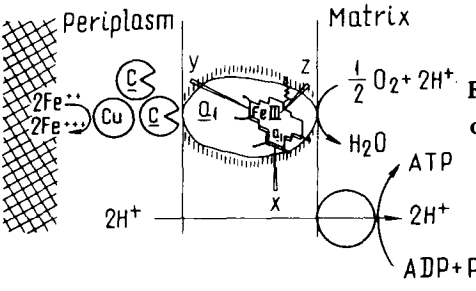


Fig. 9. Schematic representation of Fe^{2+} oxidation by *T. ferrooxidans*

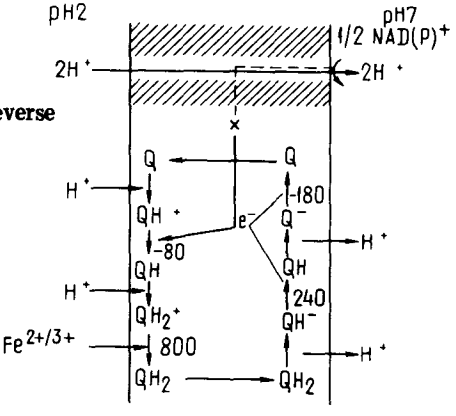


Fig. 10. Schematic representation of reverse electron flow in *T. ferrooxidans*

oxidans is an energy-yielding reaction coupled with carbon dioxide fixation [102]. Its ability to be oxidized by rusticyanine proves that electrons of U^{4+} can be directly incorporated into the electron transfer chain. Experiments with the use of specific inhibitors seem to indicate a resemblance of this chain composition to that involved in the oxidation of ferrous iron [102]. The energy output of the reaction oxidizing U^{4+} has been calculated to equal 130.4 kJ/M [102].

Oxidation of reduced sulfur compounds. The process of the oxidation of reduced sulfur compounds consists of two steps, viz. the oxidation of sulfur components by corresponding oxidases and the transport of electrons along the electron transfer chain from an oxidized substrate to terminal electron acceptors, i.e. oxygen and nitrate if *T. denitrificans* is used. With regard to *T. neapolitanus* and *T. thioparus* ATP synthesis was demonstrated to be coupled with electron transfer [103—106]. Fluorescence measurements brought out the association between electron transfer and proton transport in *T. denitrificans* [107]. These data confirm that oxidation of reduced sulfur compounds by *thiobacilli* involves a chemiosmotic mechanism of energy generation.

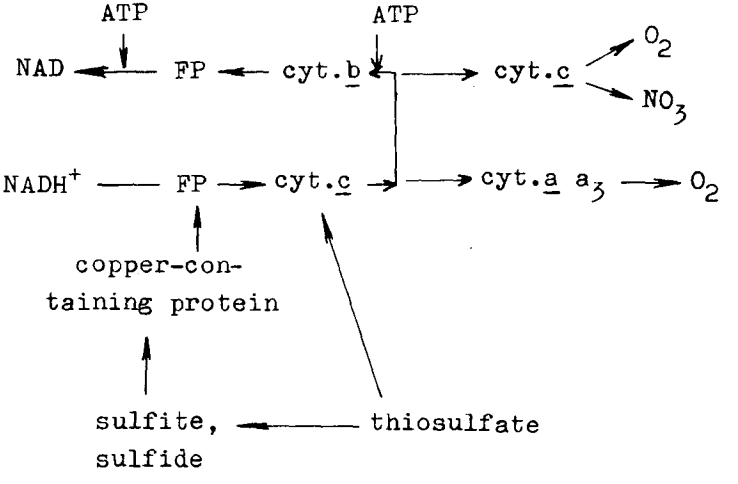
The electron transfer chain includes several types of cytochrome *c* [108—113], cyt. *b* [108, 110, 114], and cyt. *a* [108, 110—112].

Ubiquinone has been found in *T. thiooxidans* and *T. thioparus* [115, 116] and flavoproteids — in *T. denitrificans* [117].

Many questions are still to be answered concerning localization of enzymes facilitating this process, sites of electron "intrusion" into the transfer chain, and the innate properties of primary and intermediate products.

The nature of the substrate to be oxidized seems to determine the rate of electron incorporation into the respiratory chain and the "triggerring" of one or another pathway of electron transfer. For instance, during oxidation of sulfide to sulfur by *T. concretivorus** electron transfer is effected via a copper-containing protein, flavine, and cytochromes *b*, *c*, and *a*. In sulfite oxidation, flavines, ubiquinone, and cytochromes *b*, *c*, and *a* were involved [110].

The same conclusion was arrived at in the studies of the electron transfer chain in *T. denitrificans* [111]. Terminal fragments of the chain are also subject to variations depending on the last electron acceptor as can be clearly seen in *T. denitrificans* [117]:



Reduction of NAD occurs by means of reverse electron transfer as in Fe^{2+} oxidation [118—120].

Oxidation of sulfur. Elemental sulfur undergoes bacterial action before enzymatic oxidation. Many authors reported that oxidation of sulfur by *thiobacilli* is accompanied by the accumulation of lipoid substances [121—123]. Lipids are most actively released into the medium and concentrated on the surface of sulfur at the beginning of the log phase [124]. The exogenous lipoid products were identified as phospholipids. Studies of fractionation patterns of the stable sulfur isotopes have demonstrated that the action of *T. ferrooxidans* on elemental sulfur is a step-wise process generating colloid products that

* To date it is referred to as *T. thiooxidans* [4].

chiefly contain light isotopes. Phospholipids play an important role in the solubilization and fractionation of sulfur isotopes [125].

Three systems have been described to be responsible for the oxidation of sulfur. The first one is located in cytosol and requires glutathione to be present in the amount sufficient to catalyze the process [126]. The second system contains a cell wall — membrane complex [116, 127], and the third one consists of cytosolic and membrane fractions [128, 129].

Sulfite was shown by the formaldehyde trapping method to be the first product of sulfur oxidation in *T. thiooxidans* and *T. thioparus* [130]. The following mechanism of the oxidation of elemental sulfur has been suggested [131]:

$$S_n + GSH \rightarrow GSS_nH$$
$$GSS_nH + O_2 + H_2O \rightarrow GSS_{n-1}H + SO_3^{2-} + 2H^+$$

The sulfite thus produced is oxidized to sulfate via sulfite-oxidizing pathway.

Cytosolic system produces glutathione-polysulfide and oxidizes it to SO_3^{2-} [126]. Complete oxidation to sulfate is accomplished by the membrane-containing system [116, 127—129]. The total system is believed to include a sulfur-oxidizing enzyme and a sulfite-oxidizing system. The former is likely to be located in a periplasmatic interspace, and is therefore detectable in the membrane and cytosolic fractions. The sulfite-oxidizing system is to be found in the membrane. If sulfhydryl groups are lacking in the system, GSH or analogous components need be added.

Oxygenase is a sulfur oxidizing enzyme containing a ferric ion as a cofactor [130]. No evidence is thus far available as to its coupling with the respiratory chain. It follows from the theory of Mitchell and enzyme localization data that energy is unlikely to be generated at the first stage of sulfur oxidation. ATP synthesis is coupled with the second stage of this process, i.e. sulfite oxidation.

Oxidation of sulfide. Electrometric studies as well as the use of labelled sulfide indicate that the first oxidation products are contained in the membrane fraction [111, 131]. They are absorbed at greater wavelength than colloid sulfur. The authors have inferred from these observations that the first product of sulfide oxidation is polysulfide.

In case of *T. neapolitanus* sulfide oxidation was observed to be a two-step process [132]. The first stage is characterized by a low rate while the second one proceeds rapidly. At the first stage soluble sulfide disappears, the reaction being inhibited by copper-binding substances. This step is supposed to be promoted in the presence of cytosol and is similar to the reaction catalyzed by the sulfur-oxidizing system [133]. A copper-containing protein seems to be the primary sulfide acceptor.

The second stage of sulfide oxidation is associated with the membrane fraction, electrons of polysulfide being transferred along the respiratory chain which comprises cytochromes *b*, *c*, and *a* [115, 132]. Thus the reaction of sulfide oxidation may be presented as follows:

$$\underbrace{S \rightarrow \text{Cu-containing protein}}_{\text{First stage}} \rightarrow \underbrace{\text{flavines ?} \rightarrow \text{cyt. } b \rightarrow \text{cyt. } c \rightarrow \text{cyt. } d \rightarrow O_2}_{\text{Second stage}}$$

T. denitrificans can utilize No_3^{2-} as the terminal electron acceptor.

Oxidation of sulfite. This process constitutes the final stage of the oxidation of reduced sulfur compounds and is shown to proceed via two alternative pathways: adenosine phosphosulfate synthesis (APS) and direct oxidation. The former pathway of sulfite oxidative conversion to sulfate has been elucidated by Peck [134]. It appears to occur at the substrate phosphorylation level:

$$2SO_3^{2-} + 2AMP \xrightarrow{\text{APS-reductase}} 2APS + 4e^-$$

$$2APS + 2P_i \longrightarrow 2ADP + 2SO_4^{2-}$$

$$ADP \xrightarrow[\text{kinase}]{\text{adenylate}} AMP + ATP$$

Further studies of APS-reductase have shown that SO_3^{2-} reduces FAD contained in the enzyme to $FADH_2$. Partial oxidation of $FADH_2$ is observed upon addition of AMP [134]:

$$E\text{-}FAD\text{-}Fe^{3+} + SO_3^{2-} + H_2O \rightarrow E\text{-}FADH_2Fe^{3+}(SO_4^{2-})$$

$$E\text{-}FADH_2\text{-}Fe^{3+}(SO_4^{2-}) + AMP \rightarrow E\text{-}FADH\text{-}Fe^{2+}(APS) + OH^-$$

APS-reductase amounts to 4—5% of the total protein in the cell. The data obtained point to a significant contribution of the sulfite oxidation via adenosine phosphosulfate to the energy metabolism in *thiobacilli* [133, 136, 137].

Direct oxidation of sulfite is effected by sulfite-cytochrome *c* oxidoreductase. This enzyme was isolated from *T. thioparus*, *T. intermedius*, and *T. ferrooxidans* [137—139]. Sulfite oxidase was shown to be associated with the cytoplasmic membrane in various species of *thiobacilli* [110, 111, 116, 128]. The use of inhibitors enabled some authors to demonstrate that SO_3^{2-} oxidation involved a total electron transfer system, i.e. cytochromes *a*, *b*, and *c* and possibly flavines [128, 140, 141]. Thus, different *thiobacilli* possess a similar mechanism of direct sulfite oxidation coupled with oxidative phosphorylation.

Oxidation of thiosulfate. Thiosulfate is oxidized either directly or through cleavage. The former reaction is catalyzed by a thiosulfate

oxidizing enzyme found in *T. neapolitanus*, *T. ferrooxidans*, and *T. thioparus* [113, 142, 143]:

$$2\ S_2O_3^{2-} \rightarrow O_3SSSSO_3^- + 2\ e^-$$

Electrons are accepted by cytochrome *c* or ferricyanide [113, 137, 142—144]. Further oxidation of tetrathionate is supposed to proceed via polythionates according to the following scheme [145—148]:

$$S_4O_6^{2-} \rightarrow S_3O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$

However, this assumption has failed to be confirmed experimentally. No enzymes catalyzing thiosulfate oxidation via this pathway were found. Besides, *thiobacilli* have been reported to be incapable of polythionate conversion [134, 149, 150].

Thiosulfate oxidation through cleavage is effected either directly or after reduction. The reductive cleavage is catalyzed by thiosulfate reductase and occurs in the presence of reduced glutathione [124, 151, 152]:

$$S_2O_3^{2-} + 2\ GSH \rightarrow GSSG + H_2S + SO_3^{2-}$$

$$H_2S + 1/2\ O_2 \rightarrow S^O + H_2O$$

The course of thiosulfate cleavage under the influence of rhodanase is represented by the following equations [153]:

$$R\text{-}SH + S\text{-}SO_3^{2-} \xrightarrow[\text{rhodanase}]{} RS\text{-}SH + SO_3^{2-}$$

$$R\text{-}S\text{-}SH + O_2 + H_2O \xrightarrow[\text{sulfuroxidase}]{} SO_3^{2-} + 2\ H^+$$

S^O and SO_3^{2-} produced during thiosulfate oxidation through cleavage are oxidized by systems oxidizing either sulfur or sulfite.

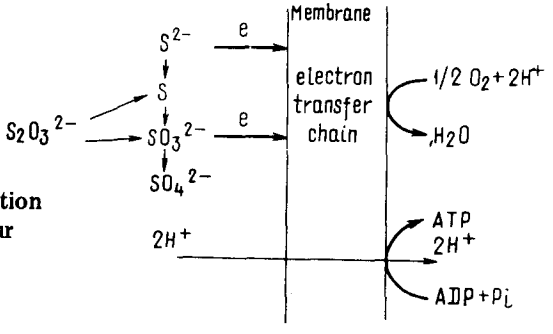


Fig. 11. Schematic representation of oxidation of reduced sulfur compounds by thiobacilli

Rhodanase was shown to be present in *T. denitrificans*, *T. ferrooxidans*, and *Thiobacillus* A2 [154—158]. It was identified in the membrane fraction [150, 153], and appears to be coupled with the systems oxidizing sulfur and sulfite. Therefore, the cleavage products, S^0 and SO_3^{2-}, are eventually converted to sulfate.

The general course of the sulfur compound oxidation is presented in Fig. 11. It can be seen that oxidation of reduced sulfur compounds is a step-by-step process, proceeding in stages corresponding to compounds of different levels of reduction. Some energy is generated during substrate phosphorylation. But its main portion accumulates as membrane potential that is formed in accordance with Mitchell's theory as a consequence of spatial uncoupling of initial and terminal oxidative reactions by the membrane.

CONSTRUCTIVE METABOLISM

Autotrophic fixation of carbon dioxide by acidophilic organisms oxidizing ferrous iron, elemental sulfur, and sulfide minerals has only been investigated in *thiobacilli* and *Sulfolobus*. The Calvin reductive ribulosodiphosphate cycle is the main pathway of carbon dioxide fixation both in *thiobacilli* and the majority of other chemolithotrophic Eubacteria. Upon uptake by the cell carbon dioxide interacts with ribulosodiphosphate under the influence of ribuloso-1,5-diphosphate carboxylase and is converted to 3-phosphoglyceric acid, a product of autotrophic assimilation. High activity of ribuloso-1,5-diphosphate carboxylase has been recorded in many species of *thiobacilli* [159, 160]. It is worth mentioning that in *T. intermedius* the enzyme exists as the t-type and is thus similar to that in higher plants [161].

T. novellus has been reported to possess hydroxypyruvate reductase along with enzymes for autotrophic fixation. This enzyme catalyzes the assimilation of c_1-compounds via serine pathway, its metabolic function being obscure [162].

The overall reaction of the cycle may be represented as follows:

$$3\,CO_2 + 9\,ATP + 5\,H_2O + 6NADH \rightarrow HCOCHOHCH_2OH_2PO_3 +$$

$$+ 9\,ADP + 6NAD + 8H_3PO_4$$

Obviously, ATP and CO_2-NADH reducing complex are necessary for the cycle to be operating. They are synthesized in the course of energy metabolism and are responsible for its coupling with constructive metabolism in *thiobacilli*.

Carboxylation of phosphoenole pyruvate constitutes a by-way of carbon dioxide assimilation. In obligate autotrophic organisms the Krebs cycle is disengaged at the level of α-keto-glutaric acid conversion to succinic acid [163] while in *thiobacilli* it performs a purely synthetic function [164].

Autotrophic assimilation of carbon dioxide in *Sulfolobus* proceeds via reductive tricarboxylic acid cycle rather than the Calvin cycle, with malic, aspartic, glutamic, and citric acids, as well as an unidentified substrate X, playing a key role in the process [18].

CONCLUSION

It is concluded from the data presented in this paper that oxidation of ferrous iron, reduced sulfur compounds, and sulfide minerals can be accomplished by microorganisms belonging not only to different genera but also to different kingdoms. Notwithstanding the differences of their morphology, ultrastructure, and some physiological properties, these organisms are similar in that they share one important feature, viz. the ability to obtain energy by oxidizing the above mentioned substances. Presumably, this similarity is based on the capacity to accumulate the proton potential generated during the oxidation of these substrates. In such a case the microorganisms in question should be expected to have much in common as regards the composition of the electron transfer chain and the enzyme pool catalyzing the transformation and oxidation of reduced sulfur compounds and ferrous iron. The convergent nature of physiological and, perhaps, biochemical similarity of these organisms is obvious.

REFERENCES

1. Karavaiko, G.I., Kuznetsov, S.I., and Golomzic, A.I., Role of Microorganisms in Metal Leaching in Ores, Nauka, Moscow (1972) (in Russian).
2. Brierley, C.L., *CRC Critic. Rev. Microbiol., 6,* (3), 207 (1978).
3. Murr, L.E., *Minerals Sci. Eng., 12,* (3), 121 (1980).
4. Bergey's Manual of Determinative Bacteriology, 8th Ed., Baltimore (1971).
5. Woese, C.R., *Sci. Amer., 244,*(6), 9 (1981).
6. Brierley, J.A., and Le Roux, N.W., In: Conference Bacterial Leaching, W. Schwartz (Ed.), Verlag Chemie, New York, 55, (1977).
7. Le Roux, N.W., Wakerley, D.S., and Hunt, S.D., *J. Gen. Microbiol, 100,* 197 (1977).
8. Karavaiko, G.I., and Avakyan, A.A., *Mikrobiologiya, 39,* 950 (1970).
9. Markosyan, G.E., *Biologicheskii zhurnal Armenii, 25,* 26 (1972).
10. Balashova, V.V., Vedenina, I.Ya., Markosyan, G.E., and Zavarzin, G.A., *Mikrobiologiya, 43,* 581 (1974).
11. Pivovarova, T.A., Markosyan, G.E., and Karavaiko, G.I., *Mikrobiologiya, 50,* 482 (1981).
12. Golovacheva, R.S., and Karavaiko, G.I., *Mikrobiologiya, 47,* 815 (1978).
13. Golovacheva, R.S., *Mikrobiologiya, 48,* 528 (1979).
14. Golovacheva, R.S., *Mikrobiologiya, 48,* 681 (1979).
15. Golovacheva, R.S., *Mikrobiologiya, 48,* 863 (1979).
16. Brock, T.D., Brock, K.M., Belly, R.T., and Weiss, R.L., *Arch. Microbiol., 84,* 54 (1972).

17. Brierley, C.L., and Brierley, J.A., *Can. J. Microbiol., 19*, 183 (1973).
18. Fuchs, G., and Stupperich, E., *Zbl. Bacteriol. Hyd.*, I abt., C3, 536 (1982).
19. De Rosa, M., Gambacorta, A., and Bu'Lock, J.D., *J. Gen. Microbiol., 86*, 156 (1975).
20. Millong, G., de Rosa, M., Gambacorta, A., and Bu'Lock, J.D., *J. Gen. Microbiol., 86*, 165 (1975).
21. Zillig, W., Stetter, K.O., Wunderl, S., Schutz, W., Priess, H., and Scholz, J., *Arch. Microbiol., 125*, 259 (1980).
22. Brock, T.D., Thermophilic Microorganisms and Life at High Temperatures, Springer Verlag, New York — Heidelberg — Berlin (1978).
23. Weiss, R.L., *J. Gen. Microbiol., 77*, 501 (1973).
24. Gromova, L.A., Pereverzev, N.A., and Karavaiko, G.I., *Mikrobiologiya, 47*, 2, 293 (1978).
25. Karavaiko, G.I., and Pivovarova, T.A., In: Conference Bacterial Leaching, W. Schwartz (Ed.), Verlag Chemie, New York, 37 (1977).
26. Pivovarova, T.A., and Karavaiko, G.I., *Mikrobiologiya, 44*, 269 (1975).
27. Tcherni, N.E., Solovyeva, Zh.V., Fedorov, V.D., and Kondratyeva, E.N., *Mikrobiologiya, 38*, 479 (1969)
28. Remsen, C., Watson, S., Waterbury, J., and Trüper, H., *J. Bacteriol., 95*, 2374 (1968).
29. Watson, S., *Arch. Microbiol., 75*, 179 (1971).
30. Weiss, R.L., *J. Bacteriol., 118*, 275 (1974).
31. Avakyan, A.A., and Karavaiko, G.I., *Mikrobiologiya., 39*, 855 (1970).
32. Shively, J.M., Decker, G.L., and Greenawalt, J.W., *J. Bacteriol., 101*, 2, 618 (1970).
33. Holt, S.C., Shively, J.M., and Greenawalt, J.M., *Can. J. Microbiol., 20*, 1347 (1974).
34. Caeseele, L., and Lees, H., *Can. J. Microbiol., 15*, 7, 651 (1969).
35. Shively, J.M., Ball, F.L., Brown, D.H., and Saunder, R.E., *Science, 182*, 584 (1973).
36. Fitz-James, P., and Young, E., In: The Bacterial Spore, G.W. Gold and A. Hurs (Eds.), London—New York, 39 (1969).
37. Ohye, A.D., and Murrell, W.G., *J. Cell Biol., 14*, 11 (1962).
38. Warth, A.D., Ohye, D.F., and Murrell, W.G., *J. Cell Biol., 16*, 579 (1963).
39. Waksman, S.A., and Ioffe, I.S., *J. Bacteriol., 7*, 2, 239 (1922).
40. Parker, C.D., and Prisk, T., *J. Gen. Microbiol., 8*, 13, 344 (1953).
41. Kodama, A., and Mori, T., *Plant Cell Physiol., 9*, 707 (1968).
42. Barton, L.L., and Shively, J.H., *J. Bacteriol., 95*, 2, 720 (1968).
43. Colmer, A.E., and Hinkle, M.E., *Science, 106*, 252 (1947).
44. Lyalikova, N.N., In: Application of Bacterial Leaching of Non-Ferrous Metals from Submarginal Ores, Tsvetmetinformatsia, Moscow, 5 (1968) (in Russian).
45. Silver, M., *Can. J. Microbiol., 16*, 9, 845 (1970).
46. Guay, R., and Silver, M., *Can. J. Microbiol., 21*, 3, 281 (1975).
47. Brierley, J.A., and Lockwood, E.J., *FEMS Microbiol. Letters, 2*, 163 (1977).
48. Brierley, J.A., *Appl. Environment. Microbiol., 36*, 3, 523 (1978).
49. Brierley, J.A., Norris, F.R., Kelly, D.P. and Le Roux, N.W., *Europ. J. Appl. Microbiol. Biotechnol., 5*, 4, 291 (1978).
50. Norris, P.R., and Kelly, D.P., *FEMS Microbiol. Letters, 4*, 143 (1978).
51. Norris, P.R., Brierley, J.A., and Kelly, D.P., *FEMS Microbiol. Letters, 7*, 119 (1980).

52. Karavaiko, G.I., Kovalenko, T.A., ıd Golovacheva, R.S., In: Proceedings Internat. Conference on Use of Microorganisms in Hydrometallurgy, Pécs, Hungary, 95 (1980).
53. Golovacheva, R.S., and Karavaiko, G.I., *Mikrobiologiya., 50,* 6, 1113 (1981).
54. Brock, T.D., Thermophilic Microorganisms and Life at High Temperatures, M.P. Starr (Ed.), Springer Verlag, New York — Heidelberg — Berlin (1978).
55. Brierley, C.L., *J. Less Common Metals, 36,* 237 (1974).
56. Lu, M., Matin, A., and Rittenberg, S., *Arch. Microbiol., 79,* 354 (1971).
57. Tuovinen, O.H., and Kelly, D.P., Z. *Allg. Mikrobiol., 12,* 4, 311 (1972).
58. Trivedi, N.C., and Tsuchia, H.M., *Internat. J. Mineral Processing, 2,* 1 (1975).
59. Shaffia, F., and Wilkinson, R.F., *J. Bacteriol., 97,* 1, 256 (1969).
60. Shaffia, F., Brinson, K.R., Heinzman, M.W., and Brady, J.B., *J. Bacteriol., 111,* 1, 56 (1972).
61. Harrison, A.P., Jarvis, B.W., and Johnson, J.L., *J. Bacteriol., 143,* 1, 448 (1980).
62. Brock, T.D., and Gustafson, J., *Appl. Environ. Microbiol., 32,* 567 (1976).
63. Chakrabarty, A., in: Metallurgical Application of Bacterial Leaching and Related Microbiological Phenomena, L. Murr and A. Torma (Eds.), London, 137 (1978).
64. Hoffman, L., and Hendrik, J., *Biotechnol. Bioeng., 18,* 1161 (1976).
65. Kelly, D.P., and Jones, C.A., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L. Murr and A. Torma (Eds.), London, 19 (1978).
66. Kovalenko, T.V., and Karavaiko, G.I., *Mikrobiologiya, 50,* 2, 326 (1981).
67. Kovalenko, T.V., Karavaiko, G.I., and Piskunov, V.P., *Mikrobiologiya, 51,* 1, 156 (1982)
68. Dave, S.R., Natarajan K.A., and Bhat, J.V., Institution of Mining Metallurgy Section C, *88,* 234 (1979).
69. Sugio, T., Tano, T., and Imai, K., *Agric. Biol. Chem., 45,* 9, 2037 (1981).
70. Mao, M.W., Dugan, P.R., Martin, P.A., and Tuovinen, O.H., *FEMS Microbiol. Letters, 8,* 121 (1980).
71. Skulatchev, V., Essays on Bioenergetics, Molodaya Gvardia, Moscow (1982) (in Russian).
72. Mitchell, P., *Bioenergetics, 3,* 5 (1972).
73. Cook, T.M., *J. Bacteriol., 88,* 620 (1964).
74. Karavaiko, G.I., and Pivovarova, T.A., *Mikrobiologiya, 42,* 389 (1973).
75. Baldensperger, J., Guarraia, L.J., and Numhreys, W.J., *Arch. Microbiol., 99,* 323 (1974).
76. DiSpirito, A.A., Dugan, P.R., and Tuovinen, O.H., *Biotechnol, Bioeng., 25,* 4, 1163 (1983).
77. Weiss, R.L., *J. Gen. Microbiol., 77,* 2, 501 (1973).
78. Wang, W.S., Korczynsky, M.S., and Lundgren, D.G., *J. Bacteriol., 104,* 1, 556 (1970).
79. Gromova, L.A., Karavaiko, G.I., Sevtsov, L.V., and Pereverzev, N.L., *Mikrobiologiya, 52,* 3, 455 (1983)
80. Vestal, J.R., and Lundgren, D.G., *Can. J. Microbiol., 19,* 11, 1335 (1973).
81. Tano, T., and Lundgren, D.G., *Appl. Environ. Microbiol., 35,* 6, 1198 (1978).

82. Tuovinen, O.H., and Kelly, D.P., *Z. Allg. Microbiol., 12,* 311 (1972).
83. Tikhonova, G.V., Lissenkova, L.L., Doman, N.G., and Skulatchev V.P., *Biokhimiya, 32,* 725 (1967).
84. Ingledew, W.J., and Cobley, J.G., *Biochem. Biophys. Acta, 590,* 141 (1980).
85. Dugan, P.R., and Lundgren, D.G., *J. Bacteriol., 89,* 825 (1965).
86. Short, A.S., White, D.C., and Aleem, M.I., *J. Bacteriol., 99,* 142 (1969).
87. DiSpirito, A.A., Loh, W.H., and Tuovinen, O.H., *Arch. Microbiol., 135,* 77 (1983).
88. Cobley, J.G., and Haddoc, B.A., *FEBS Lett., 61,* 29 (1975).
89. Cox, J.C., and Boxer, D.H., *Biochem. J., 174,* 497 (1978).
90. Vernon, L.P., Mangum, J.H., Beck, J.V., and Shaffia, F.M., *Arch. Biochem. Biophys., 88,* 227 (1960).
91. Anderson, K.J., and Lundgren, D.G., *Can. J. Microbiol., 15,* 73 (1969).
92. Lundgren, D.G., Pfister, R.M., and Merrick, J.M., *J. Gen. Microbiol., 33,* 441 (1964).
93. Aleem, M.I., Lees, H., and Nicholas, D.J., *Nature, 200,* 759 (1963).
94. Imai, K., and Konno, H., *J. Biochem.* (Jap), *49,* 29 (1975).
95. Case, G.D., and Leigh, J.S., *Biochem. J., 160,* 768 (1976).
96. Case, G.D., Ohnishi, T., and Leigh, J.S., *Biochem. J., 160,* 785 (1976).
97. Ingledew, W.J., *Bioch. Biophys. Acta, 683,* 89 (1982).
98. Rich, P.R., *FEBS Lett., 130,* 173 (1981).
99. Rich, P.R., and Bendall, D.A., *Biochem. Biophys. Acta, 542,* 506 (1980).
100. Nielsen, A.M., and Beck, V., *Science, 175,* 1124 (1972).
101. Lewis, A.J., and Miller, J.D., *Can. J. Microbiol., 23,* 319 (1977).
102. DiSpirito, A., and Tuovinen, O.H., *Arch. Microbiol., 133,* 1, 28 (1982).
103. Cole, J.S., and Aleem, M.I., *Biochem. Biophys. Res. Commun, 38,* 736 (1970).
104. Ross, A.J., Schoenhoff, R.L., and Aleem, M.I., *Biochem, Biophys. Res. Commun, 32,* 301 (1968).
105. Saxena, J., and Aleem, M.I., *Can. J. Biochem., 51,* 560 (1973).
106. Kelly, D.P., and Syrett, P.J., *Nature, 202,* 597 (1964).
107. Tuovinen, O.H., Nicholas, D.J., and Aleem, M.I., *Arch. Microbiol., 113,* 11 (1977).
108. Blaylock, B., and Nason, A., *J. Biol. Chem., 238,* 10, 3453 (1963).
109. London, J., *Science, 140,* 409 (1963).
110. Moriarty, D.J., and Nicholas, D.J., *Biochem. Biophys. Acta, 197,* 143 (1970).
111. Aminuddin, M., and Nicholas, D.J., *J. Gen. Microbiol., 82,* 115 (1974).
112. Aleem, M.I., *J. Bacteriol., 90,* 95 (1965).
113. Trudinger, P.A., *Biochem. J., 78,* 673 (1961).
114. Peeters, T., and Aleem, M.I., *Arch. Microbiol., 71,* 319 (1970).
115. Cook, T.M., and Umbreit, W.W., *Biochemistry, 2,* 194 (1963).
116. Adair, F.W., *J. Bacteriol., 92,* 899 (1966).
117. Peeters, T., and Aleem, M.I., *Arch. Microbiol., 71,* 319 (1970).
118. Aleem, M.I., *J. Bacteriol., 91,* 729 (1966).
119. Aleem, M.I., *Antonie van Leeuwenhoek J. Microbiol. Serol., 35,* 379 (1969).
120. Saxena, J., and Aleem, M.I., *Arch. Microbiol., 84,* 317 (1972).
121. Cook, T.M., *J. Bacteriol., 88,* 620 (1964).
122. Schaeffer, W., and Umbreit, W., *J. Bacteriol, 85,* 2, 492 (1963).
123. Shively, I., and Benson, A., *J. Bacteriol., 94,* 5, 1679 (1967).
124. Karavaiko, G.I., and Pivovarova, T.A., *Mikrobiologiya, 42,* 389 (1973).

125. Pivovarova, T.A., Miller, Yu.M., Krasheninnikova, S.A., Kapustin, O.A., and Karavaiko, G.I., *Mikrobiologiya, 51*, 4, 552 (1982)
126. Suzuki, I., *Biochem. Biophys. Acta, 104*, 359 (1965).
127. Taylor, B.F., *Biochem. Biophys. Acta, 170*, 112 (1968).
128. Kodama, A., and Mori, T., *Plant Cell Physiol., 9*, 725 (1968).
129. Kodama, A., *Plant Cell Physiol., 10*, 645 (1969).
130. Suzuki, I., and Silver, M., *Biochem. Biophys. Acta, 122*, 22 (1966).
131. Moriarty, D.J., and Nicholas, D.J., *Biochem. Biophys. Acta, 184*, 114 (1969).
132. Saxena, J., and Aleem, M.I., *Can. J. Biochem., 51*, 560 (1973).
133. Suzuki, I., *Ann. Rev. Microbiol., 28*, 85 (1974).
134. Peck, H., *Bacteriol. Rev., 26*, 1, 67 (1962).
135. Adachi, K., and Suzuki, I., *Can. J. Biochem., 55*, 91 (1977).
136. Bowen, T.J., Happold, F.C., and Taylor, B.F., *Biochem. Biophys. Acta, 118*, 566 (1966).
137. Lyric, R.M., and Suzuki, I., *Can. J. Biochem., 48*, 344 (1970).
138. Charles, A.M., *Arch. Biochem. Biophys., 129*, 124 (1969).
139. Vestal, J.R., and Lundgren, D.G., *Can. J. Biochem., 49*, 1125 (1971).
140. Takakuwa, S., *Plant Cell Physiol., 17*, 103 (1976).
141. Kodama, A., Kodama, T., and Mori, T., *Plant Cell Physiol., 11*, 701 (1970).
142. Trudinger, P.A., *Biochem. J., 78*, 680 (1961).
143. Trudinger, P.A., *J. Bacteriol., 89*, 617 (1965).
144. Silver, M., and Lundgren, D.G., *Can. J. Biochem., 46*, 1215 (1968).
145. Trudinger, P.A., *Rev. Pure Appl. Chem., 17*, 1 (1967).
146. Vishniac, W., and Santer, M., *Bacteriol. Rev., 21*, 195 (1957).
147. London, J., and Rittenberg, S.C., Proc. Natl. Acad. Sci. USA, *52*, 1183 (1964).
148. Trudinger, P.A., *Biochem. J., 90*, 640 (1964).
149. Peck, H.D., *Ann. Rev. Microbiol., 22*, 489 (1968).
150. Charles, A.M., and Suzuki, I., *Biochem. Biophys. Acta, 128*, 510 (1966).
151. Peck, H.D., Proc. Natl. Acad. Sci. USA, *46*, 1053 (1960).
152. Peck, H.D., and Fisher, E.J., *J. Biol. Chem., 237*, 190 (1962).
153. Oh, J.K., and Suzuki, I., *J. Gen.Microbiol., 99*, 413 (1977).
154. Smith, A.J., and Lascelles, J., *J. Gen. Microbiol., 42*, 357 (1966).
155. Yoch, D.C., and Lindstrom, E.S., *J. Bacteriol., 106*, 700 (1971).
156. Bowen, T.J., Butler, P.J., and Happold, F.C., *Biochem. J., 97*, 651 (1965).
157. Tabita, R., Silver, M., and Lundgren, D.G., *Can. J. Biochem., 47*, 1141 (1969).
158. Silver, M., and Kelly, D.P., *J. Gen. Microbiol., 97*, 277 (1976).
159. Rao, G.S., and Berger, L.R., *J. Bacteriol., 102*, 462 (1970).
160. Tabita, R., and Lundgren, D.G., *J. Bacteriol.,108*, 328 (1971).
161. Bowman, L.H., and Chollet, R., *J. Bacteriol., 141*, 652 (1980).
162. Chandra, T.S., and Shethna, Y.I., *J. Bacteriol., 181*, 389 (1977).
163. Kelly, D.P., *Sci. Progr., 55*, 31 (1967).
164. Zavarzin, G.A., Lithotrophic Microorganisms, Nauka, Moscow (1972) (in Russian).

SOME CHARACTERISTICS OF IRON-OXIDIZING THIOBACILLI ISOLATED FROM URANIUM MINE LEACH LIQUORS

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INTRODUCTION

The present paper summarizes recent work undertaken to elucidate morphological and physiological characteristics of acidophilic iron-oxidizing thiobacilli isolated from the Agnew Lake uranium mine leach liquors. The Agnew Lake Mines Ltd. started full-scale uranium leaching operation in January 1977 in northern Ontario, Canada. The cyclic solution flow has been presented [1] and involves the following stages. The acidic leach liquor (pH < 2) is sprayed on the surface heap and the seepage is collected in a holding pond. The leach circuit also involves the treatment of underground stopes in a successive cascade type fashion. Both percolation (drip) and flood leaching have been practiced with the underground stopes at various times during the life of the mine. Uranium is separated from the leach liquor by ion exchange and the barren solution is recycled to the leach circuit. To maintain a suitable water balance and to lower the concentration of other solubilized metals (e.g., iron, thorium), some of the barren solution is drawn off to the tailings. The concentrations of uranium, thorium, radium, Fe^{2+}, and total iron in various points of the leach cycle have been presented [1]. The leach circuit does not accommodate a separate stage for the microbiological and chemical oxidation of ferrous iron. The data for soluble iron show that ferric iron predominates in all parts of the leach circuit, indicating that (i) some iron oxidation occurs concurrently with the leaching of uranium from the ore material; and (ii) ferric iron is not completely reduced by the ore.

The deposit contains on average less than 2 lbs. U_3O_8 per ton ore. Uranium is mineralized mainly as uranothorite (Th/U $\sim$ 4/1) in the ore body. Uranium also occurs as uraninite associated with pyrite conglomerates. In addition to pyrite, pyrrhotite occurs in some areas of the deposit. The gangue contains quartz with silica matrix and there is no clay in the ore.

The leaching of uranium at Agnew Lake is effected by ferric iron in sulfuric acid, as shown for uraninite in the following equation:

$$UO_2 + 2Fe^{3+} \rightarrow UO_2^{2+} + 2Fe^{2+} \qquad (1)$$

Ferrous iron thus produced is a carrier for electrons from uranium oxidation and is re-oxidized by thiobacilli (*Thiobacillus ferrooxidans):*

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \qquad (2)$$

$$\Delta G_{30^\circ C} = -9.1 \text{ kcal/mole}$$

The redox potential of the leach liquor has been consistently $>$ +400 mV (range +413 to +645 mV) which is also indicative of the predominantly oxidized form of iron in the solution. Speciation of dissolved sulfur anions in Agnew Lake leach liquors has not been reported. The sulfate concentration has usually varied between 5 and 10 g of SO_4—S per liter. It is likely that local areas of low oxygen tension may exist in the ore piles, giving rise to the formation of elemental sulfur and thionates especially from the incomplete oxidation of pyrrhotite.

IRON-OXIDIZING THIOBACILLI IN AGNEW LAKE LEACH LIQUORS

Iron-oxidizing thiobacilli were enumerated in leach liquor samples collected from various stages of the leach circuit [1, 2]. The viable numbers ranged from < 1 to over 10^6 bacteria per ml leach solution. There was no seasonal pattern of fluctuation in the viable counts. Attempts were not made to enumerate iron-oxidizers attached on ore material but in view of work reported elsewhere [3], a significant proportion of the iron-oxidizing population can be presumed to be associated with the surfaces and fractures of ore particles. Currently, there are no adequate methods to enumerate thiobacilli attached on ore material. Activity estimates based on the iron-oxidation, oxygen uptake, or carbon dioxide fixation seem possible but their relationship with bacterial density would be difficult to assess for environmental samples. For the Agnew Lake ore, pyrite (and pyrrhotite) is the source of soluble iron and sulfuric acid. The microbiological oxidation of pyrite requires a contact between the mineral and thiobacilli but the reversibility of the contact is not known. Thus, the iron-oxidizers found in the leach liquors are not only involved in ferrous iron oxidation but they may also have been in prior close association with pyrite and ore surface before sloughing off.

Eleven isolates of iron-oxidizing thiobacilli derived from leach liquor samples were maintained for laboratory studies. The concentration of uranium and thorium in the respective leach liquor samples varied in the range of 0.07—0.92 mM U and 3.28—5.99 mM Th.

Fig. Electron micrograph of negatively stained *Thiobacillus ferrooxidans* TFI-1 isolated from the Agnew Lake uranium mine. Both a polar flagellum and several pili can be seen on the cell.

Bar, 0.5 μm

All isolates were motile under a light microscopic examination but the relative abundance of motile cells in each culture varied greatly. To enhance the flagellation, cultures growing in a ferrous iron liquid medium [2] were incubated in test tubes without shaking. The presence of flagella was confirmed by transmission electron microscopic examination of bacteria collected from the surface layer of the cultures in test tubes [2]. Samples were negatively stained with uranyl acetate before examination.

Two different types of flagellation were observed among the isolates [2]. One isolate, designated as TFI-1, had one polar flagellum per cell (Fig.), conforming to the taxonomic description of *Thiobacillus ferrooxidans* [4]. Peritrichous flagella were detected in isolates TFI-9 and TFI-10. Flagella of TFI-1 had a maximum length of 9 μm and an average diameter of 19.9 nm. The average diameter of peritrichous flagella was 19.3 nm for TFI-9 and 18.2 nm for TFI-10.

Pili were observed both in TFI-1 and TFI-10; their average diameter was 8.9 nm and 4.9 nm, respectively.

Flagella and pili are known to facilitate the attachment of microorganisms to surfaces. Scanning electron microscopic studies in general have indicated that thiobacilli can be found in large numbers on mineral surfaces immersed in bacterial cultures. In addition, as for example in enteric bacteria, pili may be involved in genetic functions but this has not been demonstrated with thiobacilli. Glycocalyx-type extracellular polysaccharides, acting as an adhesive and protective layer, may also be produced by iron-oxidizing thiobacilli in their natural environment, as commonly found with bacteria in aquatic habitats, but remain to be characterized for *T. ferrooxidans*.

TOXICITY OF UO_2^{2+} AND Th^{4+}

Because both uranium and thorium are concurrently solubilized from the Agnew Lake ore material, it was of interest to compare their relative toxicities to the *T. ferrooxidans* isolates derived from leach

liquor samples. At pH 1.5, the toxic concentrations varied between 0.5 and 3.5 mM UO_2^{2+} and 1 and 2.5 mM Th^{4+}. At pH 2.5 the respective concentrations were between 1 and > 5 mM UO_2^{2+} and 1 and > 5 mM Th^{4+}. Isolates that were resistant to uranium (> 5 mM) were also resistant to thorium [5].

The toxic concentrations of uranium and other metal ions vary depending on the test method used. For example, in addition to pH-related effects, the toxicity of a metal ion is influenced by the substrate, cell age and density, aeration, length of incubation, and prior growth history of the organisms. There is a need to standardize procedures for testing metal toxicity to thiobacilli before valid interlaboratory comparisons can be made.

The toxic mechanism of uranium and other metals is not known but probably involves several causes. Uranium has a tendency to bind with nucleic acids which alone would suffice to impair cell growth. In general, carbon dioxide fixation by whole cells of *T. ferrooxidans* is more sensitive to uranium than is oxygen uptake coupled with ferrous iron oxidation [6], suggesting an uncoupling effect by uranium. It is also possible that metal ions such as UO_2^{2+} bind on the cell surface, thereby interfering with substrate (e.g., Fe^{2+}) oxidation and transport processes.

PLASMID PROFILES OF THE AGNEW LAKE ISOLATES

All *T. ferrooxidans* isolates from the Agnew Lake uranium mine contained plasmid DNA but each isolate had a different plasmid composition. A plasmid of about 13 ± 0.5 megadaltons was detected in *T. ferrooxidans* isolates TFI-1, TFI-4, TFI-5, TFI-7, and TFI-10. A 40 megadalton plasmid was present in both TFI-4 and TFI-8 and a 46 megadalton plasmid was found in TFI-5 and TFI-11 [1].

RESPONSE OF IRON-OXIDIZING ISOLATES TO URANYL ION

Isolate TFI-7 was resistant to 2 mM uranium and had a 13 megadalton plasmid when grown in the presence or absence of 2 mM uranyl sulfate in the growth medium [7]. Isolate TFI-13 was originally sensitive to 2 mM uranium but became resistant upon subculturing in the presence of uranium. Upon initial exposure to uranyl sulfate the cells of TFI-13 did not appear to have a 13 megadalton plasmid and a massive cell death occurred within a matter of hours. In the survivors, a 13 megadalton plasmid was detected. Upon re-exposure of

the plasmid-bearing population of TFI-13 to 2 mM uranium, no further cell death occurred [7].

When subcultured repeatedly in the absence of uranium, the 13 megadalton plasmid became undetectable (by agarose gel electrophoresis) and the bacteria were rendered sensitive to 2 mM UO_2^{2+}. When the plasmid-carrying TFI-13 population was subcultured repeatedly in the presence of 2 mM UO_2^{2+}, the resistance and the 13 megadalton plasmid persisted.

These results indicated that the 13 megadalton plasmid may have been responsible for conferring uranium resistance in TFI-13, but a direct causative relationship remains to be determined by techniques of plasmid curing and gene transfer. In the non-resistant population of TFI-13 the plasmid may be present in a very low copy number or only in very few cells, and in the presence of uranium the plasmid DNA is probably increased in copy number. Alternatively, this DNA may become undetectable by the technique used, due to its transposition in the absence of uranium in the growth medium. Moreover, 2 mM uranyl sulfate in the medium may select for the fraction of the resistant, plasmid-carrying bacteria from the total population.

In isolate TFI-4 the 13 megadalton plasmid was also detected only when the culture was challenged with uranium in the growth medium, suggesting a relationship between uranium resistance and plasmid DNA.

OXIDATION OF URANOUS ION

Manometric studies with washed cell suspensions indicated the following stoichiometry of oxygen uptake coupled with uranous ion oxidation [5]:

$$2U^{4+} + O_2 + 2H_2O \rightarrow 2UO_2^{2+} + 4H^+ \qquad (3)$$

$$\Delta G_{30^\circ C} = -31.2 \text{ kcal/mole}$$

Experimental values of O_2-uptake agreed within 107% for the theoretical ratio of $2U^{4+}/1\ O_2$. Ferrous iron was used separately as a reference substrate with an average of 92.5% agreement with the ratio of $4Fe^{2+}/1\ O_2$ (equation 2).

Uranous oxide (UO_2; $U/O < 0.5$) was also oxidized by washed cell suspensions of *T. ferrooxidans* but at slower rates than the soluble uranous sulfate [8].

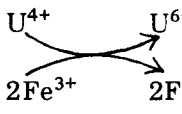
The fixation of $^{14}CO_2$ was used as a measure of energy conservation coupled with the oxidation of U^{4+} and Fe^{2+} [5]. The ef-

ficiency per electron pair transferred in the transport chain ($2e^-$ from 2 Fe^{2+}; $2e^-$ from 1 U^{4+}) was in the range of about 11% for both ions. Since the ΔG values indicate that more energy is available from uranous ion oxidation compared with that of ferrous iron, it is apparent that the energy from Fe^{2+}-oxidation is used more efficiently on a thermodynamic basis.

Both substrates are likely to couple at the cytochrome *c* level of the electron transport chain, with rusticyanin as an intermediate carrier protein. By use of selective inhibitors of carbon dioxide fixation and oxygen uptake, evidence was obtained for the reverse electron flow from cytochrome *c* to cytochrome *b*, quinones, and flavoprotein before the reduction of NAD^+ during the oxidation of either one of the substrates [5].

Major differences were established in the kinetic parameters of uranous ion and ferrous iron oxidation. The V_{max} and K_M^{app} for uranous ion were 0.06—0.08/μmoles/min/mg protein and 0.031—0.132 mM, respectively, and for ferrous iron these values were 2.13—2.47 and 1.41—1.58, respectively [8]. These results indicated that the rates of uranium oxidation, expressed as V_{max}, did not vary much in different isolates. For ferrous iron, the K_M^{app} values were more uniform than those for uranous ion.

Mixed substrate studies with U^{4+} and Fe^{2+} indicated that at a fixed concentration of 0.25 mM U^{4+}, no enhancement of oxygen uptake was obtained with the addition of < 0.5 μM Fe^{2+} [8]. Above this Fe^{2+}-concentration, there was an increased rate of oxygen uptake. The mixed substrate studies in general indicated that uranous ion was a competitive inhibitor of ferrous iron oxidation. However, because of the redox reactions involved, i.e.,



U(IV)/U(VI) = +344 mV

Fe(II)/Fe(III) = +770 mV

the interactions between oxidized and reduced forms of iron and uranium cannot be separated to individual reactions in mixed substrate studies, particularly because also inhibitory interactions are involved.

The direct oxidation of reduced compounds of uranium has been suggested previously [9, 10], but no firm conclusions could be made previously before the kinetic evaluation of the oxidation. The diffe-

rences in the kinetic parameters of uranium and iron oxidation strongly suggest that in the experiments described, uranium was oxidized directly by *T. ferrooxidans* without a detectable contribution by the cyclic involvement of trace levels of iron. Moreover, added trace levels of iron (< 0.5 mM Fe^{2+}) did not accelerate the rate of uranium oxidation. Another acidophile, *T. acidophilus* AFK-1 (grown with $S_4O_6^{2-}$), has been shown to oxidize both U^{4+} and Fe^{2+}, whereas after growth on glucose, this strain oxidized glucose and Fe^{2+} but not U^{4+} [11]. This observation suggests that uranium oxidation is not associated with the iron oxidation system. Growth of *T. ferrooxidans* in a chemically defined medium with uranium as the substrate has not been reported.

CONCLUDING REMARKS

The ability of *T. ferrooxidans* to derive energy from uranium oxidation demonstrates its respiratory versatility. In addition to iron, sulfur, and uranium, *T. ferrooxidans* has been reported to oxidize inorganic compounds of Sn(II), Cu(I), and Se (as selenide) [12, 13, 14]. It is not known whether these microbiological oxidation systems for uranium, tin, and copper are of significance in microbiological leaching situations in view of the recycling role of ferric and ferrous iron in acid leach solutions.

The role of multiple plasmids in *T. ferrooxidans* from the Agnew Lake uranium mine and from other sources [1, 15] has not been characterized. The genetic system in *T. ferrooxidans*, and generally in chemolithotrophic bacteria, is in its incipient stage of research. Genetic techniques such as transformation and chemical curing of plasmids that have been developed with heterotrophic bacteria, do not readily avail themselves to *T. ferrooxidans* owing to the acidophilic and lithoautotrophic nature of this organism. Cloning of thiobacillus plasmids in better characterized systems (for example, *Escherichia coli)* has been accomplished [16], but the lack of knowledge of plasmid borne traits in thiobacilli must be resolved before the genetic system can be better explored.

REFERENCES

1. Tuovinen, O.H., Silver, M., Martin, P.A.W., and Dugan, P.R., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi et al. (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 59 (1980).

2. DiSpirito, A.A., Silver, M., Voss , L., and Tuovinen, O.H., *Appl. Environ. Microbiol., 43,* 1196 (1982).
3. Bhappu, R.B., Johnson, P.H., Brierley, J.A., and Reynolds, D.H., *Trans. Soc. Min. Engin. (AIME), 244,* 307 (1969).
4. Vishniac, W.V., *Thiobacillus,* In: Bergey's Manual of Determinative Bacteriology, 8th ed., R.E. Buchanan and N.E. Gibbons (Eds.), 456, Williams and Wilkins, Baltimore (1974).
5. DiSpirito, A.A., and Tuovinen, O.H., *Arch. Microbiol., 133,* 28 (1982).
6. Tuovinen, O.H., and Kelly, D.P., *Arch. Microbiol., 95,* 165 (1974).
7. Martin, P.A.W., Dugan, P.R., and Tuovinen, O.H., Annual Meeting of the American Society for Microbiology, Abstract I 34, 92 (1981).
8. DiSpirito, A.A., and Tuovinen, O.H., *Arch. Microbiol., 133,* 33 (1982).
9. Ivarson, K.C., *Curr. Microbiol., 3,* 253 (1980).
10. Soljanto, P., and Tuovinen, O.H., In: Biogeochemistry of Ancient and Modern Environments, P.A. Trudinger, M.R. Walter, and B.J. Ralph (Eds.), Australian Academy of Sciences and Springer Verlag, Canberra, New York, 469 (1980)
11. DiSpirito, A.A., and Tuovinen, O.H., *Geomicrobiol. J., 2,* 275 (1981).
12. Nielsen, A.M., and Beck, J.V., *Science, 175,* 1124 (1972).
13. Torma, A.E., and Habashi, F., *Can. J. Microbiol., 18,* 1780 (1972).
14. Lewis, A.J. and Miller, D.J.D., *Can. J. Microbiol., 23,* 119 (1977).
15. Martin, P.A.W., Dugan, P.R., and Tuovinen, O.H., *Can. J. Microbiol., 27,* 850 (1981).
16. Holmes, D.S., Lobos, J.S., and Bopp, L.H., Microbial Chemoautotrophy Colloquium, The Ohio State University, Columbus, Abstracts, 10 (1982).

A STUDY OF GROWTH AND DEVELOPMENT OF *THIOBACILLUS FERROOXIDANS* WITH REGARD TO OXIDATION OF Fe^{2+} AND SULFIDE MINERALS BY MEANS OF MATHEMATICAL MODELLING

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INTRODUCTION

Production processes and their control cannot be optimized without more or less complete mathematical models of the processes involved. Mathematical modelling is now widely used in various branches of science, such as physics, chemistry and biology, as well as in industry and agriculture. Leaching of metals from ores involves processes of biological and chemical nature coupled with a physical process of diffusion. The present work deals with some aspects of the theory of growth and development of microorganisms and attempts to use it as a basis for mathematical modelling of microorganism-mediated leaching.

INTRODUCTION TO THE THEORY OF GROWTH AND DEVELOPMENT OF MICROORGANISMS

The foundations of mathematical modelling of microbiological processes have been laid down in 1942 by the studies of the outstanding French scientist Monod. He has experimentally established that specific growth rate of *Escherichia coli* cells is a hyperbolic function of substrate concentration (Fig. 1a) and proposed to express this function, now called the Monod equation, as follows:

$$\mu(S) = \frac{dx}{xdt} = \frac{\mu_m \cdot S}{K_s + S} \qquad (1)$$

Variables that enter this and following equations are explained in the Appendix.

A theoretical growth curve derived from the Monod model has an exponential growth phase, a deceleration phase and a stationary

phase (Fig. 1b). However, when a culture is transferred to a different environment the exponential phase will often be preceded by the lag phase, a delay of growth, whose duration depends upon the environment, the state of the culture, the amount of the inoculate, etc.

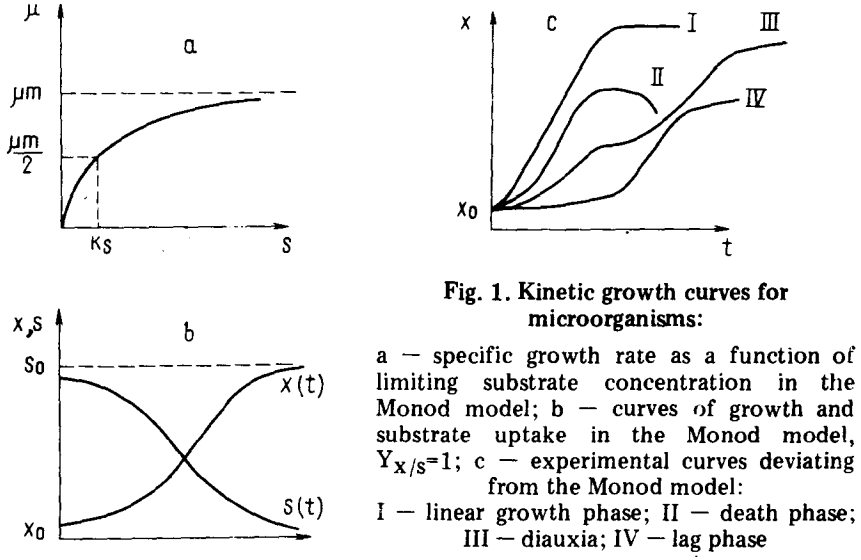

Fig. 1. Kinetic growth curves for microorganisms:

a — specific growth rate as a function of limiting substrate concentration in the Monod model; b — curves of growth and substrate uptake in the Monod model, $Y_{x/s}=1$; c — experimental curves deviating from the Monod model:
I — linear growth phase; II — death phase; III — diauxia; IV — lag phase

(Fig. 1c, curve IV). The stationary phase can be followed by a death phase characterized by a decrease in the number of viable cells and the total cell mass (Fig. 1c, curve II). The growth of a real culture may also exhibit other deviations from growth curves predicted by the Monod model. A typical feature of growth curves of microorganisms, having gas as their substrate (i.e. hydrogen-oxidizing, methane-oxidizing bacteria) is the presence of a long interval of linear growth (Fig. 1c, curve I). Some cultures, including *Thiobacillus ferrooxidans*, may, under certain conditions, show a recurrence of intensive growth following a deceleration phase, the so-called "false diauxia" (Fig. 1c, curve III). Neither these, nor certain other deviations from theoretical growth curves can be described by merely choosing the values of parameters of the Monod model.

It is noteworthy that the relationship between the specific growth rate and the substrate concentration (equation 1) is similar to the

relationship between the specific rate of enzymatic reaction and the concentration of the rate-limiting substrate:

$$V = \frac{V_m \cdot S}{K_s + S} \qquad (2)$$

Such a resemblance between eq. (1) and (2) has subsequently prompted the development of the "bottle neck" theory for enzymatic catalysis chain. It has been found [1] that the rate of end product formation in any system of enzymatic reaction is governed by the rate of the slowest reaction of the system. The "bottle neck" theory has stimulated a rapid development in the mathematical modelling of microbial growth in 1960—1970. By analogy with the well-known kinetics of enzymatic reactions, new, distinct from the classical Monod's type, relationships between specific growth rate and concentrations of substrate, product, inhibitor, activator, and acidity of the medium [2, 3] have been looked for (and found!).

To incorporate into the model the dependence of specific growth rate upon concentrations of product, inhibitors, etc., additional equations for these new variables have to be introduced. This extends the range of applications of the model but makes it much less straightforward.

In some works the Monod model was modified by revising its second postulate, the invariability of the yield coefficient $Y_{x/s}$. This coefficient was assumed to vary due to some portion of energy being used outside the synthesis process (maintenance energy), or alternatively, the equation system was merely supplemented with $Y_{x/s}$ as a function of some parameter of the process [3].

A probability approach to growth of microbial populations is used in the so-called stochastic models which consider distribution of cells in age, length or size.

The most promising approach to mathematical modelling of microbial growth involves the development of structured models [4, 5]. The formulation of a structured model is preceded by a phenomenological description of the process of intracellular synthesis of the substance in question or the biomass, based on the available biochemical and physiological information. This phenomenological verbal description is then translated into a system of mathematical equations, usually differential. The results obtained by solving this system are compared with experimental data, thus either confirming or disproving the initial assumptions on the mechanism of synthesis regulation. The

next step in developing an adequate structured model involves refining or even changing the initial hypothesis. This may suggest what further experimental research is needed and, at the same time, expands our knowledge on the object under study. One of the first fundamental structured models was that by Fredrickson and Ramkrishna which considered the interaction between cell structure elements: nucleic acids and proteins.

Structured models were advanced still further in the studies [5, 6] in which the kinetic equations of the population growth were derived by considering the induction and repression of synthesis of relevent enzymes.

The research into the behaviour of a microbial population under multifactor action has brought attention to another postulate inherent in the Monod model, namely the existence of a single "bottle neck" in a chain of enzymatic reactions.

A hypothesis suggesting that, in the course of transformation of the initial substrate into the biomass, one "bottle neck" may be gradually replaced by another has offered a relatively simple way to account for almost all microbial growth curves observed. According to a model proposed by Petrova et al. [7], this transformation proceedes in two steps: a limiting exogeneous substrate, S, is converted into an intermediate product, P, which can be represented by an endogenous product, by ATP, by intermediate metabolites, etc. At the second step P serves as a substrate for the reaction of biomass, X, formation: $S \rightarrow P \rightarrow X$.

Most of microbial growth curves found in experiments can be at least qualitatively described by solutions obtained by simulating this model [7] (Fig. 1c). Plainly, such solutions will depend upon the initial concentration of the intermediate product P and therefore upon the history of the culture, i.e., the inoculum preparation. As a special case, the model can also produce the classical S-shaped curves of the Monod model.

The introduction of continuous cultivation of microorganisms gave birth to a theory of this process based on mathematical models of the growth of batch cultures. If the relationship between the specific growth rate and the concentration of a single growth-limiting substrate follows the Monod equation (equation 1), the steady-state concentrations of biomass and substrate in the chemostat will vary with the dilution rate as shown in Fig. 2a. The curve $\bar{x}(D)$ shown in Fig. 2a is called the classical chemostat Monod curve; such curves are observed when the main growth-limiting enzymatic reaction obeys equation (2).

When the specific growth rate depends on substrate concentration in some other way, or is affected by some other factors, or else the replacement of "bottle necks" is possible, the chemostat curve may differ from the classical one (Fig. 2b): multiple steady states may occur (Fig. 2c), or stable sustained oscillations may be generated around a certain equilibrium point (Fig. 2d). These types of deviations from the chemostat Monod model have been studied both theoretically and experimentally [3, 8].

A turbidostat cultivation, as distinct from a chemostat method, is based of the concentration of the biomass rather than dilution rate as the control parameter. Comparative advantages and shortcomings of the two cultivation methods have been discussed elsewhere [3].

Concluding this brief review on the existing mathematical models for microbiological processes we would like to emphasize that in the recent years most workers have abandoned, quite rightly, the generalized mathematical models and turned to developing models that describe a particular process accomplished by particular species or populations of bacteria under given conditions of culturing. Comprehensive surveys covering the existing models, their areas of application, methods of their study and also their utilization in the microbiological industry have been published [2—6, 8, 9].

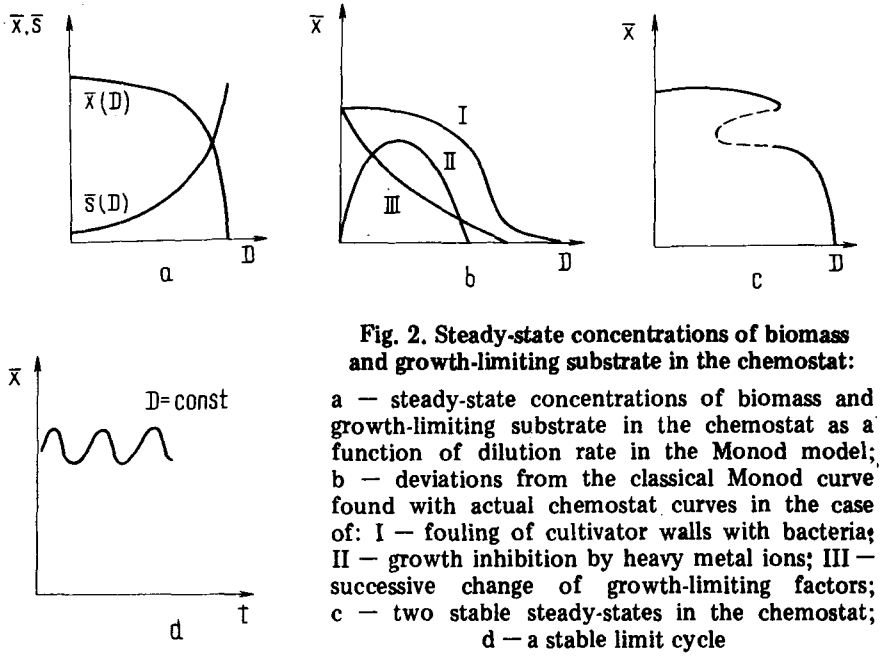

Fig. 2. Steady-state concentrations of biomass and growth-limiting substrate in the chemostat:

a — steady-state concentrations of biomass and growth-limiting substrate in the chemostat as a function of dilution rate in the Monod model; b — deviations from the classical Monod curve found with actual chemostat curves in the case of: I — fouling of cultivator walls with bacteria; II — growth inhibition by heavy metal ions; III — successive change of growth-limiting factors; c — two stable steady-states in the chemostat; d — a stable limit cycle

MODELS OF LEACHING

Let us consider in more detail the few existing mathematical models of bacterial oxidation of iron and sulfide minerals. The process of bacterial oxidation presents many features to be described, explained and predicted with the aid of a mathematical model. The first objective is a description which should not be at variance with well established experimental data. This should be followed by explanation of those relations between interacting factors that are not obvious from the experiment, and by refutation or confirmation of the adopted hypothesis about the intracellular nature of synthesis regulation. Finally, the results offered by the model should be suggestive as to further experimental work and allow prediction of its results.

Developing mathematical models of oxidation of iron and of oxidation of sulfide-containing minerals are two tasks which are different, although interrelated. They are interrelated because microbiological leaching of metals from ores always involves both oxidation of sulfide minerals (chalcopyrite, pyrite, arsenopyrite, etc.) and regeneration of Fe^{3+}. The basic difference between these processes is the fact that a limiting substrate in oxidation of sulfide minerals is a substance incorporated into solid particles while in regeneration of Fe^{3+} it is a soluble substrate (Fe^{2+}) that is oxidized.

Of all possible oxidation processes that accompany leaching we shall consider bacterial oxidation mediated by *T. ferrooxidans*. Moreover, it can be argued that the bacterial leaching is merely a result of growth and development of this microorganism which derives the energy it requires from iron and sulfide sulfur oxidation reactions:

$$Fe^{2+} \xrightarrow{\text{bacteria}} Fe^{3+} + \bar{e};\ S^{2-} \xrightarrow{\text{bacteria}} SO_4^{2-} + 6\bar{e} \qquad (3)$$

In agreement with the available experimental evidence, we shall assume that both iron and sulfur compounds can serve as a limiting substrate for the growth of *T. ferrooxidans*.

Thus the problem of developing a leaching model reduces to two tasks: modelling of *T. ferrooxidans* growth on insoluble substrates in a heterogeneous medium, and modelling of Fe^{2+} oxidation in a liquid homogeneous suspension.

Two approaches to modelling microbial growth on poorly soluble substrates are known. In both cases these models are similar to that of Monod, with a replacement of substrate concentration, S, in equation (1) by "concentration" of finely ground solid particles or assuming S to be proportional to the total surface area of these particles. In the former case S does not depend on particle size, in the latter it is a function of the square of particle radius.

Studying *T. ferrooxidans* growth on various sulfides, Torma [10] has found that the relationship between the metal extraction rate and the pulp density (or the initial total accessible surface area of particles) follows the Michaelis-Menten equation. He warned against incorporating such a relationship directly into a model of the Monod type since the kinetic parameters V_m and K_s in equation (2) are not quite identical with the parameters μ_m and K_s of the Monod model.

In another study [11] the leaching of copper from chalcopyrite is considered as a chain of successive reactions: chemical oxidation, direct microbial oxidation, microbial oxidation of Fe^{2+}, and hydrolysis of Fe^{3+} compounds. The kinetics of oxidation of ferrous iron ions by *T. ferrooxidans* cells was assumed to be similar to the Monod kinetics, while the chemical reactions were assumed to proceed with the first order kinetics and obey the rules of chemical kinetics.

The mathematical model for nickel leaching, developed by Hendy and Rogers [12], is very much the same as in the case of copper leaching [11]. The authors point out that the model satisfactorily describes nickel extraction only in the course of the first 30 hours of cultivation when the growth of microorganisms is not much different from the ordinary exponential growth. The difference between predictions based on the model and the actual process is accounted for, in their opinion, by changes in the acidity and redox potential (Eh) of the medium, i.e. by a shift of the equilibrium of certain reactions or by some other changes in their course.

When *T. ferrooxidans* grows on pyrite, the biomass growth depends on both iron and sulfur oxidation. It may be assumed that similar processes take place in oxidation of chalcopyrite and other sulfide-containing minerals. Hence, an appropriate assumption reflecting the possible dependence of biomass growth rate on the other substrate, sulfur, would be worth introducing into the models discussed above [11, 12].

Both these models ignore heterogeneity of the medium and insolubility of the growth-limiting substrate, and deal instead with "concentrations" of finely ground solid particles.

Gormely et al. [13] have adopted a different approach to modelling the growth of a continuous culture of *T. ferrooxidans* on a zinc sulfide concentrate. The proposed model was based on the model due to Erickson treating insoluble substrates in heterogeneous fermentation processes. Substrate insolubility is the starting point of the latter model, which distinguishes it from the Monod model.

The authors produce an equation for a dynamic balance between the number of microbial cells in contact with solid zinc sulfide and a fraction of the population for which the solid substrate is not available.

The growth of cells is assumed to be possible only for the former portion of the population. Another important assumption of the Gormely model consists in taking the rate of bacterial growth to be directly proportional to the number of cells in contact with the solid substrate. As a result, the specific rate of population growth depends on both the concentration of bacteria and parameters pertaining to heterogeneity of the medium.

Using the condition of a stable steady-state in a continuous culture (μ = D), one arrives at an expression for $\overline{x}$(D). Strictly speaking, it is not to be considered as an expression for a chemostat curve since it should incorporate only the parameters of the process that for any rate of dilution are constant with time t. The Gormely's equation for $\overline{x}$(D) [13] contains a variable S, the "concentration" of available surface area of zinc sulfide, which stands for concentration of the growth-limiting substrate. During leaching S is naturally subject to variation due to chemical leaching which always takes place in such a medium, although at a very low rate.

Along with the dynamic parameters of population growth, the equation for $\overline{x}$(D) proposed by Gormely et al. [13] allows one to take into account the distribution of the concentrate particles in size, and a possible change in their geometry and in the equilibrium between the fractions of population in contact with the solid substrate and in the liquid phase. By using this equation, from an experimentally established relationship between the steady-state in a continuous culture of *T. ferrooxidans* and its cultivation conditions, many important physiological characteristics relating to growth of *T. ferrooxidans* on zinc sulfide and to the entire leaching process have been obtained. These are the rate of bacterial leaching, the yield coefficients for carbon and nitrogen, the value of free energy of oxidation, the rates of uptake of oxygen and nitrogen, etc. The stoichiometry of the main equation for zinc sulfide leaching was also confirmed.

The first mathematical model dealing with the growth of *T. ferrooxidans* on ferrous iron, and, hence, with Fe^{3+} regeneration, was proposed in 1970 [14]. The assumption was that the growth-limiting substrate was Fe^{2+} and the limiting stage of the process was associated with the $Fe^{2+} \rightarrow Fe^{3+} \rightarrow e^{-}$ reaction with the Monod kinetics. The resulting model was completely identical with the Monod model. A study of the exponential growth phase at constant pH 2.2 and various temperatures ranging from 20° to 31°C has shown the maximum growth rate and the Monod saturation constant to be temperature-dependent.

The entire curve of iron oxidation in the course of regeneration at two different temperatures 28° and 12°C, has been described by the model proposed by Petrova et al. [15], which will be considered in detail below.

CHOOSING AN ADEQUATE MODEL. FINDING KINETIC CHARACTERISTICS

Our approach to development of a mathematical model of Fe^{2+} oxidation was based on preliminary experiments on cultivation of a batch culture of *T. ferrooxidans* on ferrous iron at various temperatures. Fe^{2+} has been revealed to be growth-limiting substrate [14]. It has been found that *T. ferrooxidans* showed a usual S-shaped growth curve at 28°C while displaying the "false diauxia" at 12°C [16]. It was apparent that a model of the Monod type, assuming the existence of a single "bottle neck" in the chain of enzymatic reactions, namely the model described in ref. [14], was unable to describe both these curves. This, however, could be done on the basis of a model of the S → P → X type [7], and hence we made the following principal assumptions:

1) The process of growth of *T. ferrooxidans* on ferrous iron has two successive "bottle necks": one in the reaction of iron oxidation:

$$2FeSO_4 + 1/2\ O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O + 11\ kcal; \quad (4)$$

and the other in the hydrolysis reaction:

$$Fe_2(SO_4)_3 + 6H_2O \rightleftarrows 2Fe(OH)_3 + 3H_2SO_4 \quad (5)$$

2) The relationship between the specific rate of the former reaction (μ_1) and the concentration of its substrate, $FeSO_4$, is the one typical for enzymatic reactions and is given by the Monod equation (2). We had no experimental data as to the way in which μ_1, and therefore the bacterial growth rate, depend on acidity of the medium, that is on the concentration of H_2SO_4, the second substrate of the reaction. This relationship had to be found by means of mathematical modelling.

We assumed that oxygen concentration does not limit the rate of oxidation of Fe^{2+} in the experiment but temperature does affect it, that is

$$\mu_1 = \mu_1(Fe^{2+}, H_2SO_4, T) = \frac{\mu_1 m(T) Fe^{2+}}{K_s(T) + Fe^{2+}} \cdot f_1 \cdot (H_2SO_4, T) \quad (6)$$

(the symbols of variables are explained in the Appendix and in ref. [15]).

3) The relationship between specific rate of hydrolysis of ferric sulfate (μ_2) and concentration of Fe^{3+} had not been established in previous experiments and had to be found by mathematical modelling. It was only clear that in the presence of *T. ferrooxidans* and at concentrations of Fe^{3+} studied this rate was different from that of an ordinary chemical hydrolysis and that it was a function of pH of the medium and temperature, i.e.

$$\mu_2 = \mu_2(Fe^{3+}, H_2SO_4, T) = \mu_2(Fe^{3+}, T) \cdot f_2(H_2SO_4, T) \qquad (7)$$

4) The specific rate of Fe^{2+} oxidation in the range of temperatures under consideration is proportional to the specific growth rate. This assumption was based on experimental curves for growth and oxidation of iron by *T. ferrooxidans* at different temperatures and allowed only three variables characterizing the process to be considered in the initial version of the model: the concentration of ferrous iron (Fe^{2+}), the concentration of ferric iron (Fe^{3+}), and pH value. It should be noted that in the pH range considered (pH 2—3) the H_2SO_4 concentration is approximated as a linear function of the medium acidity measured in pH units.

Schematically, the process of Fe^{2+} oxidation can be presented as follows:

$$Fe^{2+} + H_2SO_4 \rightarrow Fe^{3+} \rightarrow Fe(OH)_3 + H_2SO_4 \qquad (8)$$

By taking into account the mass balance of each substance in the appropriate reactions, we arrive at the following system of equations:

$$\begin{aligned} \frac{dFe^{2+}}{dt} &= -\mu_1 \cdot Fe^{3+} \\ \frac{dFe^{3+}}{dt} &= \left(\frac{1}{Y_1}\mu_1 - \mu_2\right) Fe^{3+} \\ \frac{dpH}{dt} &= -\left(-\frac{1}{Y_2}\mu_1 + \frac{1}{Y_3}\mu_2\right) Fe^{3+} \end{aligned} \qquad . \quad (9)$$

Several different functions have been tried as $\mu_2(Fe^{2+})$, $f_1(pH)$, $f_2(pH)$, and the adequacy of the models obtained has been tested (Fig. 3a, b, c). All these functions have been chosen of particular physiological and biochemical grounds.

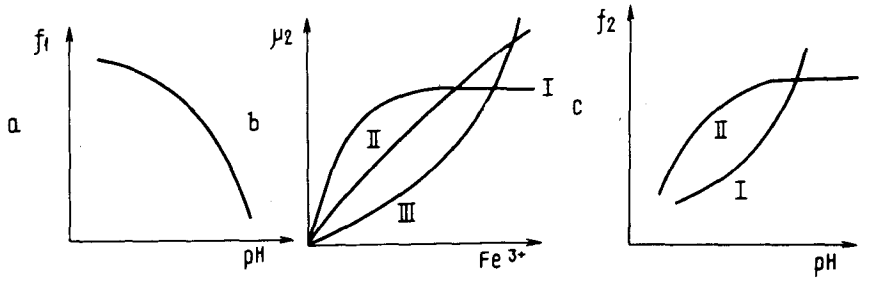

Fig. 3. Versions of hypothetical kinetic characteristics considered in the model of ref. [15]:

a — the specific rate of Fe^{2+} oxidation as a function of the H_2SO_4 concentration (plotted in pH coordinates); b — the specific rate of hydrolysis as a function of the Fe^{3+} concentration; c — the specific rate of hydrolysis as a function of acidity

The relationship between specific rate of Fe^{2+} oxidation and concentration of the second substrate of the reaction, H_2SO_4, was assumed to be the one typical for enzymatic reactions — a hyperbolic curve of the Monod type — which when plotted against pH looks as shown in Fig. 3a. Three different relationships between specific rate of hydrolysis and its substrate Fe^{3+} were considered: that of an ordinary enzymatic reaction (Fig. 3b, curve I); the one of a chemical reaction of the first order (Fig. 3b, curve II); the one of the chemical reaction affected by the action of biocomplexes (Fig. 3b, curve III). In the latter case this relationship is described by the following formula:

$$\mu_2(Fe^{3+}) = K_2(Fe^{3+})^{K_3} \qquad (10)$$

The effect of pH of the medium on the rate of hydrolysis of ferric sulfate was accounted for by the Le Chatelier-Braun principle. Apparently, with increased concentration of H_2SO_4 (a product of hydrolysis), the rate of hydrolysis should decrease. We considered two possible forms of this relationship, $f_2(H_2SO_4)$, which are shown in Fig. 3c plotted against pH.

An adequacy test of these assumptions showed that only one their combination can account satisfactorily for experimental curves describing iron oxidation by microorganisms.

Simulation results are presented in Figs. 4, 5, 6. Figs. 4a, b show, respectively, experimental and theoretical ferric and ferrous iron concentrations and pH of the medium as functions of time.

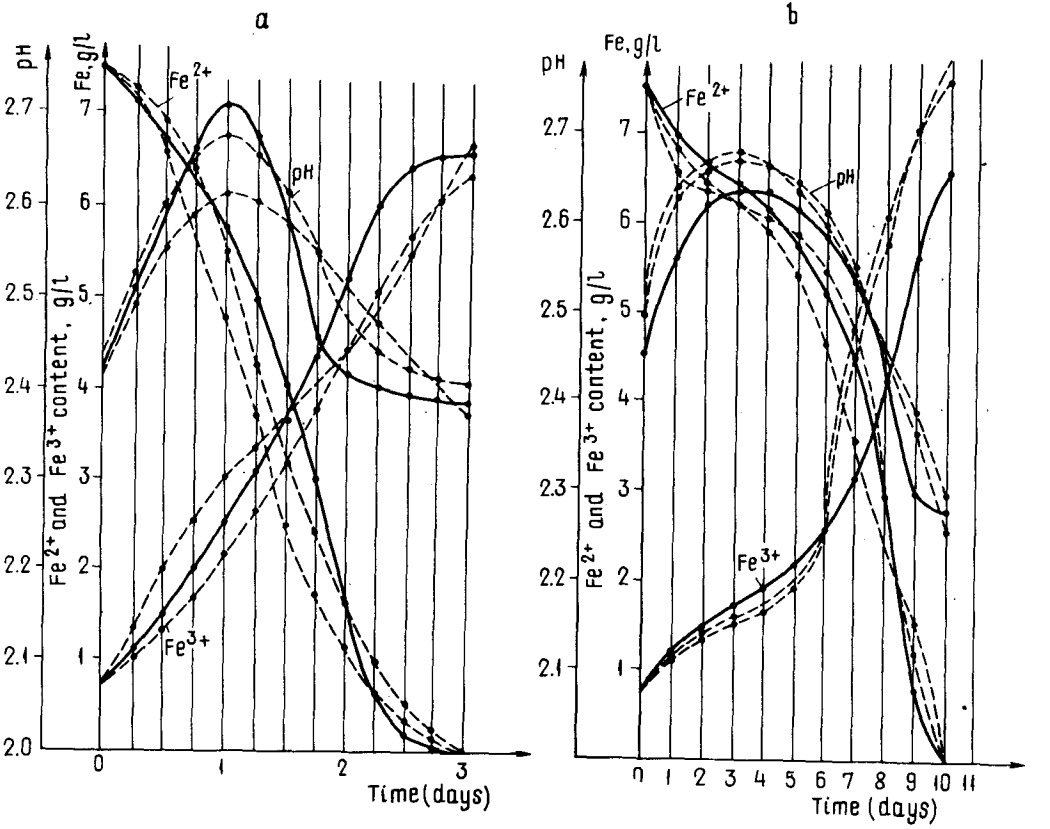

Fig. 4. Concentrations of ferrous iron, of ferric iron and pH of the medium as functions of time:

a — at 28 °C; b — at 12 °C, theory (solid line) and experiment (dashed line)

As can be seen from Fig. 5a, a decrease in substrate concentration does not affect specific growth rate of *T. ferrooxidans* until the concentration drops below 4—5 g/l, the saturation constant happens to be roughly the same for the two temperatures tested.

Nor do the maximum specific growth rate values differ much for the two cases — 1.42 l/day and 0.997 l/day at 28°C and at 12°C, respectively. With the second reaction (Fig. 5b) the principal difference in the graphs of $\mu_2(Fe^{3+})$ at 28°C and 12°C is the presence in the latter case of an interval $0 < Fe^{3+} < 4$ (g/l) where μ_2 shows very little variation. It is indeed the occurrence of this quasi-horizontal region that gives rise to the three-phase growth curves.

Figs. 6a and 6b show the effect of pH on the specific rates of Fe^{2+} oxidation and Fe^{3+} hydrolysis, respectively. The model suggests that hydrolysis of ferric sulfate depends on Fe^{3+} concentration as a chemical reaction (K_3 = 1.76) and on pH of the medium as an enzymatic reaction.

As can be inferred from presented figures, temperature has no gross effect on the overall pattern of the curves but merely changes their shape. This change is of a certain biological significance indicating a narrower or a wider range of pH values that have no effect on specific rates of the reactions. It can be seen from Fig. 6 that for the first reaction at 28°C such pH values should be below 2.8, while at 12°C they should not exceed roughly 2.4. For the second reaction this range will be given by pH > 2.5 at 28°C and by pH > 2.3 at 12°C. It can be concluded, therefore, that maximum reaction rates will be obtained for pH values within 2.5 to 2.8 at 28°C and within 2.3 to 2.4 at 12°C, i.e. at a lower temperature the optimum pH range seems to be shifted leftwards. The model implies that provided pH of the

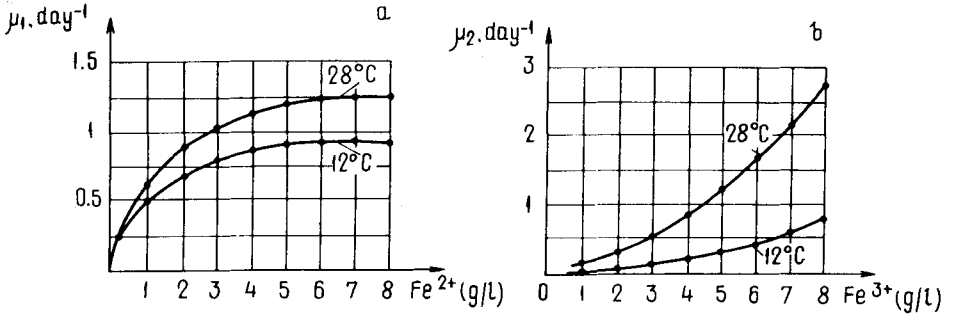

Fig. 5. Specific reaction rate as a function of substrate concentration:
a — for the first reaction (oxidation); b — for the second reaction (hydrolysis)

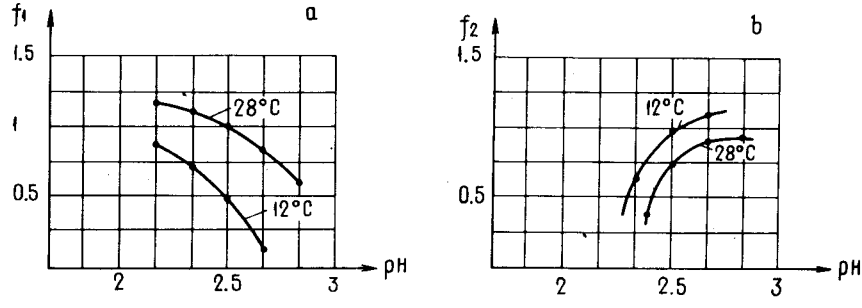

Fig. 6. Effect of pH of the medium and of temperature on the first (a) and second (b) reactions

medium at low temperatures is maintained within the optimum range, the curves of *T. ferrooxidans* growth and Fe^{2+} oxidation should have no deceleration phases. This thesis has been confirmed experimentally [16].

SOME MATHEMATICAL MODELS INCORPORATING INHIBITION BY THE PRODUCT AND EXPLICITLY TAKING INTO ACCOUNT THE CONCENTRATION OF MICROORGANISMS

Even though experimental and simulation results are in a good qualitative and quantitative agreement, there is some experimental evidence suggesting a possible inhibition of growth of *T. ferrooxidans* in a liquid medium by a product of its activity, i.e. by ferric iron, Fe^{3+} [17, 18].

So we had to introduce additional terms in the model equations that would account for action of the inhibiting product. The inhibition kinetics was assumed to be similar to the competitive inhibition kinetics in enzymatic reactions. In this case the specific growth rate is given by the equation:

$$\mu_1(Fe^{2+}, Fe^{3+}) = \frac{\mu_{1\ max} Fe^{2+}}{K_s + Fe^{2+} + Fe^{3+}/K_i} \qquad (11)$$

We assumed ferric iron, i.e. the reaction product, to inhibit the first step of the regeneration process since inhibition by a product is a characteristic feature of enzymatic reactions.

The system of equations incorporating inhibition differs from system (9) presented above only in that μ_1 has now to be described by equation (11).

No qualitative difference was observed between approximation of experimental data provided by earlier model and that provided by the model that accounts for inhibition in a direct way. The parameter values of the novel model calculated from experimental data showed only insignificant deviations from those of the first one. Comparing these models by relevant adequacy criterion showed that the model with inhibition produces somewhat better results. It appears that inhibition by the reaction product, of the competitive inhibition type, indeed exists under our cultivation conditions but its effect on oxidation is not very significant.

A further analysis of the performance of the model led us to the conclusion that an adequate model should explicitly take into account the biomass concentration. The equation system then should include another differential equation for biomass, while in the other three equations the factor proportional to concentration of Fe^{3+} must be replaced by a factor proportional to biomass (B).

By solving this system of equations it was found that no further improvement of approximation as compared to that offered by the first model can be attained. Therefore, the most convenient and adequate model turned out to be the one represented by the equation system (9) with the kinetic characteristics shown in Fig. 5 and Fig. 6.

A MATHEMATICAL MODEL OF A CONTINUOUS CULTURE

For continuous cultivation of *T. ferrooxidans*, mathematical model (9) can be reformulated as follows [19]:

$$\frac{dFe^{2+}}{dt} = -\mu_1 \cdot Fe^{3+} + D \cdot (Fe_0^{2+} - Fe^{2+})$$

$$\frac{dFe^{3+}}{dt} = \left(\frac{1}{Y_1}\mu_1 - \mu_2\right) Fe^{3+} - D \cdot Fe^{3+} \qquad (12)$$

$$\frac{dpH}{dt} = -\left[\left(-\frac{1}{Y_2}\mu_1 + \frac{1}{Y_3}\mu_2\right) \cdot Fe^{3+} + D \cdot (pH_0 - pH)\right]$$

In the above model, biomass and Fe^{3+} concentrations are assumed to be directly proportionate.

Of particular interest is the relationship between the steady-state concentration of biomass and the dilution rate. This relationship

can be found by setting to zero the derivatives on the left-hand side of eq. (12):

$$\frac{dFe^{2+}}{dt} = \frac{dFe^{3+}}{dt} = \frac{dpH}{dt} = 0 \qquad (13)$$

Although the analytical solution of the obtained system of three algebraic equations in three unknows is difficult since it leads to equations of higher orders, this system can be solved on a computer. In a real chemostat continuous cultivation, acidity of the medium can be readily kept constant by applying an automatic pH control. This makes our task of studying the system of algebraic equations much easier since now this system will reduce to the equations of the second order.

System of equations (12), with (13) and pH = const. taken into account, has two solutions for a steady-state concentration of biomass. One which is trivial corresponds to washing out of the culture from the chemostat. The other is the chemostat curve we are concerned with. An analysis of these curves showed that when pH was within the permissible range each curve had a characteristic peak, the location and magnitude of which were temperature dependent [19]. With pH corresponding to the left margin of the permissible range we obtain a chemostat curve similar to the classic Monod chemostat curve. When the concentration of ferrous iron at the inlet of the chemostat is in-

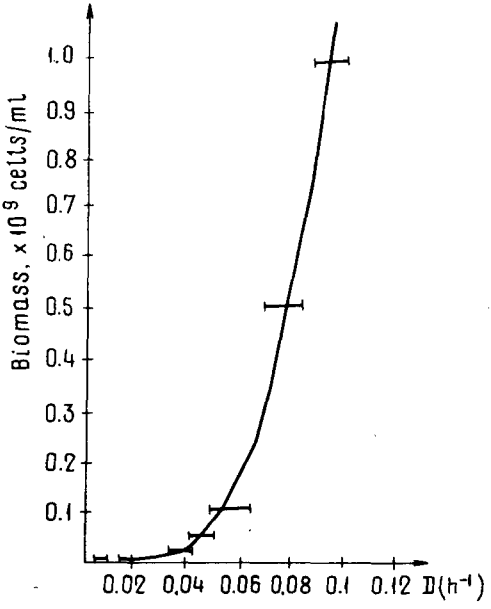

Fig. 7. The experimentally-obtained portion of the chemostat curve

creased, the chemostat curve maximum is also increased. With increasing acidity of the medium, the maximum is decreased.

In ref. [19] we also considered the capacity of fermentor as a function of temperature, acidity of the medium, and input concentration of ferrous iron.

The predictions suggested by the continuous cultivation model have been partially confirmed in appropriate experiments (Fig. 7). The section of a chemostat curve obtained has a clear-cut ascending character which corresponds to the initial section of the theoretical curve. The results of the study can be used in optimization of Fe^{3+} regeneration processes under continuous cultivation conditions.

UNDERSTANDING THE EFFECTS OF INITIAL CONDITIONS OF THE ENVIRONMENT ON THE BATCH CULTIVATION PROCESS

We used our model (equation system 9) to study changes in growth curves, uptake of the substrate and pH of the medium resulting from variations in initial concentrations of the oxidation substrates: Fe^{2+} and H_2SO_4 [20]. It has been shown that changes in initial pH value have the most profound effect on the shape of oxidation curve and the process duration.

So far the experiments were concerned only with the effect of initial concentration of ferrous iron on the oxidation process. The predictions of the model [20] and the experiment results obtained for various temperatures [21] were in qualitative agreement. Yet, for a long time we were unable to achieve a good quantitative agreement that would demonstrate the adequacy of our model. All the three versions of the batch cultivation model (i.e., without inhibition, with inhibition, and the one with the equation for biomass) have been tested under new initial conditions.

It seemed that a quantitative agreement between the model predictions and experimental findings could be arrived at only at the expense of radically changing the kinetic relationships incorporated into the model. Such a change following a change in initial conditions contradicts the "bottle-neck" principle underlying the adopted approach to mathematical modelling.

A thorough analysis of the postulates incorporated into the physiological-biochemical model has revealed that it ignores a possible shift of hydrolysis equilibrium, i.e., a possible sedimentation of some of the ferric iron in the form of $Fe(OH)_3$. To allow for this circumstance, the model should consider two forms of ferric iron: Fe^{3+} in sediment and Fe^{3+} in solution.

Preliminary results obtained with this modification of the basic model suggest a high degree of correspondence between predictions offered by it and the experimental data reported in ref. [21].

Mathematical modelling of ferrous iron oxidation by *T. ferrooxidans* has led to understanding the growth and development of this bacteria in media with various physical and chemical characteristics.

CONCLUSION

A recent progress in mathematical modelling of microbial growth has provided models for oxidation of iron and sulfide minerals. It is difficult so far to compare these models with one another as all of them are but a first step to developing a controlled industrial process of leaching of ores and concentrates. A relatively small number of such models is due, on the one hand, to scarcity of experimental data on the subject resulting from a relatively recent rise of interest to microbiological leaching of metals. On the other hand, there are objective hindrances in this way, related to a comparatively high complexity of microbiological oxidation processes in the ore body or in dense pulp. Apart from insolubility of the substrates there is a large variety of oxidation processes that take place depending on the environment.

A joint effort of theoretical and experimental scientists should be aimed at revealing most important factors affecting the growth of *T. ferrooxidans*, establishing the role of these factors under different cultivation conditions, and understanding the most general regularities of bacterial oxidation. The development and investigation of mathematical models of the process is a necessary step on the way to creating a controlled bioleaching technology.

APPENDIX

Notation of the Variables Used in the Present Paper

S — concentration of a growth-limiting substrate or "concentration" of available surface in model (13);
X — biomass concentration;
$\bar{x}$ — steady-state biomass concentration in the chemostat;
V — rate of formation of enzyme-substrate complex;
V_m — maximum rate of formation of enzyme-substrate complex, attained at "infinite" concentration of a limiting substrate;
μ — specific rate of microbial growth;
μ_m — maximum specific rate of microbial growth, attained at "infinite" concentration of a limiting substrate;

K_s — saturation constant which characterizes affinity to substrate and is equal to substrate concentration for which $\mu = \frac{1}{2}\mu_m$;

K_i — inhibition constant;

D — dilution rate in the chemostat;

t — time;

T — temperature;

$Y_{1, 2, 3}$ — stoichiometric coefficients in model (15).

REFERENCES

1. Chernavsky, D.S., and Yerusalimsky, N.D., *Izv. AN SSSR, ser. biol.*, No. 5, 665 (1965).
2. Basnakyan, I.A., Biryukov, V.V., and Krylov, Yu.M., *Itogi nauki i tekhniki, Ser. Mikrobiologiya, 5*, 5 (1976).
3. Pert, C.J., Principles of Microbe and Cell Cultivation, Blackwell Scientific Publications, Oxford (1975).
4. Fredrickson, A.G., *Biotechnol. Bioeng., 18*, 1481 (1976).
5. Muzychenko, L.A., and Valyev, V.I., *Folia Microbiol., 25*, 259 (1980).
6. Moreira, A.P., van Dedem, G., and Moo-Jong, M., *Biotechnol. Bioeng. Symp., 9*, 179 (1979).
7. Petrova, T.A , Knorre, W.A., Gutke, R., and Bergter, F., *Z. Allg. Mikrobiol., 17*, 531 (1977).
8. Bergter, F., Wachstum von Mikroorganismen, Experimente und Modelle, Veb G.F. Verlag (1972).
9. Barford, J.P., and Hall, R.J., *Processes Biochem., 13*, 8, 22 (1978).
10. Torma, A.E., *Rev. Can. Biol., 30*, 209 (1971).
11. Yukawa, T., Vanselow, D., Ralph, B.J., and Rogers, P.L., *J. Ferment. Technol., 56*, 1, 45 (1978).
12. Hendy, N.A., and Rogers, P.L., *J. Ferment. Technol., 56*, 4, 435 (1978).
13. Gormely, L.S., Duncan, D.W., Branion, R.M.R., and Pinder, K.L., *Biotechnol. Bioeng., 17*, 31 (1975).
14. Lacey, D.T., and Lawson, F., *Biotechnol. Bioeng., 12*, 29 (1970).
15. Petrova, T.A., Galaktionova, N.A., Karavaiko, G.I., Krylov, Yu.M., and Moshnyakova, S.A., *Mikrobiologiya, 48*, 235 (1979).
16. Moshnyakova, S.A., and Karavaiko, G.I., *Mikrobiologiya, 48*, 49 (1979).
17. Pozmogova, I.N., Pronina, T.V., and Petrova, T.A., *Izv. AN SSSR, ser. biol.*, No. 4, 574 (1982).
18. Kelly, D.P., and Jones, C.A., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York — San Francisco — London (1978).
19. Petrova, T.A., Shul'ts, F., Stepanova, N.V., Pozmogova, I.N., and Galaktionova, N.A., In: Mathematical Models of Cell Population, V.D. Goryachenko (Ed.), Gor'kii, Gor'kii State University, 146 (1981) (in Russian).
20. Galaktionova, N.A., Petrova, T.A., Karavaiko, G.I., Krylov, Yu.M., and Moshnyakova, S.A., In: Mathematical Models in Ecology, V.D. Goryachenko (Ed.), Gor'kii State University, 43 (1980) (in Russian).
21. Kovalenko, T.V., and Karavaiko, G.I., *Mikrobiologiya, 50*, 326 (1981).

DIFFERENCES BETWEEN STRAINS OF *THIOBACILLUS FERROOXIDANS* WITH RESPECT TO THEIR ABILITY TO OXIDIZE SULPHIDE MINERALS

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INTRODUCTION

Many papers have been published on the ability of *T. ferrooxidans* to oxidize different sulphides and most data have recently been summarized in an excellent review [19]. In general, the susceptibility of the different sulphide minerals and even the susceptibility of the different specimens of a given sulphide mineral to bacterial oxidation by *T. ferrooxidans* is quite different [2, 3, 5—11, 13, 14, 19, 20]. This is undoubtedly due to their different mineralogical nature [3, 4, 14, 22, 23]. On the other hand, it has been shown that different metal extraction rates are achieved when one and the same mineral specimen is leached by different strains of *T. ferrooxidans* [7, 9]. These differences are connected with the specific characteristics of the strains. It is known that from a taxonomic point of view the species *T. ferrooxidans* can be regarded as a rather heterogeneous population characterized by marked physiological elasticity [4, 17]. It is very important to know which peculiarities of these bacteria are needed to achieve a more rapid oxidation rate. The present study aims at elucidating on this problem of biological leaching by means of a comparison between strains with respect to their oxidizing activity and various physiological and biochemical properties which were determined during the microbial growth on different sulphide minerals.

MATERIALS AND METHODS

370 different strains of *T. ferrooxidans* isolated from acid mine drainage waters and ore lumps of various mines and dumps in Bulgaria were used. The strains were maintained on 9K medium [18] with Fe^{2+}, S^0 or sulphide minerals as a source of energy.

The following mineral substrates were used: two natural pyrite specimens (FeS_2 I and FeS_2 II), three natural chalcopyrite specimens ($CuFeS_2$ I, $CuFeS_2$ II and $CuFeS_2$ III), a natural covellite specimen (CuS I), a synthetic covellite specimen (CuS II), a natural sphalerite specimen (ZnS), a synthetic nickel sulphide (NiS), a synthetic cobalt sulphide (CoS) and a natural arsenopyrite specimen (FeAsS).

The two pyrite specimens were of a high degree of purity. The FeS_2 I contained 43.0 % iron, 49.2 % sulphur and 7.8 % impurities,

and the FeS_2 II contained 42.8 % iron, 48.8 % sulphur and 8.4 % impurities. The $CuFeS_2$ I was the main mineral in a concentrate containing 16.52 % copper, 29.06 % iron and 31.20 % sulphur. The $CuFeS_2$ II and $CuFeS_2$ III were magnetically separated from the gangue of the respective ores and the repetition of the magnetic cleaning procedure several times produced extremely pure chalcopyrite mineral specimens. The CuS I contained only 1.9 % impurities. The impurities consisted mainly of silicate gangue and pyrite (0.23 % iron in the sample), no other copper minerals were represented. The CuS II was prepared by the method described by Dutrizac and MacDonald [3]. The ZnS contained 62.80 % zinc, 32.72 % sulphur and 1.71 % iron. The FeAsS contained 43.77 % arsenic, 30.02 % iron and 18.54 % sulphur. The NiS and CoS were analytically pure.

The leaching of minerals was carried out in 300 ml Erlenmeyer flasks containing 95 ml iron-free 9K medium, 5—20 g substrate (crushed to —400 mesh, with the exception of the FeS_2 I and FeAsS which were crushed to —325 mesh) and 5 ml of an active culture of *T. ferrooxidans*, previously adapted to the mineral being leached, and containing 2×10^8 cells/ml. pH of the leach suspensions was adjusted to 2.3, with the exception of the pH of the pyrite suspensions which was adjusted to 1.65. This low pH was needed to avoid iron precipitation. The flasks were cultivated on a reciprocal shaker with 160 strokes per minute, in the dark, at 35 °C. The water losses due to evaporation were compensated by addition of distilled water, and pH was readjusted with 1 N sulphuric acid or 1 N sodium hydroxide. In the sterile control flasks, 5 ml of a methanol solution containing 2 % of thymol were added instead of the inoculum.

The kinetics of metal extraction was followed by periodic determinations of the dissolved metal concentration in aliquots taken from the leach suspensions. The volume of these suspensions was compensated by a 9K iron-free medium. The rate of metal extraction was estimated from the linear part of a plot representing the dissolved metal concentration as a function of time, use being made of the linear method of least squares fitting.

The physiological and biochemical characterization of the strains was carried out accordingly the methods described by Groudev [4]. The ferrous- and sulphur-oxidizing activities were determined by shake-flask technique. The activity of the enzymes tested was expressed as nmoles of substrate transformed per minute per milligram of protein. Cell fractions S_{10} of crude extracts of cells grown on different sulphide minerals were used. All analytical, microbiological and biochemical procedures used have been described earlier [4, 12].

RESULTS AND DISCUSSION

Data about the metal extraction rates achieved during the biological and chemical oxidation of the sulphide minerals are shown in Table 1. Each sulphide was oxidized by the different strains under identi-

cal conditions which seemed to be the optimal or, at least, the closest to the optimal for most strains. The pyrite specimens only were oxidized at pH which obviously was too low for achieving the highest possible oxidation rates.

Table 1

Metal extraction rates achieved during biological and chemical oxidation of sulphide minerals

Sulphide mineral	Biological oxidation		Chemical oxidation
	rate achieved by the most active strain	rate achieved by the least active strain	
	Metal extraction rate, mg/l · h*		
FeS_2 I	12.4	6.7	0.3
FeS_2 II	48	19	0.9
$CuFeS_2$ I	194	12	7.9
$CuFeS_2$ II	86	7.1	6.0
$CuFeS_2$ III	10.8	2.5	2.5
CuS I	170	44	11
CuS II	52	17	5.1
ZnS	387	156	24
NiS	431	118	14
CoS	408	88	12
FeAsS	44	14	3.7

* — The copper and arsenic extraction rates were determined during the oxidation of chalcopyrite and arsenopyrite, respectively.

The histograms of the strains distribution in accordance with their metal extraction rates during the oxidation of the different sulphide minerals are shown in Figures 1—5.

The FeS_2 I was much more unsusceptible to oxidation than the FeS_2 II. The pH of the leach solution decreased during the oxidation and when dropped below 1.3 was the most important rate-limiting factor. However, in some tests only the pyrite was leached not to completion in spite of the fact that the pH was lowered to about 1.0 at the end of leaching. It must be noted that most strains were inhibited below pH 1.3 during growth in soluble ferrous iron media. There was not a clear relationship between the levels of the pyrite oxidation rates, on the one hand, and those of the ferrous iron and elemental sulphur oxidation rates, on the other (Table 2). Nevertheless, the strains which oxidized the pyrite at the highest rates oxidized the ferrous iron and elemental sulphur also very rapidly.

Table 2

Ferrous-oxidizing and sulphur-oxidizing activities of different ferrobacilli classes formed on the basis of bacterial oxidation rates versus FeS_2 I

Class	Fe^{2+}-oxidizing activity	S^0-oxidizing activity
	Substrate oxidation rate, mg/l · hr	
I	301	4.27
II	309	3.71
III	243	3.92
IV	263	3.20
V	218	2.47
VI	210	2.61
VII	227	2.71

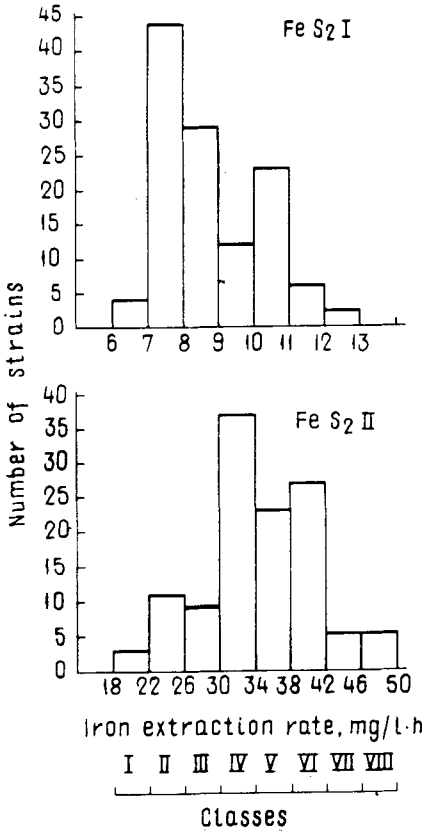


Fig. 1. Histograms of the ferrobacilli strains distribution in accordance with their iron extraction rates during the leaching of pyrite specimens

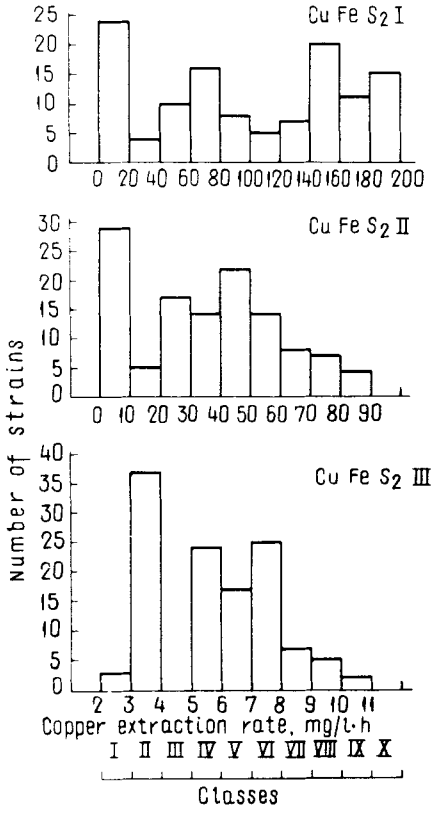


Fig. 2. Histograms of the ferrobacilli strains distribution in accordance with their copper extraction rates during the leaching of chalcopyrite specimens

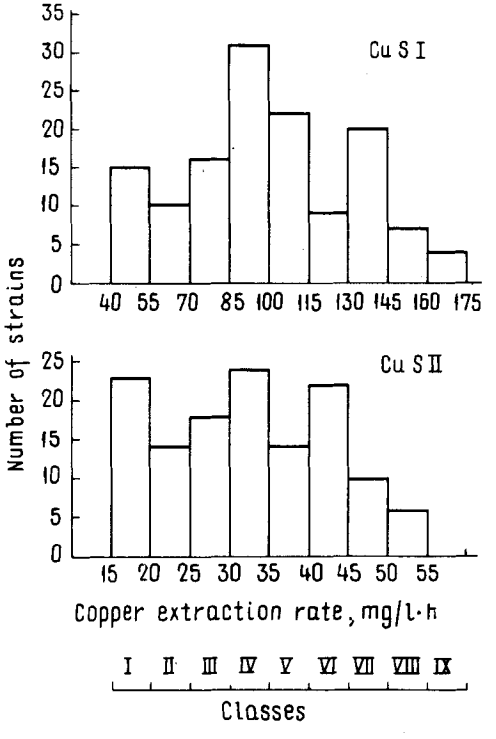


Fig. 3. Histograms of the ferrobacilli strains distribution in accordance with their copper extraction rates during the leaching of covellite specimens

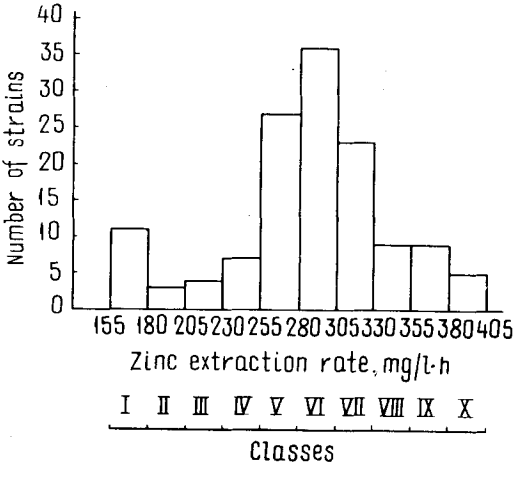


Fig. 4. Histogram of the ferrobacilli strains distribution in accordance with their zinc extraction rates during the leaching of sphalerite

The oxidation rates highly depended on the ferric iron content and the Fe^{3+}/Fe^{2+} ratio in the leach solution. The maximum pyritic iron extraction rates were achieved at ferric content above 10 g/l and Fe^{3+}/Fe^{2+} ratio above 8.5 : 1. The pyrite oxidation in the presence of different initial concentrations of Fe^{3+} was characterized by an

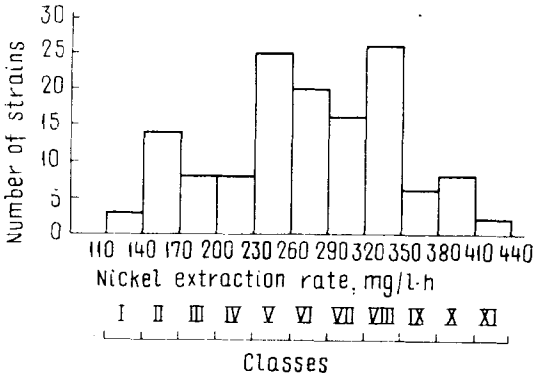


Fig. 5. Histogram of the ferrobacilli strains distribution in accordance with their nickel extraction rates during the leaching of synthetic nickel sulphide

increased rate as well as by a shortened lag-phase in comparison with the oxidation carried out without addition of Fe^{3+}

Norris and Kelly [15] suggested that the progressive release and/or oxidation of iron from pyrite rather than the rate of sulphide oxidation or release was the limiting factor in growth of *T. ferrooxidans*. However, the coating of sulphur on the pyrite surface was a more pronounced in the tests with strains which oxidized pyrite at low rates. This was an indication that high sulphur-oxidizing activity was also needed to achieve a fast pyrite oxidation rate.

In general, the strains which oxidized the first pyrite specimen (FeS_2 I) more rapidly oxidized also the second pyrite specimen (FeS_2 II) at higher rates than the other strains.

The susceptibility of the three chalcopyrite specimens to oxidation by *T. ferrooxidans* was quite different. There was manifest relationship between the electrode potentials and the oxidation rates of the different specimens. The oxidation rates were also related to the level of the difference between the mineral electrode potential and the oxidation reduction potential of the leach solution [7, 9]. These data are in support of the hypothesis about the operation of an electrochemical oxidation system during the leaching of sulphide minerals by *T. ferrooxidans* [23]. However, it was found that some strains were not capable of promoting the leaching of chalcopyrite.

The strains which oxidize the chalcopyrite at the highest rates were characterized by their higher sulphur-oxidizing activity (Table 3) and activity of the enzymes participating in the transformation of sulphur compounds (Table 4). The activity of the rhodanese was particularly high. This enzyme is involved in the cleavage of thiosulphate and probably facilitates the action of the sulphite oxidase. It is also known that thiosulphate inhibits the biological oxidation of ferrous iron and sulphide minerals [16], and that the sensitivity of ferrobacilli versus heavy metal ions increases during the oxidation of thiosulphate [21]. The adenosine phosphosulphate (APS) metabolic pathway, connected with a substrate phosphorylation, was not important during the chal-

copyrite oxidation. The most active strains usually possessed a high ferrous-oxidizing activity but there was not manifest relationship between the levels of the chalcopyrite oxidation rates, on the one hand, and those of the ferrous iron oxidation rates, on the other (Table 3). The addition of exogenous iron did not accelerate the oxidation of chalcopyrite. Some strains possessing a high ferrous-oxidizing activity but a relatively low sulphur-oxidizing activity oxidized the $CuFeS_2$ I at high rates (above 150 mg/l · h). However, their activity towards the other chalcopyrite specimens was low. It is possible that the oxidation of $CuFeS_2$ I by those strains is carried out by means of the mechanism suggested by Dugan and Randles [1] for the pyrite oxidation, i.e. the strains oxidize the iron atoms in the crystal lattice and these atoms act as intermediary electron acceptors during the sulphide atoms oxidation.

The two covellite specimens markedly differed from each other with respect to their susceptibility to bacterial oxidation. It must be noted, however, that the two specimens were oxidized under different conditions. The synthetic covellite (CuS II) was oxidized in absence of iron. Therefore, its oxidation was carried out by the direct mechanism. On the other hand, most of the iron contained in the natural covellite specimen dissolved during the leaching, i.e. all experiments were carried out in the presence, though at different concentrations of iron ions (some final solutions used in the tests assayed up to 75—80 mg/l iron).

The electrode potentials of one and the same covellite specimen were different in the presence of different strains of *T. ferrooxidans.* The electrode potential minus oxidation reduction potential differences were also different. The electrode potentials were lower and the electrode potential minus oxidation reduction potential differences were greater when a given specimen was oxidized by a more active strain [9].

The strains which oxidize the covellite most rapidly are characterized by their high sulphur-oxidizing activity (Table 5) as well as by the activity of those enzymes which participate in the sulphur compounds transformations (Table 6). The activity of the rhodanese is very high and the APS-metabolic pathway is not important during the covellite oxidation.

The exogenous addition of soluble iron exerted a different effect on the oxidation rates of the various strains. The rates of most strains were accelerated more significantly versus the synthetic covellite but only slightly versus the natural covellite. The accelerations of the oxidation rates of the least active strains were the most significant. Elemental sulphur was always detected by X-ray diffraction and mass spectrometry on the surface of covellite leached by these strains. It is clear that their low sulphur-oxidizing activity, for which they are not able to remove the protective sulphur films, is an obstacle to the indirect (Fe^{3+}-dependent) mechanism to proceed to a greater extent.

Exogenous iron impeded the oxidation rates versus the natural covellite of some of the most active strains (of classes I, II and III), and was without noticeable effect on the rates of the other strains of

Table 3

Ferrous-oxidizing and sulphur-oxidizing activities of different ferrobacilli classes formed on the basis of bacterial oxidation rates versus $CuFeS_2$ I

Class	Fe^{2+}-oxidizing activity	S^0-oxidizing activity
	Substrate oxidation rate, mg/l · h	
I	266	4.39
II	275	4.12
III	257	3.54
IV	251	3.25
V	220	3.19
VI	243	3.05
VII	200	2.73
VIII	214	2.44
IX	182	1.78
X	205	1.51

Table 4

Activity of enzymes participating in transformations of inorganic sulphur and iron compounds by *Thiobacillus ferrooxidans

Enzyme	Specific activity (nmoles/min · mg protein)	
	Class I	Class X
Sulphur-oxidizng enzyme	189	2.18
Sulphite oxidase	268	9.11
APS-reductase	4.12	0.84
ADP-sulphurylase	3.24	0.97
Adenylate kinase	14.4	11.0
Thiosulphate-oxidizing enzyme	5.32	0.61
Rhodanese	325	27.1
Fe^{2+}-cytochrome *c* reductase	3.42	1.31
Cytochrome oxidase	3.31	1.35

* — Cell fractions S_{10} of crude extracts of $CuFeS_2$ I-grown cells were used; the classes were formed on the basis of the bacterial oxidation rates versus $CuFeS_2$ I.

Abbreviations: APS — adenosine phosphosulphate
ADP — adenosine diphosphate

this group as well as on the rates of some moderate strains (of classes IV—VI). A possible explanation of this finding is the action of the direct mechanism only. A simultaneous action of the indirect mechanism, however, cannot be excluded with certainty.
indirect mechanism, however, cannot be excluded with certainty.

Although it is known that only minor concentrations of ferric iron are needed for the indirect mechanism to proceed efficiently [2, 4],

Table 5

Ferrous-oxidizing and sulphur-oxidizing activities of different ferrobacilli classes formed on the basis of bacterial oxidation rates versus CuS I

Class	Fe^{2+}-oxidizing activity	S^0-oxidizing activity
	Substrate oxidation rate, mg/l · h	
I	257	4.57
II	248	4.40
III	268	3.61
IV	243	3.32
V	251	3.11
VI	231	3.14
VII	211	2.61
VIII	219	1.80
IX	199	1.56

Table 6

Activity of enzymes participating in transformations of inorganic sulphur and iron compounds by *Thiobacillus ferrooxidans

Enzyme	Specific activity (nmoles/min · mg protein)	
	Class I	Class IX
Sulphur-oxidizing enzyme	208	3.56
Sulphite oxidase	270	11.09
APS-reductase	3.92	0.80
ADP-sulphurylase	3.81	0.77
Adenylate kinase	11.01	9.12
Thiosulphate-oxidizing enzyme	5.55	0.59
Rhodanese	301	23.00
Fe^{2+}-cytochrome *c* reductase	3.20	1.98
Cytochrome oxidase	3.09	1.81

* — Cell fractions S_{10} of crude extracts of CuS I-grown cells were used; the classes were formed on the basis of the bacterial oxidation rates versus CuS I.

Abbreviations: APS — adenosine phosphosulphate
ADP — adenosine diphosphate

the large spectrum of different oxidation rates observed during the leaching of the natural covellite suggests that the copper solubilization from this specimen is not due solely to the indirect mechanism. It seems that both the direct and indirect mechanisms operate simultaneously but the character of this simultaneous action can be different: either synergic or competitive. In any event, the level of the strain sulphur-oxidizing activity is the most important rate-controlling factor. On the one hand, this activity is the basis of the direct mechanism, and, on the other hand, to some extent it is also beneficial to the indirect mechanism. It is logical to be assumed that the direct to indirect mechanism ratio during the natural covellite oxidation probably increases with the increase of the sulphur-oxidizing ability of the strains.

It was found that the high rates of sphalerite oxidation were also connected with the high levels of sulphur-oxidizing activity of the respective strains (Table 7) as well as with the high activities of their sulphur-compound transforming enzymes (Table 8). The particularly high activity of the rhodanese and the low activities of the enzymes of the APS-metabolic pathway were also observed.

The effect of iron added to the leach system on the zinc solubilization rates depends on its concentration as well as on the peculiarities of the strains used. The rates of the most active strains (of classes I—III) were not or were only slightly accelerated by the addition of ferrous iron in concentrations up to 3 g/l. It must be noted, however, that most iron contained in the sphalerite dissolved during the leaching, and this endogenous iron raised but only slightly and temporarily the total iron concentrations. In fact, a significant part of the soluble iron, endogenous and exogenous, precipitated as basic ferric sulphates. The iron added in higher concentrations (from 4 to 27 g/l) had no effect or only slightly retarded the leaching rates of those strains. The retarding effect was probably due to the greater extent of iron precipitation on the sphalerite surface.

The exogenous iron accelerated the zinc solubilization rates of most of the moderate strains (of classes IV—VII) as well as the rates of all least active strains (of classes VIII—X). The concentrations from 9 to 15 g/l of iron added were most efficacious. The surface of the sphalerite leached by those strains, especially at higher iron concentrations, was partially covered by elemental sulphur. This sulphur was not removed in time because of the relatively low sulphur-oxidizing activities of the respective strains, and, together with the iron precipitates, retarded the zinc extraction rates.

The exogenous ferric iron had a similar effect on zinc leaching, and only the initial zinc extraction rates were accelerated in its presence.

The estimations of the cell yields (Y_m), i.e. the cell mass per mole of sphalerite oxidized, by the net nitrogen concentrations showed that the yields were much larger than those predicted by oxidation with ferric iron only. Furthermore, the increase of the leaching rates was

connected with an increase of the cell yields (values of Y_m from 1.412 to 4.311 g/mole were found).

It can be assumed from the above data that both direct and indirect biocatalytic mechanisms operate simultaneously during the sphalerite

Table 7

Ferrous-oxidizing and sulphur-oxidizing activities of different ferrobacilli classes formed on the basis of bacterial oxidation rates versus ZnS

Class	Fe^{2+}-oxidizing activity	S^0-oxidizing activity
	Substrate oxidation rate, mg/l · hr	
I	259	4.59
II	253	4.37
III	263	3.86
IV	251	3.40
V	239	3.10
VI	228	2.69
VII	210	1.99
VIII	214	1.60
IX	199	1.72
X	196	1.49

Table 8

Activity of enzymes participating in transformations of inorganic sulphur and iron compounds by *Thiobacillus ferrooxidans

Enzyme	Specific activity (nmoles/min · mg protein)	
	Class I	Class X
Sulphur-oxidizing enzyme	187	3.13
Sulphite oxidase	226	10.02
APS-reductase	4.11	0.97
ADP-sulphurylase	4.01	0.92
Adenylate kinase	13.04	9.83
Thiosulphate-oxidizing enzyme	3.81	0.77
Rhodanese	262	18.06
Fe^{2+}-cytochrome *c* reductase	3.69	2.28
Cytochrome oxidase	3.59	2.11

* — Cell fractions S_{10} of crude extracts of ZnS-grown cells were used; the classes were formed on the basis of the bacterial oxidation rates versus ZnS.

Abbreviations: APS — adenosine phosphosulphate
ADP — adenosine diphosphate

oxidation by *T. ferrooxidans*. High sulphur-oxidizing activity is needed to achieve a fast leaching rate, and the direct to indirect mechanism ratio probably increases with the rise of this activity.

The rates of metal solubilization from the synthetic NiS and CoS depended on the levels of sulphur-oxidizing activity of the strains. These sulphides were oxidized in absence of iron. It is known that some sulphides including NiS, CoS and ZnS can be leached following oxidation of elemental sulphur or free sulphide rather than of the sulphur moiety of the solid substrate in situ, and that any acidophile capable of oxidizing the "free" sulphur, e.g. *T. thiooxidans*, is capable of promoting the leaching [13]. However, the leaching effect is not due solely to the oxidation of the elemental sulphur being formed, since *T. thiooxidans*, which is a more efficient S^0 oxidizer than *T. ferrooxidans*, oxidized these sulphides more slowly than some strains of *T. ferrooxidans*. It seems that at least some strains of *T. ferrooxidans* are capable of dissolving metals from NiS, CoS and ZnS also by direct attack on the sulphide moiety of the minerals.

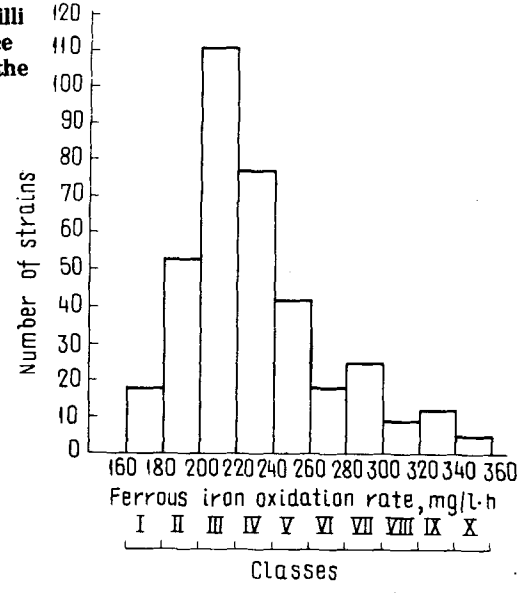
The arsenic solubilization rates were accelerated by the addition of iron ions. This finding indicates that the indirect Fe^{3+}-dependent oxidative mechanism plays a significant role in the oxidation of arsenopyrite, and that the concentrations of iron dessolved during the leaching of this mineral were lower than the optimal ones needed for achieving maximum oxidation rates. It must be noted that most iron contained in the arsenopyrite dissolved during the leaching. However, its concentrations in solution were always below 1 g/l since a considerable part of the soluble iron precipitated as iron arsenites and arsenates and as basic iron sulphates.

The elemental sulphur generated during the oxidation of arsenopyrite formed passivation films on the mineral surface. The inability of *T. ferrooxidans* to prevent the formation of these films and/or to remove them from the surface was due to the low sulphur-oxidizing activity of bacteria in the presence of arsenic. It is a well known fact that the sensitivity of *T. ferrooxidans* to heavy metal ions during elemental sulphur oxidation is greater than during ferrous iron oxidation [21]. For those reasons, a high ferrous-oxidizing activity was needed to achieve rapid arsenic solubilization.

The ability of *T. ferrooxidans* to oxidize sulphide minerals depends on its ability to oxidize Fe^{2+} and S^0. The histograms of the strains distribution in accordance with their ferrous iron and elemental sulphur oxidation rates are shown in Figures 6 and 7, respectively. It is clear that the differences between strains are more pronounced in relation to their ability to oxidize S^0 than in relation to their ability to oxidize Fe^{2+}.

An analysis of all data showed that some strains were able to oxidize all sulphide minerals tested at high rates, while some other strains oxidized all sulphides at low rates. However, a third group of strains was the most numerous. This group includes strains whose c idation

Fig. 6. Histogram of the ferrobacilli strains distribution in accordance with their oxidation rates during the oxidation of ferrous iron



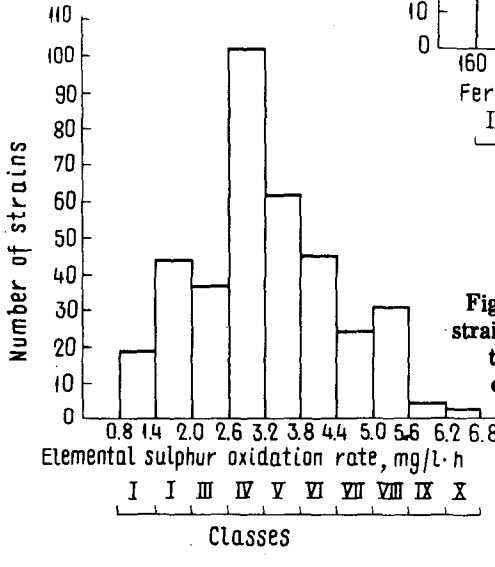


Fig. 7. Histogram of the ferrobacilli strains distribution in accordance with their oxidation rates during the oxidation of elemental sulphur

activities versus the different sulphides markedly differed from each other, i.e. a given strain oxidized one or more sulphides at high rates but oxidized the other sulphides at moderate or even low rates. More comprehensive studies are needed to elucidate the biological bases of the specific bacterial "predilections" for given mineral substrates.

REFERENCES

1. Dugan, P., and Randles, C., In: Acid Mine Drainage Formation and Abatement, The Ohio State University Research Foundation, Columbus, 43 (1971).
2. Dutrizac, J., and MacDonald, R., *Minerals Sci. Engng., 6,* 59 (1974).
3. Dutrizac, J., and MacDonald, R., *Can. Metall. Quart., 13,* 423 (1974).

4. Groudev, S., Microbiological Processes Catalyzed by *Thiobacillus ferrooxidans* during the Bacterial Leaching of Sulphide Minerals, M. Sci. Thesis, Institute of Microbiology of the Bulgarian Academy of Sciences, Sofia, 288 p. (1974).
5. Groudev, S., *Mikrobiologija* (Belgrade), *16*, 1, 75 (1979).
6. Groudev, S., *Compt. rend. Acad., Bulg. Sci., 33*, 659 (1980).
7. Groudev, S., *Compt. rend. Acad. Bulg. Sci., 33*, 829 (1980).
8. Groudev, S., *Compt. rend. Acad. Bulg. Sci., 33*, 1119 (1980).
9. Groudev, S., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi et al. (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 193 (1980).
10. Groudev, S., *Compt. rend. Acad. Bulg. Sci., 34*, 217 (1981).
11. Groudev, S., *Compt. rend. Acad. Bulg. Sci., 34*, 1139 (1981).
12. Groudev, S., and Genchev, F., *Mikrobiologija* (Belgrade), *15*, 2, 139 (1978).
13. Kelly, D., Norris, P., and Brierley, C., In: Microbial Technology, Society for General Microbiology Symposium 29, A. Bull, D. Ellwood and C. Ratledge (Eds.), Society for General Microbiology Ltd., 263 (1979).
14. Khalid, A., and Ralph, B., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York, 165 (1977).
15. Norris, P., and Kelly, D., *FEMS Microbiology Letters, 4*, 143 (1978).
16. Razzell, W., and Trussell, P., *J. Bacteriol., 85*, 595 (1963).
17. Silver, M., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L. Murr, A. Torma and J. Brierley (Eds.), Academic Press, New York — San Francisco — London, 3 (1978).
18. Silverman, M., and Lundgren, D., *J. Bacteriol., 77*, 642 (1959).
19. Torma, A., and Bosecker, K., *Progr. Ind. Microbiol., 16*, 77 (1982).
20. Torma, A., and Sakaguchi, H., *J. Ferment. Technol., 56*, 173 (1978).
21. Tuovinen, O., Niemela, S., and Gyllenberg, H., *Antonie van Leeuwenhoek, 37*, 489 (1971).
22. Wyckoff, R., *Bull. Soc. fr. Minéral. Cristallogr., 93*, 120 (1970).
23. Yakhontova, L., Sergeev, V., Karavaiko, G., and Sukhantseva, V., In: Ecology and Geochemical Activity of Microorganisms, M. Ivanov (Ed.), Puschino, 99 (1976) (in Russian).

PART II

ROLE OF MICROORGANISMS IN CHEMICAL ELEMENT CYCLES

KINETICS OF THE SULFUR CYCLE

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1. INTRODUCTION

In common with most of the terrestrial elements, sulfur is continually recycled between different chemical forms and between geospheric reservoirs. Since organisms are involved at several stages of sulfur recycling the process is often referred to as the *biogeochemical sulfur cycle*. Several quantitative assessments of the biogeochemical sulfur cycle have been made over the last decade or so [1—5], further references are cited by Bremner and Steele [6]. The most recent and comprehensive assessment is the SCOPE 19 Report on the Biogeochemical Sulfur Cycle [7] and, except where indicated, data on reservoir magnitudes and fluxes in this paper are cited from that report.

A simplified version of the sulfur cycle is illustrated in Fig. This shows the principal geospheric reservoirs of sulfur, with estimates of their magnitudes, and the various routes by which the element is transferred between reservoirs. In this diagram the lithosphere includes sediments and magma as well as the crust. All but about 0.01 % of the total world sulfur is present in the lithosphere (as metal sulfides, mainly pyrite, or sulfate evaporites) or as dissolved sulfate in the oceans and seas.

The quantitatively minor reservoirs — the atmosphere, pedosphere and freshwaters — have special significance, however, since they are highly mobile and interact directly with the biosphere. Changes in these reservoirs, therefore, lead to almost immediate effects on floral and faunal ecosystems.

Because of the buffering effects of the oceans and lithosphere the overall sulfur cycle, in the short-term, approximates a steady state. Over geological time, however, it appears that there have been episodic shifts in steady state which resulted in major redistributions of sulfur between the main reservoirs [8]. There is also concern for the

effects that modern industrial practices exert on the mobile sulfur reservoirs and the short and long-term consequences of these effects for the terrestrial environment [9, 10].

This paper is an attempt to summarize briefly the kinetics of the sulfur cycle as we know it today and, where possible, to indicate where Man's activities have a significant impact. It should be recognized that all estimates quoted are subject to varying degrees of uncertainty due to the limitations of available data and to the many assumptions on which calculations are based. Discussion of these uncertainties is outside the scope of this paper but will be found in [7] and other publications cited in the text.

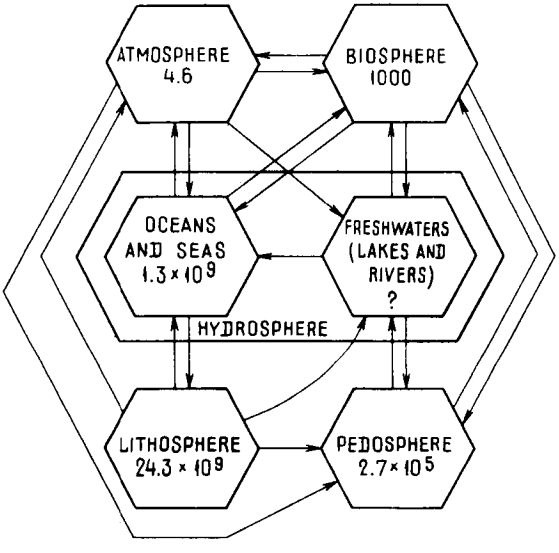

Fig. A simplified version of the biogeochemical cycle of sulfur

The magnitudes (Tg) of the atmospheric, hydrospheric, pedospheric and lithospheric reservoirs are cited from Ivanov and Freney [7]. Eriksson's value of 600 Tg for biospheric sulfur in land plants has been upgraded to 1000 Tg to allow for an oceanic contribution [1]. This is probably an underestimate. Recent estimates of the total land phytomass (5.6×10^5 Tg C [29]) would correspond to ~6000 Tg S assuming an average C:S ratio of 100:1

2. PRINCIPAL CHEMICAL PROCESSES INVOLVED IN SULFUR TRANSFER BETWEEN RESERVOIRS

2.1. Weathering. Oxidative weathering of rocks is accompanied by solution of sulfate evaporites and oxidation to sulfate of sulfidic minerals, principally pyrite. The latter reaction can be purely chemical

in character but can be accelerated by the action of microorganisms, particularly chemolithotrophic bacteria, such as *Thiobacillus* spp. and *Sulfolobus*, that have the ability to oxidize both ferrous iron and reduced sulfur [11]. Information is lacking, however, on the relative contributions of chemical and biological processes to *in situ* weathering [cf. 12].

2.2. **Reduction of sulfate. Abiological.** At temperatures in excess of about 300 °C, sulfate is reduced by organic matter (equation 1) [13],

$$\underset{\text{(organic matter)}}{2\,CH_2O} + SO_4^{2-} \rightarrow 2\,CO_2 + H_2S + 2OH^- \qquad (1)$$

or ferrous iron [14]. The reaction with ferrous iron has been extensively studied in relation to high-temperature interactions between seawater and basalt (equation 2) and appears to account for the

$$\underset{\text{(basalt)}}{11\,Fe_2SiO_4} + 2SO_4^{2-} + 4H^+ \rightarrow 7Fe_3O_4 + FeS_2 + 11SiO_2 + 2H_2O \qquad (2)$$

formation of sulfide in hydrothermal solutions generated during circulation of seawater and other waters through the crust [15].

Biological. At the prevailing temperatures of most terrestrial environments sulfate is reduced biologically. The majority of microorganisms and plants carry out *assimilatory* sulfate reduction to provide reduced sulfur for the synthesis of cysteine, methionine, iron-sulfur proteins and other molecules that are essential for an organism's structure and physiology.

Certain bacteria (Table 1), however, utilize sulfate in place of oxygen as an electron acceptor in the oxidation of hydrogen or organic

Table 1

Genera of sulfate-reducing bacteria [16, 17]

Genus	Habitat
Desulfobacter	Anaerobic mud of brackish water, marine environments
Desulfobulbus	Anaerobic mud of fresh and brackish water, marine environments
Desulfococcus	Anaerobic mud of fresh and brackish water, marine environments
Desulfonema	Anaerobic marine and brackish water muds
Desulfosarcina	Anaerobic brackish water muds, marine environments
Desulfotomaculum	Soil, freshwater muds, geothermal regions, polluted waters
Desulfovibrio	Soil, fresh and brackish water muds, marine environments

matter for energy production. This is termed *dissimilatory* sulfate reduction. It is a strictly anaerobic process and is limited to environments where restricted circulation and the presence of organic matter favour depletion of oxygen. Such environments include marine and freshwater sediments, marine basins and lakes, and waterlogged soils [18].

Sulfate-linked oxidation of organic matter yields about 10 % of the energy released in analogous oxygen-linked oxidations [19]. As a consequence large amounts of sulfate are reduced to provide the energy requirements of the dissimilatory sulfate-reducing bacteria.

2.3. **Sulfide oxidation.** Sulfide oxidation by chemolithotrophic sulfur bacteria has been mentioned in section 2.1 in relation to weathering. Much of the sulfide generated in the natural environment by dissimilatory sulfate reduction and other biological processes (see section 2.4), or introduced by volcanism and hydrothermal activity, however, is not fixed but is rapidly oxidized to sulfate and other products.

Both chemical and biological processes are involved in the oxidation with the relative contributions depending upon the physical characteristics of the boundary between anoxic and oxic conditions [20]. In large deep basins such as the Black Sea, the initial stages of sulfide oxidation appear to be almost exclusively chemical. At the other extreme, biological factors predominate in sulfide oxidation in cyanobacterial or algal dominated shallow water sediments.

As well as the chemolithotrophic bacteria several heterotrophic organisms including *Beggiatoa* spp., and photosynthetic sulfur bacteria. (e.g. *Chromatium* spp., *Chlorobium* spp. and *Ectothiorhodospira* spp.) are involved in sulfide oxidation. Several cyanobacteria also have this ability in addition to their normal capacity for oxygenic photosynthesis (Table 2).

Intermediate oxidation states are often encountered during sulfide oxidation. Elemental sulfur is a common intermediate product of the reaction catalysed by photosynthetic bacteria, cyanobacteria and beggiatoa, while thiosulfate and polythionates are formed during oxidation by chemolithotrophic bacteria. Thiosulfate is also formed during chemical oxidation of sulfide. Further oxidation of these intermediate sulfur compounds to sulfate appears to be largely biological.

Sulfide oxidation by photosynthetic bacteria and cyanobacteria is a strictly anaerobic process, and when they are associated with dissimilatory sulfate reducers a complete cycle of sulfate reduction and sulfide oxidation is possible in the total absence of oxygen.

2.4. **Degradation of organic matter and formation of volatiles.** Degradation of sulfur amino acids in organic matter by microorganisms is accompanied by the release of a variety of sulfur-containing products. Hydrogen sulfide may be released directly from cysteine by the action of cysteine desulfhydrase, or the sulfur moiety can be oxidized to the level of sulfite while still attached to the carbon skeleton [22].

Table 2

Organisms involved in oxidation of reduced sulfur compounds [16, 21]

Genus	Comments
	Aerobic*
Beggiatoa	Aerobic to microaerophilic, heterotrophic
Sulfolobus	Facultative autotroph, thermophilic, acidophilic
Thiobacillus	Genus includes strict autotrophs, facultative autotrophs, mixotrophs and acidotrophs. Some species also oxidize ferrous iron. *T. denitrificans* grows anaerobically with nitrate
	Anaerobic (Photosynthetic)
Chromatium *Thiocapsa* *Thiocystis* *Thiosarcina* *Thiospirillum*	Elemental sulfur is deposited intracellularly during sulfide oxidation
Amoebobacter *Lamprocystis* *Thiodictyon* *Thiopedia*	Contain gas vacuoles: sulfur deposited in the gas-vacuole-free peripheral part of the cells
Chlanthrochloris *Chlorobium* *Ectothiorhodospira* *Pelodictyon* *Prosthecochloris*	Elemental sulfur deposited extracellularly
Aphanocapsa *Chlorogloeopsis* *Lyngbya* *Oscillatoria* *Plectonema*	Also carry out oxygenic photosynthesis in absence of sulfide

* — Other organisms associated with sulfidic environments whose metabolism is poorly defined include members of the following genera: *Thioploca, Thiothrix, Achromatium, Thiobacterium, Macromonas, Thiovulum, Thiospira.*

Sulfite-oxidizing systems are present in plants and animals as well as in the chemolithotrophic and photolithotrophic sulfur bacteria. Carbon-bonded volatile sulfur compounds are also formed from cysteine and other sulfur amino acids when incubated with soils (Table 3). Many of these compounds have been detected during decomposition of plant tissues and in aquatic systems [6]. Carbonyl sulfide (COS) can also be produced from pesticides such as nabam (disodium ethylene-bisdithiocarbamate) both in soil and fungal cultures.

Table 3

Volatile sulfur compounds evolved from soils treated with sulfur-containing amino acids [6]

Volatile S compound	Amino acid source
CS_2	cysteine, lanthionine, djenkolic acid, homocysteine
CH_3-SH	methionine, methionine sulfoxide, methionine sulfone, S-methyl cysteine
CH_3-S-CH_3	methionine, methionine sulfoxide, methionine sulfone, S-methyl cysteine, homocysteine
CH_3-S-S-CH_3	methionine, methionine sulfoxide, methionine sulfone, S-methyl cysteine
CH_3CH_2SH	ethionine, S-ethylcysteine
CH_3CH_2-S-S-CH_3	ethionine, S-ethylcysteine
CH_3CH_2-S-CH_3	ethionine, S-ethylcysteine
COS	lanthionine, djenkolic acid

2.5. **Oxidation of volatile sulfur compounds.** Volatile sulfur compounds undergo rapid oxidation in the troposphere. The chemistry is complex but it is thought that a major process is gas-phase oxidation by photochemically produced hydroxyl radicals. Rate constants for experimental oxidation of volatile sulfur compounds by OH radicals range from about 10^{-10} to 10^{-14} $cm^3 \cdot molecule^{-1} \cdot s^{-1}$ (Table 4).

Table 4

Rate constants for OH + volatile sulfur compounds [23]

Gas	K_s*
CH_3-S-S-CH_3	223
CH_3SH	90.4
CH_3-S-CH_3	9.1
H_2S	5.0
SO_2	0.72
CS_2	0.43
COS	$\leqslant 4 \times 10^{-2}$

* — $(cm^3 \cdot molecule^{-1} \cdot s^{-1}) \times 10^{-12}$

Volatile sulfur gases are also absorbed by soil where oxidation may take place, perhaps mediated by microorganisms [6].

3. MAJOR FLUXES OF THE BIOGEOCHEMICAL SULFUR CYCLE

Estimates of the overall rates of sulfur transfer between the principal reservoirs of the modern biogeochemical sulfur cycle are listed in Table 5.

Table 5

Fluxes of the modern biogeochemical sulfur cycle

Route	Section in text	Total flux (Tg yr^{-1})	Anthropogenic contribution (Tg yr^{-1})
Lithosphere → pedosphere	3.1	71	33
Lithosphere → oceans & seas	3.2	16	
Lithosphere → atmosphere	3.3	141	113
Oceans & seas → lithosphere (sediments)	3.4	125	
Oceans & seas → atmosphere	3.5	163	
Oceans & seas → biosphere	3.6	500	
Pedosphere → atmosphere	3.7	72	36
Pedosphere and lithosphere → freshwaters	3.8	171	57
Pedosphere → biosphere	3.9	600	
Atmosphere → pedosphere	3.10	120	83
Atmosphere → oceans & seas	3.11	258	
Atmosphere → freshwater	3.12	72	
Atmosphere → biosphere	3.13	?	
Freshwaters → oceans, seas & inland drainage basins	3.14	243	104
Freshwaters → biosphere	3.15	30	

3.1. **Lithosphere to pedosphere.** Most of the sulfur in the pedosphere is derived from weathering of plutonic and sedimentary rocks. Estimates of this flux have been made from calculations of global weathering rates and average sulfur concentrations of rocks. According to [5], it amounts to about 66 TgS yr^{-1} of which roughly 50 % is due to increased weathering resulting from Man's activities. An additional 5 TgS yr^{-1} is added directly to the pedosphere by volcanism.

3.2. **Lithosphere to oceans and seas.** Direct routes (with estimated fluxes in TgS yr^{-1}) by which sulfur is transferred from the lithosphere to the oceans and seas are by groundwater (9.0), abrasion of shorelines (2.0) and glacial weathering (5.0). An additional source is underwater hydrothermal activity. Exhalation of sulfurous waters is associated with some oceanic spreading centres (e.g. the East Pacific Rise system [24]) and rift-related inland seas (e.g. the Red Sea [25]) but, at present, no realistic estimate can be made of the contribution from these sources to the sulfur of oceans and seas.

3.3. **Lithosphere to atmosphere.** Sulfur is emitted to the atmosphere from the lithosphere in the form of hydrogen sulfide and sulfur dioxide in volcanic gases. The estimate flux from this source is 28 TgS yr^{-1}.

However, roughly four times this amount (113 TgS yr^{-1}) is currently contributed by Man through smelting and the combustion of solid, liquid and gaseous fuels. The rate of increase in this anthropogenic flux from 1950 onwards is approximately four times that in the period 1880 to 1950 [10].

3.4. **Oceans and seas to lithosphere.** Sulfur is fixed in the sediments of oceans and seas as evaporites, as sulfate in clays and biogenic carbonate, and as metal sulfides, mainly those of iron. Evaporite deposition is mostly limited to arid, shallow water environments where intense evaporation and concentration of seawater causes the solubility products of gypsum and aragonite to be exceeded. The average sulfate-sulfur in the solid phase of ocean sediments is about 9.55 % on a dry mass basis with roughly equal proportions of $CaSO_4$ and barite. The average concentration of sulfate-sulfur in biogenic carbonate is about twice that of the overall sediment. Most of the reduced sulfur incorporated into ocean sediments appears to be of biogenic origin. Incorporation is most intense on the continental shelves, slopes and rises where authigenic and terrestrial organic carbon required for bacterial sulfate reduction is most abundant. Most of the reduction takes place in the near surface layers of sediment. Additional factors are necessary for the *fixation* of sulfide which is not necessarily a function of sulfate reduction intensity. Generally fixation is controlled by the supply of available metals, notably iron [26] while anaerobic conditions must be maintained within the sediment to allow *preservation* of fixed sulfide. From the average rate of sedimentation and average reduced sulfur content of sediments, the flux of reduced sulfur to the ocean sediments has been estimated to be of the order of 97 TgS yr^{-1}. Similar calculations for incorporation of solid phase sulfate yield a flux of about 20 TgS yr^{-1}. To this should be added an estimated 8 TgS yr^{-1} of sulfate-sulfur trapped in pore water on the assumption that most of this sulfur will eventually be incorporated into sedimentary rock.

No estimates are available for the present rates of evaporite deposition or incorporation of hydrothermal sulfides into sediments. However, Holzer and Kaplan [27] calculate that, over geological time, the rate of evaporite formation has been close to twice that of sulfide fixation.

3.5. **Oceans and seas to atmosphere.** Emission of sulfate in sea spray is the major mechanism of transfer of oceanic sulfur to the atmosphere. It is estimated to amount to 140 TgS yr^{-1}.

Additional sulfur is introduced by volatile gases (H_2S, COS, dimethyl sulfide, etc.) generated by biological reduction of sulfate and the decomposition of organic matter. The magnitudes of these fluxes are

difficult to evaluate globally. Analytical data are sparse and there are uncertainties regarding the extent to which hydrogen sulfide is oxidized before any significant mixing with the atmosphere takes place. Direct determinations on coastal sediments have demonstrated that maximum H_2S emissions occur at night when the activities of photosynthetic sulfide-oxidizing bacteria are suppressed [28].

From limited data on emission of reduced sulfur gases from sandy sediments and coastal marshlands the estimated annual flux from littoral zones is 5 TgS. An additional flux of 15 TgS · yr^{-1} has been calculated for the open oceans from estimates of the reduced sulfur content of the atmosphere above the oceans and its average resident time. Both flux estimates, however, have an order of magnitude error of uncertainty.

3.6. **Oceans and seas to biosphere.** An estimate of the flux of sulfur transfer from the oceans to the biosphere can be made from calculated rates of primary organic matter production which vary from about 1.5 to 12.5 x 10^4 TgC · yr^{-1} with an average of ~5 x 10^4 Tg [29, 30]. The last author calculated an annual primary production of 4.35 x 10^4 TgC which included a contribution of 20.3 % for extracellular excretion of organic matter.

Assuming a value for primary production of 5 x 10^4 TgC · yr^{-1} and average carbon to sulfur ratio of 100 : 1, the flux of sulfur from the oceans to biosphere would be in the order of 500 TgS · yr^{-1}.

3.7. **Pedosphere to atmosphere.** Sulfur enters the atmosphere from the pedosphere in aeolian dust and as volatile gases of biogenic origin. Dust emissions vary considerably with space and time and are concentrated largely in arid zones with limited ground cover. They have been considered by some to be an insignificant source of atmospheric sulfur [5] but, in the SCOPE Report, an estimated flux of 20 TgS · yr^{-1} is reported.

It can be presumed that deforestation and other activities initiated by Man have increased aeolian dust emission but no estimates of this contribution have been made.

Biogenic sulfur-containing gases that have been reported as being emitted from soil include carbonyl sulfide, dimethyl sulfide, dimethyl disulfide, methyl mercaptan and hydrogen sulfide. It is likely, however, that most H_2S produced in soil is fixed as iron sulfides.

The flux of biogenic sulfur from the pedosphere to the atmosphere has been calculated indirectly from data on the content and resident time of reduced sulfur in the atmosphere over the continents. The calculated flux of ~16 TgS · yr^{-1} has a high degree of uncertainty.

Atmospheric sulfur is also derived indirectly from the pedosphere through natural and man-induced burning of forests and grasslands. The magnitude of this flux is uncertain. Hampicke [31] estimated that about 3.6 x 10^3 Tg of carbon are released as CO_2 annually during clearing of forests and woodlands by controlled burning. If it

is assumed that the average carbon to sulur ratio for organic matter is 100 : 1 and that all sulfur is converted to SO_2 and other volatile sulfur gases during burning, then about 26 TgS · yr^{-1} are released to the atmosphere by this anthropogenic activity.

3.8. **Pedosphere and lithosphere to fresh waters.** Weathering of rocks and soils provides the main non-anthropogenic input of sulfur to the rivers and lakes of continents. The total flux is estimated to be ~114 TgS · yr^{-1}.

Increasing amounts of sulfur are, however, being added directly from anthropogenic sources, principally washout of fertilizer sulfur from soils (28 TgS · yr^{-1}), effluents from chemical industries (28 TgS · yr^{-1}) and discharges (acid mine water) from mining operations (1 TgS yr^{-1}). When to these is added the estimated flux of anthropogenic sulfur transferred to rivers and lakes via the atmosphere and soil (see section 3.12) it is clear that about 50 % (104 TgS · yr^{-1}) of the present input of sulfur to the global freshwater system arises from the industrial activities of man.

3.9. **Pedosphere to biosphere.** As in the case of the oceans, estimates of the annual primary productivity of land plants vary widely (from about 1.0 to 10.6 x 10^4 TgC · yr^{-1} [29]). Based on the recent estimate by Ajtay et al. [29] of ~6 x 10^4 TgC · yr^{-1}, and employing the same criteria as those used to estimate the sulfur flux from oceans to biosphere, the analogous flux from pedosphere to biosphere is of the order of 600 TgS · yr^{-1}.

3.10. **Atmosphere to pedosphere.** Inputs from the atmosphere to the pedosphere are: sulfur in precipitation; dry deposition of sulfates; absorption of volatile gases by soils. Both precipitation and dry deposition include sulfur originating from sea spray, from aeolian dust and from atmospheric oxidation of sulfur dioxide and other volatile sulfur gases.

The global flux of sulfur in precipitation is estimated at 51 TgS · yr^{-1} from data on the sulfur content of rain waters. Regional fluxes vary extensively, however, due to the differing proportions of sea spray, aeolian and volatile gas sulfur in the atmosphere, and to differing rates of precipitation. For most of the continental surface the contribution from sea spray is minimal. The estimated fluxes for arid, industrial, and non-industrial continental regions are 3, 37, and 11 TgS · yr^{-1}, respectively.

Sulfate-containing particles in the atmosphere vary in size according to their origin. Particles from gas phase reactions range from 0.1 to 1 μm in diameter and those from sea spray and aeolian emission range from 0.5 to 10 μm and 1 to 100 μm, respectively. As in the case of precipitation, dry deposition on the continents involves mainly particles of aeolian and gas phase origins. Their average rates of deposition are calculated to be 0.2 and 1.5 cm · s^{-1}, respectively: the rate of deposition of sea spray particles is assumed to be ~0.5 cm · s^{-1}. The total flux of dry deposition of particulate sulfur on the continents is

estimated at ~16 TgS · yr^{-1} about half of which is deposited in zones of intensive aeolian weathering.

Most studies on the uptake of volatile sulfur gases by soils have dealt with sulfur dioxide. Rates of absorption depend upon the physical, chemical and biological properties of the soil surface and are, as expected, higher for calcareous soils than acidic soils, and increase with relative humidity. The average rate of SO_2 absorption for all continental conditions is estimated to be 0.5 to 0.6 cm · s^{-1} and the total flux of sulfur from the atmosphere to pedosphere in the order of 17 TgS · yr^{-1}.

The overall flux of sulfur from the atmosphere to the continents (soils plus freshwater systems) is ~ 84 Tgs yr^{-1} about half of which (47 TgS · yr^{-1}) is the result of anthropogenic processes.

3.11. **Atmosphere to oceans and seas.** The inputs of sulfur to the oceans and seas are similar to those to the pedosphere except that sulfur in sea spray becomes quantitatively more significant. About 230 TgS is transferred annually on a global scale half of which arises from sea spray.

Absorption of SO_2 by the oceans occurs at a rate of ~ 0.8 cm · s^{-1} and the annual input of sulfur via this route is estimated at 11 Tg. Dry deposition amounts to ~ 17 TgS · yr^{-1}.

3.12. **Atmosphere to fresh waters.** It is assumed that most of the sulfur transferred from the atmosphere to the continents eventually enters the freshwater system via rivers.

The estimated flux of sulfur from the atmosphere to fresh waters by precipitation and dry deposition is 72 TgS · yr^{-1}. As indicated in section 3.10, at the present most of this sulfur (47 TgS · yr^{-1}) is derived originally from burning of fossil fuels and industrial activities of Man and is returned to the continents as acid rain.

3.13. **Atmosphere to biosphere.** Direct transfer of sulfur from the atmosphere to the biosphere is via uptake of SO_2 and perhaps other volatile gases by plants and soil microflora. No estimate of this flux has been made and it is incorporated in the flux from the atmosphere to pedosphere.

3.14. **Freshwaters to oceans and inland drainage basins.** Sulfur in rivers is present as dissolved sulfate and as sulfates and sulfides in particulate matter. Most of this sulfur is discharged to the oceans. The estimated fluxes are 208 and 5 TgS yr^{-1} for dissolved and particulate sulfur, respectively. About 50 % of the dissolved sulfate arises from anthropogenic processes.

A proportion of the total sulfur in rivers (~ 35 TgS · yr^{-1}) is deposited in the sediments of lakes and inland drainage basins.

3.15. **Freshwater to biosphere.** Ajtay et al. estimate the annual primary productivity of lakes and streams to be ~ 400 TgC. This corresponds to a flux of sulfur from fresh waters to the biosphere of ~ 4 TgS · yr^{-1} [29].

3.16. **Biospheric sulfur.** Most biospheric sulfur is rapidly recycled to the pedosphere and hydrosphere. A small proportion, however, becomes incorporated into the lithosphere in fossil organic matter.

Fixation of sulfur during peat formation has been estimated to amount to 0.6 $TgS \cdot yr^{-1}$ while Eriksson calculated a flux of ~ 14 $TgS \cdot yr^{-1}$ for the total transfer of organic sulfur to the lithosphere [1].

4. GENERAL OBSERVATIONS

Table 6 shows the total inputs and outputs for the hydrosphere (oceans, seas and inland drainage basins), the atmosphere, and the combined lithosphere-pedosphere system. It is immediately apparent that while the atmosphere approximates homoeostasis with respect to total sulfur (note, however, that this does not reflect changes in *quality*), there is a distinct trend towards a transfer of sulfur from the lithosphere-pedosphere to the hydrosphere. The magnitude of this transfer (~ 160 $TgS \cdot yr^{-1}$) is close to that of the estimated utilization of lithospheric sulfur in fossil fuels, smelting and fertilizers (~ 170 $TgS \cdot yr^{-1}$). The general conclusion, therefore, is that the movement of sulfur from the lithosphere-pedosphere to the hydrosphere is the consequence of Man's socio-industrial activities.

From Table 5 the total flux of sulfur through the biosphere amounts to about 1130 $TgS \cdot yr^{-1}$. This is about the same as the total flux through all other reservoirs (Table 6) and highlights the importance of the biosphere in the overall cycling of sulfur in the present environment.

Table 6

Summary of major sulfur fluxes

	Fluxes, $TgS\ yr^{-1}$		
	Input	Output	Balance
Oceans, seas and inland drainage basins	517	288	229
Atmosphere	376	378	−2(0)
Pedosphere and lithosphere	245	472	−227
Total:	1138	1138	

REFERENCE

1. Eriksson, E., *Tellus, 12*, 63 (1960).
2. Junge, C.E., Air Chemistry and Radioactivity, Academic Press, New York (1963).
3. Kellog, W.W., Cadle, R.D., Allen, E.R., Lazrus, A.L., and Martell, E.A., *Science, 175*, 587 (1972).
4. Friend, J.P., In: Chemistry of the Lower Atmosphere, S.I. Rasool (Ed.), Plenum Publ. Co., New York, 177 (1973).
5. Granat, L., Rodhe, H., and Hallberg, R.O., In: Nitrogen, Phosphorus, and Sulphur Global Cycles, B.H. Svensson and R. Söderlund (Eds.), SCOPE Report No. 7, *Econ. Bull., 22*, 89 (1976).
6. Bremner, J.M., and Steele, C.G., *Adv. Microbial Ecol., 2*, 155 (1978).
7. Ivanov, M.V., and Freney, J.R., The Global Biogeochemical Sulfur Cycle, SCOPE Report No. 21, John Wiley and Sons, Chichester (1982).
8. Claypool, G.W., Holser, W.T., Kaplan, I.R., Sakai, H., and Zak, I., *Mar. Geol., 28*, 199 (1980).
9. Nriagu, J.O., Sulfur in the Environment, Parts I and II, John Wiley and Sons, New York (1978).
10. Freney, J., and Rodhe, H., In: The Interactions of the Biogeochemical Cycles, B. Bolin and R.B. Cook (Eds.), SCOPE Report No. 24, John Wiley and Sons, Chichester (1983).
11. Kelly, D.P., Norris, P.R., and Brierley, C.L., In: Microbial Technology, A.T. Bull, D.C. Ellwood and C. Ratledge (Eds.), Soc. Gen. Microbiol. Symposium 29, 263 (1979).
12. Trudinger, P.A., *Phil. Trans. R. Soc. Lond., B 298*, 563 (1982).
13. Kiyosu, Y., *Chem. Geol., 30*, 47 (1980).
14. Ohmoto, H., and Rye, R.O., In: Geochemistry of Hydrothermal Ore Deposits, H.L. Barnes (Ed.), John Wiley and Sons, New York, 509 (1979).
15. Bonatti, E., *Ann. Rev. Earth Planet. Sci., 3*, 401 (1975).
16. Buchanan, R.E., and Gibbons, N.E., Bergey's Manual of Determinative Bacteriology, 8th Edn., Williams and Wilkins, Baltimore (1974).
17. Widdel, F., Anaerober Abbau von Fettsäuren und Benzoesäure durch neue isolierte Arten Sulfat-reduzierender Bakterien, Thesis Georg-August-Universität, Göttingen (1980).
18. Postgate, J.R., The Sulphate-reducing Bacteria, Cambridge University Press (1979).
19. McCarty, P.L., In: Water Pollution Microbiology, R. Mitchell (Ed.), Wiley-Interscience, New York, 91 (1972).
20. Jørgensen, B.B., *Phil. Trans. R. Soc. Lond., B 298*, 543 (1982).
21. Garlick, S., Oren, A., and Padan, E., *J. Bacteriol., 129*, 623 (1977).
22. Trudinger, P.A., and Loughlin, R.E., In: Amino Acid Metabolism and Sulphur Metabolism, A. Neuberger (Ed.), Comprehensive Biochemistry, *19*, A. Elsevier, Amsterdam, 165 (1981).
23. Cox, R.A., and Sheppard, D., *Nature, 284*, 330 (1980).
24. Cronan, D.S., Underwater Minerals, Academic Press, London (1980).
25. Degens, E.T., and Ross, D.A., Hot Brines and Recent Heavy Metal Deposits in the Red Sea, Springer Verlag, New York (1969).

26. Trudinger, P.A., *BMR J. Aust. Geol. Geophys.*, *6*, 279 (1981).
27. Holser, W.T., and Kaplan, I.R., *Chem. Geol.*, *1*, 93 (1966).
28. Jørgensen, B.B., Hansen, M.H., and Ingovorsen, K., In: Evironmental Biogeochemistry and Geomicrobiology, W.E. Krumbein (Ed.), *1*, Ann Arbor Science, Ann Arbor, 245 (1978).
29. Ajtay, G.L., Ketner, P., and Duvigneaud, P., In: The Global Carbon Cycle, B. Bolin, E.T. Degens, S. Kempe and P. Ketner (Eds.), SCOPE Report No. 13, John Wiley and Sons, Chichester, 129 (1979).
30. De Vooys, C.G.N., In: The Global Carbon Cycle, B. Bolin, E.T. Degens, S. Kempe and P. Ketner (Eds.), SCOPE Report No. 13, John Wiley and Sons, Chichester, 259 (1979).
31. Hampicke, U., In: The Global Carbon Cycle, B. Bolin, E.T. Degens, S. Kempe and P. Ketner (Eds.), SCOPE Report No. 13, John Wiley and Sons, Chichester, 219 (1979).

ROLE OF MICROORGANISMS IN THE MIGRATION OF SOME ELEMENTS

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The universal occurrence of microorganisms in nature has long been proved by Pasteur's work. They are abundant in the lithosphere down to 2000 m depths, are present in the deepest ocean troughs, and may be found at the heights of dozens of kilometers and at temperatures ranging from below 0 °C to +300 °C. Their number often exceeds 10 million to 1 g of soil, silt or rock, or 1 ml of water. Microorganisms are endowed with the greatest energy of multiplication amidst living beings. Possessing a high biological activity, they are able to process quantities of matter hundreds of times as large as their own weight. Hence, we can easily visualize the "whirlwind of elements' migration" (Vernadsky) which they can produce.

The science of geochemical activity of microorganisms in nature has emerged from the work of S.N. Winogradsky, M.W. Beijerinck, V.I. Vernadsky, B.L. Isachenko, C.E. ZoBell, N.G. Kholodny, S.I. Kuznetsov and many other scientists and their disciples.

An especially rapid development of this science began in the second half of the 20th century after not only the crucial role of microorganisms in the cycle of chemical elements in water bodies, rocks and other ecological niches had been shown experimentally but also the scientific foundation of biogeotechnology had been laid. The principal results of these studies are summarized in a number of monographs [1—8].

In this work we shall discuss some microbiological processes in the migration of sulphur and ore-forming elements, which play an important role in the hypergenesis of ore deposits.

Realizing the transformations of chemical elements, microorganisms make use of different mechanisms. Quite a number of elements, the majority of which occur in ore deposits in the reduced state, can serve as a source of energy for autotrophic microorganisms.

This is, above all, sulphur which can serve as an energy source for thionic bacteria. Of all the species of thionic bacteria *T. ferrooxidans* alone is capable of oxidizing all sulphide minerals.

Other thionic bacteria developing in neutral media, e.g. *T. denitrificans* and *T. thioparus*, as well as the latter's ecological variety *T. thioparus* subsp. *antimoniticum*, are capable of oxidizing only a few sulphide minerals and intermediate sulphur compounds formed during

chemical oxidation processes. The easily oxidizable sulphides that can be attacked by thionic bacteria developing in a neutral medium comprise antimonite, galena, and bismuthine.

Sulphide minerals are oxidized also by *Leptospirillum ferrooxidans* in symbiotic culture with *Thiobacillus thiooxidans* or *Thiobacillus acidophilus* [9, 10] while sulphide minerals, sulphur and Fe^{2+} are oxidized also by facultative thermophilic bacteria, such as *Thiobacillus* TH 1, 2, 3 [11], *Sulfobacillus thermosulfidooxidans* [12] and *Sulfolobus brierleyi* [13]. They occur in ores and leach dumps.

As a result of the oxidizing activity of bacteria, bivalent sulphur of sulphides is converted to hexavalent sulphur of sulphates which partly migrate with water streams, finally finding their way into the ocean, and partly react with elements in the surrounding rocks, forming secondary sulphate minerals.

The process of microbiological reduction of sulphates is also sufficiently widespread in the deposits of sulphide ores. This particularly applies to deposits of a sedimentary or volcanogenic-sedimentary genesis, the host rocks of which often contain increased amounts of organic substances, up to 2—3 %. Instances illustrating the participation of sulphate-reducing bacteria in the cycle of individual elements will be dealt with further in the text.

Apart from sulphur, other energy sources for bacteria may be bivalent iron, antimony, univalent copper, molybdenum, uranium [14—19].

Quite widespread in the desposits are microbiological processes which result in microbial metabolites exerting influence on various elements. A great effect on the transfef of elements can be produced by organic acids forming complexes with various metals. Gold is known to form complexes with amino acids. Silicate minerals may disintegrate under the effect of mineral acids released by nitrifiers, as well as under the effect of organic substances forming complexes with silicon.

Furthermore, microorganisms may exert influence on the elements through their sorption and accumulation in the biomass. In other words, microorganisms act as concentrators of chemical elements as pointed out by Vernadsky [20]. Recently, extensive research in this field has been carried out by Koval'sky, Letunova et al. [21, 22]. Nearly all elements are present in the biomass in negligible quantities, but biogenic elements may attain significant concentrations. Certain microorganisms, e.g. methane-producing ones are known to produce an increased quantity of vitamine B_{12} and, consequently, they need an increased quantity of cobalt. According to Schönheit and Moll [23], apart from cobalt, these bacteria are in need of nickel and molybdenum.

Of 104 chemical elements, about 60 are subject to the effect of living organisms [8].

In discussing the role of microorganisms in the cycle of ore-forming elements, special attention should be given to ecological conditions existing in the various ore deposits. Of great importance for microbiological processes is the mineralogical composition of ores and rocks, which determines not only the presence of the energy material for microorganisms, but also one of the highly important ecological factors crucial to the composition of the microflora, namely pH. Since pyrite, being a disulphide, yields the maximum amount of acid, its presence creates favourable conditions for the growth of acidophilic thionic bacteria. In pyrite deposits the quantity of pyrite occasionally accounts for 90 % of total ore mass and therefore pyrite and copper-pyrite deposits usually containing 30—40 % of pyrite happen to be the most favourable ecological niche for the development of *T. ferrooxidans*.

Highly important ecologically is the composition of host rocks. According to Nishihara [24], carbonates, orthosilicates and pyrrhotite are particularly active in decreasing acidity. Most of sulphides, pyroxenes, amphiboles and feldspars are characterized by average activity. The inert group includes quartz, muscovite, fluorite, barite and, as regards sulphides, chalcopyrite.

All thionic bacteria, except for *T. denitrificans*, being aerobic organisms in need of oxygen, highly important ecologically becomes the jointing of the orebody. Oxygen finds its way inside the orebody not only in the form of gas, but also with ground waters. As waters penetrate deeper, they lose the greater part of oxygen which is spent on chemical and biochemical oxidation processes. Observations at the Kal'makyrsk open-cast mine showed that the oxygen content of water declined from 9.5 mg/l at the surface to some parts of mg/l at the lower horizons.

Thionic bacteria have been discovered in nature for a wide range of Eh, from —150 to +800 mV [25]. Development of *T. thiooxidans* does not proceed below rH_2 16; *T. thioparus* makes better progress under lower values of redox potential (rH_2 = 10—16), but in the presence of oxygen [3].

For a number of deposits, the host rocks of which contain organic matter, a characteristic feature is the presence of two zones: an oxidation and a reduction one which differ with respect to the redox potential value, pH, the presence of oxygen and microflora.

For the development of thionic bacteria, apart from the energy material contained in the ores, certain mineral substances are necessary. These are nitrogen and phosphorus compounds, sulphates, magnesium, iron, and traces of certain metals. As shown by Tuovinen et al. [26], who have studied the effect of sulphate on iron oxidation, the rate of this process increases until the sulphate ion concentration attains 2 g/l. If sulphates, iron, magnesium and trace metals are,

as a rule, present in sufficient quantities in mine waters, a deficiency of nitrogen and phosphate may slow down bacterial oxidation processes taking place in nature. Man's activities promote these processes not only because more oxygen is brought into deep-lying strata and the crushing of the orebody and its water content increase, but also because ore mining brings in large quantities of nitrogen compounds resulting from the use of nitrogen-containing explosives.

According to Stevenson [27] and Wlotzka [28], nitrogen is present in the rocks, its content varying from 20 g/t for magmatic rocks to 500—800 g/t for sedimentary rocks. Nitrogen is largely in the ammonium form and, possibly, can be used by microorganisms, particularly the acid-forming ones, the metabolites of which contribute to destruction of the rocks.

We shall give some instances of the transformations of ore-forming elements.

Zinc sulphide, sphalerite, is fairly easily oxidized under the effect of *T. ferrooxidans* and some other bacteria. The resulting sulphate is notable for maximum solubility among other sulphates, 531 g/l at 18 °C. Therefore, zinc is disseminated in the oxidized zone. Nonetheless, there exists in nature a mineral goslarite $ZnSO_4 \cdot 7H_2O$ formed as a result of sphalerite oxidation. It is to be mainly encountered on the walls of mine workings, e.g. in the galleries of Rammelsberg (Harz, FRG), as well as in the oxidized zone in the Upper Silesia's deposits [29].

The biological cycle of zinc was observed by us at the Kizil-Dere deposit in Dagestan. Primary ores in this deposit are represented by pyrrhotite, pyrite and chalcopyrite, sphalerite being of secondary importance. The ores are oxidized both chemically and under the effect of *T. ferrooxidans* which we found to be present in the deposits. Analysis of water samples from a borehole in a fracture zone with impregnated sulphide mineralization showed this water to contain 54 μg/l zinc, 230 mg/l sulphates, no iron having been detected in the water. Jurassic shales which happen to be the host rock contain organic matter. The water of the borehole concerned was found to contain sulphate-reducing bacteria and 1.8 mg/l hydrogen sulphide.

Below the borehole there had formed a black precipitate of sulphides in which we found 100 cells/g of sulphate-reducing and 100,000 cells/g of thionic bacteria. The spectral analysis of the precipitate showed the presence of 3 % zinc.Thus, at this deposit we could observe bacterial oxidation processes owing to which zinc was solubilized, and reduction processes as a result of which, as revealed by our experiments, as much as 0.005 mg/l hydrogen sulphide was formed daily. Despite a low intensity of the sulphate reduction process, it led to the formation of secondary zinc sulphide, i.e. the cycle was complete.

Copper being an important metal, transformations of copper minerals are dealt with in numerous works.

Chalcopyrite is one of the main copper sulphides. Torma [30] carried out X-ray studies of the solid residue following the bacterial leaching of copper from chalcopyrite. Apart from jarosite, basic ferric sulphate, he discovered a basic copper sulphate, antlerite $CuSO_4 \cdot 2Cu(OH)_2$. This is a mineral occurring in the oxidized zone of copper deposits along with chalcanthite, brochantite and other sulphates. We observed the formation of these minerals at the Kounrad copper deposit in Kazakhstan.

During the bacterial oxidation of copper sulphides, apart from the aforementioned ones, quite a number of other copper minerals may be formed. Once the solution containing copper sulphate reacts with calcite the result is the formation of malachite $CuCO_3 \cdot Cu(OH)_2$ or azurite $2CuCO_3 \cdot Cu(OH)_2$. Under the effect of acid waters these minerals can be reconverted to copper sulphate which reacts with waters rich in silicic acid forming chrysocolla $CuSiO_3 \cdot 2H_2O$.

On the evidence of Nielsen and Beck [31], the oxidation of chalcocite by the *T. ferrooxidans* culture results in the formation of digenite Cu_9S_5 and covellite CuS. According to Silver and Torma [32], this process produces also antlerite and metallic copper, the latter being widespread in the oxidized zones of copper deposits.

In the above examples copper minerals were transformed as a result of bacterial effect on sulphide sulphur. But some reports available in the literature indicate that *T. ferrooxidans* is capable of obtaining energy from oxidation of univalent copper [16].

In experiments by Imai et al. [16], intact cells of *T. ferrooxidans* oxidized pulverized chalcocite Cu_2S. Apart from the indirect mechanism involving oxidation of chalcocite by ferric sulphate, the direct mechanism of enzymatic oxidation of univalent to bivalent copper is possibly utilized, trivalent iron being in this case a co-factor. Nielsen and Beck [31] have found out that the oxidation energy of univalent copper contributes to carbon dioxide fixation. The sulphur deficiency in chalcocite accounts for copper going partly over into cuprite Cu_2O.

Vanadium is also an element with a varying valence. As pointed out in the US patent No. 3268288 for 1966, *T. ferrooxidans* is capable of oxidizing vanadium to the pentavalent state. In our experiment with the *T. ferrooxidans* culture grown on different energy substrates — on iron and on antimony sulphide without iron it was shown that vanadium oxidation occurred only with the aid of the indirect mechanism under the effect of ferric sulphate. In nature there exist minerals in which vanadium is contained in different valent forms. In Peru there occurs minasragrite containing tetravalent vanadium; in montroseite occurring in a deposit of uranium-bearing sandstones at the Colorado Plateau vanadium is likewise tetravalent. This mineral in the oxidized zone is converted to corvusite or melanovanadium in which vanadium is mostly pentavalent. Apparently, *T. ferrooxidans* contributes to vanadium transformations in nature. Ferric sulphate participating in oxidation is formed in the development of bacteria on pyrite present in uranium-bearing sandstones.

Under these conditions thionic bacteria contribute, moreover, to uranium migration, since ferric sulphate and sulphuric acid convert uranium to a soluble state, forming uranyl sulphate.

Since uranium oxidation proceeds more rapidly in the presence of iron-oxidizing thiobacilli than in the presence of ferric iron alone, it has been suggested [33] that *T. ferrooxidans* oxidizes tetravalent uranium as follows:

$$2UO_2 + O_2 + 2H_2SO_4 = 2UO_2SO_4 + 2H_2O.$$

Conversion of tetravalent to hexavalent uranium releases 20.6 kcal/mol. Recent investigations have rendered support to this assumption [18, 19].

The presence of variable valence elements presupposes the possibility of their use by microorganisms for energy purposes.

We examined conditions which, when present, could be considered favourable for the given element to maintain an autotrophic growth.

1) The energy released in the oxidation of 1g-atom of the element concerned must be sufficient for setting up a macroergic bond.

2) The element must be sufficiently abundant in nature and be able to form regular accumulations.

3) There must exist in nature minerals containing the element concerned in the reduced and in the oxidized form.

Above we have already discussed the effect of microorganisms on copper and vanadium. Among the variable-valence elements there are also antimony, molybdenum and chromium; in antimony deposits, apart from trivalent antimony minerals, either sulphide or oxide, there are present minerals containing pentavalent antimony.

We have assumed that antimony ore consisting principally of the mineral antimonite, Sb_2S_3 is oxidized by bacteria in two phases. Initially, thionic bacteria oxidize sulphur of antimonite, forming antimony trioxide. Further oxidation of the trioxide is realized by the previously unknown microorganisms for which this process is an energetic one. This assumption found experimental support. In laboratory experiments we obtained from antimonite a mineral based on antimony pentoxide using *T. ferrooxidans* and *Stibiobacter senarmontii*, a new bacterium deriving energy from antimony oxidation. The reverse process of antimony trioxide reduction to sulphide can be realized by sulphate-reducing bacteria. This process has a practical significance, since antimony trioxide treated with the culture liquid of sulphate-reducing bacteria is covered with sulphide film and may be extracted by sulphide flotation, increasing the yield of antimony. By means of bacterial treatment it is possible additionally to extract as much as 7—11 % of antimony from the tailings of sulphide flotation.

Thus, useful in ore concentration may be not only bacterial oxidation processes but also reduction processes.

Another element which, in our opinion, can provide energy for autotrophic growth is molybdenum. Oxidation of tetravalent to

hexavalent molybdenum yields about 40 kilocalories. Molybdenum forms ore deposits and is sufficiently abundant in nature. Although tetravalent molybdenum predominates in the deposits, in the hypergenesis zone there is present the mineral ilsemannite in which molybdenum is pentavalent. In powellite and ferrimolybdite molybdenum is hexavalent. These minerals can be regarded as calcium and ferric molybdates. We succeeded in obtaining an enriched culture of microorganisms developing on a mineral substrate where the only energy source was molybdenum dioxide, MoO_2. The originally obtained enriched culture was capable of developing on molybdenite, up to 78 mg/l molybdenum being solubilized in this case, i.e. 20 times as much as in the control. Presumably, molybdenite was oxidized under a joint effect of several microorganisms, since with the progress of time the enriched culture lost its capacity to oxidize molybdenite, while preserving its ability to oxidize molybdenum dioxide. During MoO_2 oxidation up to 200 mg/l molybdenum would be solubilized in two-weeks time, i.e. 9 times as much as in the control.

For *T. ferrooxidans*, molybdenum is the most toxic of metals, its toxic effect becoming manifest already at concentrations of about 10 mg/l. Cultures tolerant to 200 mg/l Mo have been obtained by means of adaptation [34]. Hence, the role of *T. ferrooxidans* in the oxidation of molybdenum deposits is evident but it is not clear yet whether it can be used for leaching this element.

In 1972 Brierley [35, 36] isolated a micoplasma-like thermophilic organism capable of oxidizing sulphur and iron. This organism was capable of high-rate oxidation of molybdenite and easily survived dissolved molybdenum in concentrations surpassing 1.5 g/l. The isolated organism can contribute to molybdenum transformations in dumps when the ore heats up. Furthermore, it can be active in tropical regions where temperatures about 40 ^{o}C are common. In regions with a moderate climate the greatest role in molybdenum transformations is played by a little-known association of microorganisms, one of which can possibly use the energy from the conversion of tetravalent to hexavalent molybdenum.

In the destruction of sulphide minerals under the effect of bacteria not only ore-forming elements and sulphur are solubilized but also rare elements which, because of the similarity of their properties to those of zinc, copper or lead, enter in the crystal lattices of minerals isomorphously replacing these metals. The behaviour of rare and trace elements in sulphide destruction is largely dependent on the chemical properties of the given element, on the solubility of its compounds under different Eh-pH values, on the presence in the environment of substances contributing to precipitation of the given element. Together with Kulikova we made a number of experiments in bacterial oxidation of different sulphides containing such elements as cadmium, thallium, gallium, indium, germanium, selenium, tellurium, and rhenium. We used in the experiments different sphalerites, geo-

cronite, galena, copper-molybdenum ore. Selenium in its chemical properties being closer to sulphur, the role of microorganisms in its leaching was studied using hypergenous minerals of one of the deposits, namely quartz-sulphur and pyrite fines. Tellurium was likewise present in these minerals. The results of the experiments are shown in Table
The obtained results depend both on the element content of the substrate and on the degree of oxidation of the substrate. The maximum concentration of a rare element in our experiments was achieved in the oxidation of sulphur containing selenium and was 18 mg/l Se. Rhenium being one of the least abundant elements in the earth's crust and its clarke being equal to $1 \cdot 10^{-7}$ %, the concentrations of the element in our experiments were also insignificant, not more than 8 μg/l.

The role of bacteria in the geochemistry of rare elements is that they reduce the latters' content of primary minerals. In most cases rare elements are not part of secondary minerals. In our experiments

Table

Leaching of rare elements from sulphides under the effect of *T. ferrooxidans*

Sulphide	Rare element content	Experiment version	Amount in solution, μg/l
Galena	Germanium	Experiment Control	30 0
Sphalerite	Germanium 0.014 %	Experiment Control	80 19.5
Geocronite	Thallium 0.03—0.06 %	Experiment Control	84.7 250
Sphalerite	Indium	Experiment Control	50 0
Sphalerite	Cadmium	Experiment Control	7600 900
Sphalerite	Gallium	Experiment Control	50 25
Molybdenite + Chalcopyrite	Rhenium 1.9 mg/t	Experiment Control	8.6 1
Pyrite	Selenium 0.044 %	Experiment Control	8000 3000
Pyrite	Tellurium 0.013 %	Experiment Control	2800 1300

an exception from this rule happened to be the inclusion of germanium into the crystal lattice of anglesite, $PbSO_4$, formed in galena oxidation.

The role of thionic and other bacteria capable of oxidizing sulphide minerals, sulphur and Fe^{2+} as a factor in the dispersion of rare elements has been confirmed by geochemical investigations showing that secondary minerals in deposits are 50—100 times as poor in them as are primary minerals [37, 38].

The problem of arsenic cycle is of special significance on account of toxic effect produced by this element on all living organisms. The role of *T. ferrooxidans* in the oxidation of arsenopyrite and the mechanism of this process have been described by Pol'kin et al. [8]. Oxidation of As^3 to As^5 by bacteria, however, presents special interest. In 1918 Green [39] isolated such an organism from an arsenic-containing liquid used for cattle desinfection. The organism called *Bacillus arsenoxidans* was capable of growing in a medium containing 1 % arsenic in the form of arsenite and could carry out its oxidation. Later Turner [40] isolated 15 strains of heterotrophic bacteria resistant to 1 % solution of arsenite which they oxidized to arsenate. The oxidation rate attained 90 μg of arsenite per hour to 1 mg of dry weight.

Arsenite oxidation was discovered in the soil [41, 42] and, under anaerobic conditions, in activated sludge [43].

The process of arsenite oxidation is very important for environment protection, since the more toxic trivalent arsenic is transformed into pentavalent arsenic.

An interesting work was carried out by Ilyaletdinov and Abdrashitova [44] who isolated an organism called *Pseudomonas arsenitoxidans* capable of oxidizing trivalent to pentavalent arsenic under autotrophic conditions. The organism developed on a mineral substrate containing 1.3 g/l trivalent arsenic. The oxidation was accompanied by the decrease in pH to 4.5—5.5. The radiocarbon method showed the inclusion of 41 % carbonate carbon.

This organism is capable of exerting effect on arsenopyrite. Thus, arsenic-containing sulphides — realgar, auripigment, arsenopyrite can be oxidized not only in acid media in the presence of *T. ferrooxidans* but also in neutral media in the presence of *Pseudomonas arsenitoxidans.*

Bacteria isolated by Mynbaeva and Ilyaletdinov [45] oxidized arsenite under the effect of peroxide and catalase. In this case the mechanism of arsenic oxidation was similar to peroxide mechanism the existence of which was proved by Dubinina [46] for bacterial processes in manganese and iron oxidation.

Common heterotrophic organisms, such as *Pseudomonas* and *Alcaligenes*, attack arsenite and arsenate, transforming them into gaseous arsenic products — arsine, methyl and dimethyl arsine. It is probable that what takes place in this case is the reduction rather than methylation of arsenate.

Microbiological reduction processes, except for sulphate reduction, have been relatively little studied in ore deposits. But the majority of processes taking place in the soil may occur under deposit conditions as well.

Koval'sky et al. [47, 48] have discovered the capacity of various species of soil microorganisms, bacteria, fungi and actinomyces to reduce selenites to elementary selenium. Selenium reduction is not constrained to a specific group of microorganisms. In ore deposits, however, there may occur specific groups of microorganisms which use oxidized compounds as donors of oxygen. This kind of process is the reduction of chromates by *Pseudomonas dechromaticans*, as described by Romanenko and Koren'kov [49, 50], and by *Pseudomonas chromatophila* isolated by Lebedeva and Lyalikova [51] from an open-cast pit at the Kempirsaisk chromite deposit. These bacteria, when grown under anaerobic conditions on a substrate containing simple organic substances (certain organic acids, sugars and alcohols), will reduce hexavalent chromium compounds to the trivalent state. We staged an experiment in which a natural mineral crocoite, $PbCrO_4$, was used as a donor of oxygen for chromium-reducing bacteria. As revealed by the X-ray analysis, crocoite was partly transformed by bacteria into a mineral of the Cr_2O_3 composition, i.e. the hypergenous mineral crocoite was transformed into chromite.

One of the ways in which heterotrophic bacteria act upon heavy metals is their methylation. This process is of great importance in view of the fact that methylation often increases the toxicity of an element dozens of times. In studying the cycle of heavy metals in water reservoirs in industrial areas it was found out [52] that about 80 % mercury was accumulated by fish organisms in the form of methyl mercury. Doran and Alexander [53] described the methylation of selenium and its compounds by soil bacteria. Earlier this process had been known for microscopic fungi.

Selenium provides an example of the participation of both autotrophic and heterotrophic species of microorganisms in the cycle of an element.

From what has been said above we may draw a conclusion that biological cycle of an element is likely to proceed on the largest scale when the compounds of this element can serve either as an energy source for microorganisms or as a source of oxygen.

The transfer and transformation of mineral forms involve the participation of metabolic products, such as organic and mineral acids, amino acids, etc. The role of organic metabolites is particularly great in sedimentary deposits with an increased organic carbon content. Thus, according to Germanov and Panteleev [54] in the waters of the Dzhezkazgan deposit 5 to 100 % of copper is in the form of organic complexes.

Microorganisms contribute to the transformation of elements through hydrogenation, methylation and other enzymatic transfor-

mations. A great effect on the mobility of elements can be produced by such products of the microbiological origin as iron hydroxide, jarosites, methane, hydrogen sulphide. The presence of "reduction barriers' formed as a result of the activity of methane-producing and sulphate-reducing bacteria significantly alters the behaviour of certain elements.

The study of the role of microorganisms in the cycle of heavy metals is highly important both for understanding their geochemistry and for solving practical problems related to their leaching and environment protection.

REFERENCES

1. Kuznetsov, S.I., Ivanov, M.V., and Lyalikova, N.N., Introduction to Geological Microbiology, Nauka, Moscow (1962) (in Russian).
2. Ivanov, M.V., Role of Microbiological Processes in the Genesis of Sulphur Deposits, Nauka, Moscow (1964) (in Russian).
3. Sokolova, G.A., and Karavaiko, G.I., Physiology and Geochemical Activity of Thionic Bacteria, Nauka, Moscow (1964) (in Russian).
4. Zajic, J.E., Microbial Biogeochemistry, Academic Press, New York-London (1969).
5. Kuznetsov, S.I., Microflora of Lakes and its Geochemical Activity, Nauka, Leningrad (1970) (in Russian).
6. Zavarzin, G.A., Lithotrophic Bacteria, Nauka, Moscow (1972) (in Russian).
7. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.I., Role of Microorganisms in the Leaching of Metals from Ores, Nauka, Moscow (1972) (in Russian).
8. Pol'kin, S.I., Adamov, E.V., and Panin, V.V., Technology of Bacterial Leaching of Non-ferrous and Rare Metals, Nedra, Moscow (1982) (in Russian).
9. Markosyan, G.E., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (ed.), Pushchino (1976) (in Russian).
10. Norris, P.R., and Kelly, D.P., *FEMS Microbiol. Letters, 4*, 1, 143 (1978).
11. Le Roux, N.W., and Marshall, V., In: Round Table Conf. Bacterial Leaching, W. Schwartz (ed.), Braunschweig-Stöckheim, 21 (1977).
12. Golovacheva, R.S., and Karavaiko, G.I., *Mikrobiologiya, 47*, 5, 815 (1978).
13. Brierley, C.L., and Brierley, J.A., *Can. J. Microbiol., 19*, 183 (1973).
14. Colmer, A.R., and Hinckle, M.E., *Science, 106*, 253 (1947).
15. Lyalikova, N.N., Reports of the Academy of Sciences of the USSR, ser. biol., *205*, 5, 1228 (1972) (in Russian).
16. Imai, K., Sakaguchi, H., Sugio, T., and Tano, T., *J. Ferment. Technol., 51*, 12, 865 (1973).
17. Lyalikova, N.N., and Lebedeva, E.V., *Geomicrobiology J.* (in press) (1984).
18. Soljanto, P., and Tuovinen, O.H., In: Biogeochemistry of Ancient and Modern Environments, P.A. Trudinger, M.R. Walter, and B.J. Ralph (eds.), Australian Academy of Sciences and Springer Verlag, 469 (1980).
19. DiSpirito, A.A., and Tuovinen, O.H., *Arch. Microbiol., 133*, 28 (1982).
20. Vernadsky, V.I., Problems of Biogeochemistry, Transactions of Biogeochemical Laboratory, Nauka, Moscow, *16* (1980) (in Russian).

21. Koval'sky, V.V., In: Modern Objectives and Problems of Biogeochemistry, Nauka, Moscow, XVII, 12 (1979) (in Russian).
22. Letunova, S.V., In: Modern Objectives and Problems of Biogeochemistry, Nauka, Moscow, XVII, 101 (1979) (in Russian).
23. Schönheit, P., Moll, J., and Thauer, R., *Arch. Microbiol., 123*, 1, 105 (1979).
24. Nishihara, G.S., *Econ. Geol., 9*, 743 (1914).
25. Karavaiko, G.I., Lyalikova, N.N., and Pivovarova, T.A., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (ed.), Pushchino (1976) (in Russian).
26. Tuovinen, O., Niemela, S., and Gyllenberg, H., *Biotechn. Bioeng., 13*, 517 (1971).
27. Stevenson, F.J., *Science, 130*, 3369, 221 (1959).
28. Wlotzka, F., *Geoch. et Cosmoch. Acta, 29*, 106 (1961).
29. Zwizycki, J., In: Geology, Paragenesis and Resources of Ores in Deposits of Foreign Countries, Inostrannaya Literatura (1953) (in Russian).
30. Torma, A.E., Proceedings of the Third International Biodegradation Symposium, J.M. Sharpley and S.M. Kaplan (eds.), London (1976).
31. Nielsen, A.M., and Beck, J.V., *Science, 175*, 1124 (1972).
32. Silver, M., and Torma, A., *Can. J. Microbiol., 20*, 141 (1974).
33. Lundgren, D.G., and Silver, M., *Ann. Rev. Microbiol., 34*, 263 (1980).
34. Kamalov, M.D., Transactions of the Academy of Sciences of Tadj. SSR, ser. biol., 1, 39 (1972) (in Russian).
35. Brierley, C.L., New Mexico State Bureau of Mines and Mineral Resources, E-2 (1972).
36. Brierley, C.L., and Brierley, J.A., *Can. J. Microbiol., 19*, 183 (1973).
37. Kulikova, M.F., *Geokhimiya*, 2, 154 (1962).
38. Kulikova, M.F., *Geokhimiya*, 10, 1233 (1966).
39. Green,H.H., 1918, cit. [40].
40. Turner, A.W., *Nature, 164*, 76, 4158 (1949).
41. Quastel, Scholefield, *Soil Science, 75*, 279 (1953).
42. Osborne, F.H., and Ehrlich, H.L., *J. Appl. Bacteriol., 41*, 295 (1976).
43. Heimbrook, M.E., and Morrison, S.M., Abstr. of Am. Meetings of Amer. Soc. Microbiol., 48 (1973).
44. Ilyaletdinov, A.N., and Abdrashitova, S.A., *Mikrobiologiya, 50*, 2, 197 (1981).
45. Abdrashitova, S.A., Mynbaeva, B.N., and Ilyaletdinov, A.N., *Mikrobiologiya, 50*, 1, 41 (1981).
46. Dubinina, G.A., *Mikrobiologiya, 47*, 4, 591 (1978).
47. Koval'sky, V.V., Yermakov, V.V., and Letunova, S.V., *Mikrobiologiya, 37*, 122 (1968).
48. Koval'sky, V.V., and Yermakov, V.V., Biological Importance of Selenium, Nauka, Moscow (1968) (in Russian).
49. Romanenko, V.I., and Koren'kov, V.N., *Inform. Bul. of Inlands Waters Biology*, USSR Academy of Sciences, 25, 8 (1975) (in Russian).
50. Romanenko, V.I., Kuznetsov, S.I., and Koren'kov, V.N., Authorship Cert. No. 521234 for an invention "Koren'kov's method of biological purification of sewage waters", Bul. No. 26 (1976) (in Russian).
51. Lebedeva, E.V., and Lyalikova, N.N., *Mikrobiologiya, 48*, 3, 517 (1979).
52. Sammers, A.O., and Silver, S., *Ann. Rev. Microbiol., 32*, 637 (1978).
53. Doran, J.W., and Alexander, M., *Appl. Environm. Microbiol., 33*, 31 (1977).
54. Germanov, A.I., and Panteleev, V.M., *Geokhimiya*, 3, 358 (1970).

ROLE OF MICROORGANISMS IN THE HYPERGENE MIGRATION OF GOLD

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INTRODUCTION

Gold is one of the most stable elements. Its characteristic feature is a propensity to occur in the native state. Its constant associates are silver and copper, besides, it contains trace amounts of some 40 other elements. In compounds it has the valency of +1 or +3. Univalent ions more readily form water-soluble complexes with oxygen- and sulfur-containing ligands under alkaline conditions. Well-known are chelate compounds and also salts of gold with ammonia and amines. Despite its amazing chemical stability gold is rather mobile in the surface layers of the lithosphere. However, the regularities and mechanism of this element's migration have been studied poorly so far.

The purpose of this work is to examine the physicochemical, microbiological and biochemical aspects of the hypergene migration of gold.

DISTRIBUTION OF GOLD IN THE BIOSPHERE

Gold is scattered in the biosphere in all types of rocks (Table 1). Higher concentrations of gold occur in the sedimentary rocks enriched with organic matter. In auriferous areas the soil layer is also characterized by increased concentrations of gold. In rivers draining gold-mining fields, the water's gold content in some cases is up to 1 g/t. The gold content of sea water is by two-three orders lower than that of the lithosphere. It is believed that gold is present in sea water in the form of $AuCl_2^-$, $AuCl_4^-$, and AuO_2^- complexes and in the form of gold-organic compounds [1, 23, 24]. In the coastal waters of sea basins there are colloidal and coarser gold suspensions. Gold has been found in living organisms (Table 2). In the following series the animal organisms are situated in the increasing order of their capacity to concentrate gold: sea urchins — starfish — crabs — bryozoanes. For their capacity to concentrate gold, microorganisms range as follows: actinomycetes > fungi > bacteria. Gold accumulates in the higher plants, in different kinds of mushrooms, in lichens and mosses [27].

The above cited data concerning the distribution of gold in the biosphere point to this element's involvement in migration processes under the impact of physicochemical and biological factors. We shall restrict this paper to the examination of the role of microorganisms in the migration and deposition of gold.

Table 1

Gold content of different geological formations

Type of geological formation	Gold content, mg/t	Gold clark	Reference number
Volcanic rock (acid, basic, intermediate, ultrabasic)	0.16—16.0	4.3	1—6
Sedimentary terrigene rock (conglomerates, sandstones, argillites, etc.)	1.0—5.5	1.0	1—6
Biogenic rocks:			
coal	up to 11000		7, 9, 10, 11, 14
laterite	up to 10000		8
sapropel	15—2500		12
phosphorites	up to 30		13
peat	5—50		15
corals	up to 8		2
kerogen	800—2200		16
coaly shales	up to 200		2, 18
soils	50—10000		13, 17, 19
Unda river	1000		20
Aldan river tributaries	11		21
Waters of rivers feeding the Baltic Sea	4.0—9.8		22
Oceanic waters	0.8—50		1, 20—22

Table 2

Gold content of living organisms

Organisms	Gold content, mg/t	Reference number
Seas and oceans		
Algae	150 (average)	26
Water suspension containing organic substances	$0.4—15.3 \times 10^{-7}$ mg/l	22
Marine animals (sea urchins, starfish, crabs, bryozoanes)	100—750	25, 26
Ore deposits		
Microorganisms	32—85	Our data

ROLE OF MICROORGANISMS IN THE DISSOLUTION OF GOLD

As is known, the oxidation of sulfide minerals in gold-sulfide deposits takes place with the participation of microorganisms. During this process gold is solubilized, as testified by its increased content of the waters draining the ores. Experimental studies also demonstrate that during the bacterial oxidation of pyrite and galenite much more gold is solubilized than in bacteria-free controls (Table 3). Thermodynamic calculations by Kakovsky [28] point to the formation of sufficiently strong thiosulfate complexes under these conditions. The reaction potential of gold dissolution $Au + 2S_2O_3^{2-} - \epsilon \rightarrow [Au(S_2O_3)_2]^{3-}$ for standard conditions is $E_0 = 0.142$ V.

Table 3

Gold content of solutions upon oxidation of sulfides by *Thiobacillus ferrooxidans* [29]

Sulfides	Presence of bacteria	Quantity of gold in solution, μg/l
Gravitation concentrate	+	270—520
	—	0—60
Pyrite	+	60—70
	—	0
Galenite	+	320—540
	—	230

Our microbiological investigations demonstrated also the presence of great quantities of heterotrophic bacteria in the ores and waters of gold ore deposits (Table 4). Here the number of bacterial cells depends on the type and degree of disintegration of ores and rocks. The life activity of heterotrophs in the ores depends on the concentration of organic matter. The ore samples and waters examined contained 0.01 to 0.1 % of total and amine nitrogen, as well as free amino acids — alanine, valine, serine, glutamic and aspartic acids, and the pH value of the samples was in the range of 5.0 to 8.4. It may be presumed that the examined ores provide favourable conditions for the development of microorganisms, while the aforementioned acids are products of their life activity. The microorganisms active in the dissolution of gold were revealed by means of studying the microbial landscapes of the ore material. For this purpose ore particles 0.25 mm in size or powdered gold were applied to the surface of a poor solid medium. After a certain time gold changed into the ionic state. The landscapes were dominated by representatives of the genera *Bacillus* and *Pseudomonas*.

Table 4

Occurrence of heterotrophic microorganisms in gold ore deposits [30]

Description	Number of cells, thousands per 1 g of ore			
	bacteria	bacilli	fungi	actinomycetes
Disintegrated kaolinized rock	4240	1300	360	140
Attrition clay	12140	2720	1320	470
Semidisintegrated conglomerate	6720	1550	1280	1380
Semidecayed coaly shales	16780	4510	2940	6900

The first scholar to experimentally study the role of heterotrophs in the dissolution of gold was Pares [31—34].

Our data pertaining to the dissolution of gold by heterotrophic bacteria are presented in Table 5.

Table 5

Dissolution of finely-dispersed gold by microorganisms [35, 36]

Bacteria	Strain	Gold content of solution (pH = 8.0—8.5), mg/l		
		80 days	110 days	140 days
Micrococcus flavus	16	0.12	0.21	0.21
B. megaterium	20	1.21	2.15	2.15
B. mesentericus	20	0.17	0.31	1.35
B. solitarium	22	0.06	0.08	0.24
B. nitrificans	29	0.20	0.22	0.47
B. megaterium	30	0.37	1.54	1.54
P. liquefaciens	9	1.62	—	—
Control		0	0	—

One can see from Table 5 that the activity of different species of heterotrophic bacteria in gold dissolution is not the same. Most active in the dissolution of gold were *B. megaterium*, *B. mesentericus* and *P. liquefaciens*. Gold dissolution activity increased with the increase in the pH.

Microscopic fungi and actinomycetes are less active in dissolving gold and its concentration in the solution in their presence did not exceed 0.02 mg/l.

This makes it clear that natural strains of autotrophs and heterotrophs are capable of dissolving native gold. If bacterial activity is viewed in geological time, they may be considered one of the substantial factors in the migration of gold in the biosphere. Yet the mechanism whereby gold was dissolved by microorganisms remained unclear for a long time.

DISSOLUTION OF GOLD BY BACTERIAL METABOLITES

The bacteria we studied secrete into the nutrient medium amino acids, nucleic acids, pyruvic, lactic, formic and acetic acids and also peroxidase, catalase and proteolytic enzymes. Our studies demonstrated that gold is most intensively dissolved by amino acids in the presence of oxidizers.

Amino acids accumulated by natural strains of bacteria in the culture fluid are presented in Table 6. It is noteworthy that bacteria secrete into the medium relatively large quantities of aspartic and glutamic acids.

In the presence of sodium peroxide, placer gold is soluble by amino acids, particularly intensively in alkaline conditions. Thus, in 30 days gold dissolved in solutions of asparagine, aspartic acid and threonine in quantities of 0.03, 0.04 and 0.11 mg/l respectively. At low pH values 0.01—0.1 mg/l of gold were solubilized during the same time.

Table 6

Amino acids in the bacterial culture fluid [37]

Amino acids	Bacteria		
	B. mesentericus 120	*B. megaterium* 20	*P. liquefaciens* 9
	% of total amino acids		
Lysine	3.4	4.7	18.3
Histidine	6.2	—	8.4
Aspartic acid	8.6	15.9	12.4
Serine	9.5	3.5	4.7
Glutamic acid	21.3	17.8	56
Glycine	12.9	17.8	—
Cysteine	—	3.6	4.3
Valine	5.7	7.8	10.1
Total amino acids, g/l	2.0	2.8	1.2

Without the peroxide, placer gold in particles 0.07 mm in size is not dissolved by amino acids.

As compared to placer gold, finely-dispersed gold dissolves in amino acids 10 to 30 times faster. During the same period 0.24 to 3.2 mg/l of gold were solubilized in the absence of Na_2O_2, while in the presence of Na_2O_2 0.67 to 18.17 mg/l of gold were solubilized. Gold dissolves most intensively under the effect of histidine (16.17 mg/l), serine (12.10 mg/l), aspartic acid (18.17 mg/l), glycine (6.7 mg/l), alanine (5.9 mg/l), methionine (5.1 mg/l) and asparagine (3.86 mg/l). Gold dissolves also in the presence of nucleic acids, particularly in the presence of peroxide. The concentration of gold in the solution reached 1.6 mg/l (DNA) and 5.0 mg/l (RNA). Less effective in the dissolution of gold are salt- and alkali-soluble, and also water- and alcohol-soluble proteins. In 20 days in their presence at a pH of 9—10 solubilization was up to 2.17—3.3 mg/l and 0.15—0.57 mg/l of gold respectively.

Dissolution of gold by individual fractions of bacterial metabolites was also studied. With the aid of vertical paper electrophoresis, ionic sorption and chemical precipitation, the components of the bacterial solvent of gold were separated. The test data are given in Table 7 and one can see that the proteins and peptides isolated chemically are less effective in the dissolution of gold, its concentration in the solution not exceeding 1.3 mg/l.

Table 7

Solubility of gold by individual fractions of bacterial metabolites

Bacterial culture	Strain	Gold dissolved in protein mixtures (μg/l), separated			
		chemically		by ion exchange resins	
		protein	amino acid	protein	amino acid
B. mesentericus niger	12	0.70	4.2	7.6	10.2
—"—	121	0.52	4.7	2.7	18.0
—"—	123	0.67	4.9	9.2	20.7
—"—	129	1.30	6.5	8.5	43.0
B. megaterium	20	0.95	4.0	3.4	9.0
—"—	201	0.47	6.5	2.5	35.9

The fraction of proteins and peptides obtained by means of ion exchange resins contained up to 9 mg/l of gold. The amino acid fractions, purified by the method of chemical fractionation, dissolved

up to 6.5 mg/l of gold and the gold content of the amino acid fractions purified by means of ion exchange resins reached 43.0 mg/l.

Thus, one may draw the conclusion that gold dissolution activity depends on the chemical and physicochemical state of the protein and amino acid fractions.

CHANGES IN GOLD UNDER THE EFFECT OF BACTERIA AND THEIR METABOLITES

Qualitative changes in gold were observed after 30 days of interaction with bacterial solutions and were noticeable, first of all, by the colour of the gold granules [38]. Under the microscope (x 300) the particles of finely-dispersed gold after 150 days of interaction with the culture fluid had a rough, pitted surface and lost their strong metallic shine. Under x 650 magnification one can see well-pronounced corrosive relief. The size of 1.0—2.0 mm gold particles subjected to bacterial leaching for 3 years decreased 1.5 to 2.0 times, in the marginal portions they had a denser golden-yellow colour than in the center. Scanning in Ag-roentgen rays revealed a decreased silver content of the marginal portions of the gold particles. A quantitative assay demonstrated that the central portion of the gold particles contained 82.1 % of gold and 17.6 % of silver, while the silver content of the marginal portions fell to 1 %.

The proteins isolated from the culture fluid which had been interacting with gold particles for 360 days, had a brick-red colouring in the zone of contact. During a longer contact the solutions turned brown, and the precipitates contained colloids of gold and silver. The data of a semiquantitative spectral analysis demonstrated that the concentration of gold in the precipitate was 0.03 % and that of silver, 0.2 %. The culture fluid contained 10 mg/l of colloidal gold.

These data demonstrate that under the effect of the culture fluid gold and silver are solubilized with the formation of organic complexes, and that with time these complexes are decomposed with the reduction of gold and silver to their metallic state.

MECHANISM OF BACTERIAL DISSOLUTION OF GOLD

With the aid of electrophoresic separation of pure and gold-bearing protein mixtures, both in alkaline and in acid media, the formation of a complex of protein with gold was revealed, the complex being charged either positively or negatively. Quantitatively predominant are anionic complexes of gold. Taking part in the compounds of gold with proteins are functional groups of amino acid remnants of polypeptide groupings in which gold is bound through the atom of the amino group. An examination of the infra-red spectra of the gold-

containing amino acid fractions revealed that the complex formation of gold with amino acids also was effected through the atom of the amino group [39].

The interaction of gold with amino acids in the acid medium at pH = 2 leads to a slow reduction of gold to the metallic state. Under alkaline conditions when the gold (III) solutions contain small quantities of amino acids there takes place the formation of its sols, while at an excess of amino acids gold (III) is reduced to gold (I). No formation of colloids and isolation of metallic gold takes place in this case [40—41].

In an alkaline medium, glycine, alanine, valine and phenylalanine form complexes in which metal coordination of amino acids takes place through the amine and carboxyl groups. In histidine the gold-to-amino acid bonds are coordinated through the nitrogen atoms of the amino group and imidazole ring. In cysteine it is the sulfhydryl group that is the most reaction-prone. The presence of two amino groups in arginine and lysine increases the alkalinity of the amino acids and lowers their capacity to form complexes.

The stability of complexes of gold (I) with amino acids varies, as testified by the values of the oxidation-reduction potentials (Table 8). Such amino acids as glycine, valine, alanine have very similar pK_2 values and form complexes of approximately equal strength. Stronger still are complexes with histidine and asparagine.

Table 8

Standard oxidation-reduction potentials of gold (I) complexes with amino acids

Amino acids	Eh, V
Glycine	0.632
Alanine	0.644
Valine	0.624
Phenylalanine	0.648
Methionine	0.537
Asparagine	0.495
Histidine	0.457
Cysteine	0.144

DEPOSITION OF GOLD

In nature, along with dissolution, reduction of gold also takes place, which under appropriate conditions may be caused by the majority of metals placed higher than gold in the electrochemical series, and also by some organic compounds, including amino acids.

During the interaction of gold (III) with amino acids in an acid medium (pH = 2) gold, as a result of oxidation-reduction reactions, is reduced to its metallic state [42]. At a pH value of 2 histidine completely reduced gold in 30 hours. The reduction reaction proceeds slowly in solutions of amino acids with a high pK_2 value. An increase in amino acid concentration leads to a higher rate of the reduction reaction. In an alkaline medium the interaction of gold (III) with amino acids also leads to its reduction, and a gold film is formed in solutions of some amino acids.

Gold is reduced also by proteins and carbohydrates. Small amounts of glucose precipitate gold during a long contact (30 days). Among the proteins it is the globulins which register the highest degree of gold precipitation (Table 9).

Table 9

Precipitation of gold from hydrochloric solutions by different proteins [42]

Protein fractions	Gold content of solution, mg/l		Degree of precipitation, %
	before test	after test	
Globulins	90	20.7	77.0
Prolamines	90	46.8	46.8
Protenoids	90	41.0	54.4

It has been indicated above (Table 2), that microorganisms are capable of accumulating gold in the biomass. Thus, the activity of *Aspergillus* sp. biomass exceeds activated carbon 10 to 12 times in gold absorption from colloidal solutions, which is apparently due to the electrostatic interaction of colloidal particles with protein molecules [36, 43]. Along with colloidal gold microscopic fungi are capable of absorbing gold from true solutions with the formation of complex compounds.

Other microorganisms also have the capacity to concentrate gold. Thus, the gold content of bacterial cells is 15 to 600 times higher than that of mine waters (Table 2). The gold concentration coefficient in the algae of the Barents Sea in relation to the gold content of sea water is 2×10^4 [25].

The gold concentration in land plants in relation to its clark in the lithosphere reaches 1.2×10^5 [44]. Individual tissues of animal organisms are also capable of accumulating gold.

In the plant and animal organisms gold forms complex compounds. Taking part in the formation of complexes are free amine, carboxyl and sulfhydryl groups.

EFFECT OF GOLD ON MICROORGANISMS

It is known that gold and its compounds may be toxic to macro- and microorganisms [45]. We have studied the influence of gold ions on changes in the composition of the basic polymers of the cell of *B. mesentericus niger* 120 (Table 10). It has been established that a bacterial contact with gold ions for 24 hours results in a decrease in the quantity of protein and RNA in the cell. Changes in DNA content are insignificant. It has also been revealed that gold is absorbed by bacteria in negligible quantities during the first 24 hours. The survival of bacteria at a gold concentration of 3×10^{-5} to 1.6×10^{-4} g-at/l was about 90 %, and at a concentration of the metal of 1 to 5×10^{-3} g-at/l, the number of viable cells fell to 80—85 %. However, bacterial cells may retain their viability for a long time even in a culture fluid containing 7.5 to 28.6 mg/l of gold. The contamination of the cells with gold ions is apparently due to the precipitation of the gold-protein complexes.

Thus, microorganisms are capable of dissolving gold, reducing it to its elemental state and accumulating it in the biomass and thereby intensifying its complete cycle in the biosphere.

Table 10

Influence of gold ions on the polymer composition of cells

Gold concentration, g-at/l	Content, μg/mg		
	protein	RNA	DNA
0.00	540.5	80.6	2.10
3×10^{-5}	510.6	77.2	1.92
6×10^{-4}	482.2	72.6	1.80
1×10^{-4}	442.5	68.4	1.69
5×10^{-3}	410.8.	62.8	1.68
1×10^{-3}	392.3	60.5	1.65

THE HYPERGENE MIGRATION OF GOLD

It has been demonstrated above (Tables 1 and 2) that gold undergoes redistribution in nature. However, the conditions of this process and its scale have not yet been adequately assessed.

The hypergene migration of gold can be clearly traced under conditions of gold ore deposits. During microbiological and chemical oxidation of sulfides in an aerobic zone gold undergoes dissolution and is transferred by sulfate waters in the form of thiosulfate, haloid and gold-organic complexes to the lower horizons of the deposits.

At some deposits hydrogen sulfide waters containing sulfate-reducing bacteria occur below the zone of oxidation [46]. The sulfates coming in from the oxidation zone are reduced under the effect of sulfate-reducing bacteria with the formation of H_2S, HS^- and S^{2-}. The metals contained in the acid sulfate solutions precipitate in the form of secondary copper and iron sulfides which leads to the formation of a zone of secondary sulfide enrichment. In this zone the thiosulfate complex of gold is destroyed, the gold being reduced to its metallic state and forming a horizon of the ores' secondary enrichment with gold. Al'bov [47] believes that taking place in the zone of secondary enrichment is an enlargement of gold particles, right up to the formation of large nuggets.

Meanwhile, established in the hydrogen sulfide solutions is the presence of dissolved gold in amounts from 70 to 400 μg/l. Its presence may be explained by the action of the HS^- ion on the reduced metal in the zone of secondary enrichment, with the formation of an AuS^- ion in the following reaction: $2Au + 2HS^- = 2AuS^- + H_2$.

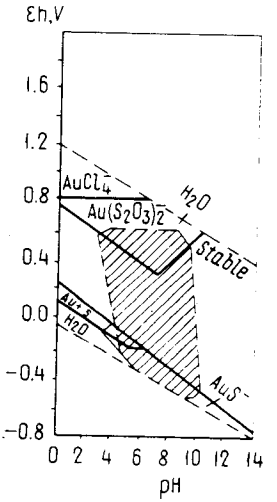

Fig. Correlations of stability between some gold compounds in water at 25 °C and 1 atm of undirect pressure. The sum total of dissolved chloride components is 10^0; the sum total of dissolved sulfur is 10^{-1}. The cross-hatching indicates the field of stable gold-organic complexes in natural waters

Some investigators point to the involvement of haloid complexes in the migration of gold [48]. Their role in the migration of gold is demonstrated in the Eh-pH diagram (Fig.). The data appearing in the diagram describe the activity of gold ions at different sulfur and chloride contents of the system, from which it follows that the $AuCl_4^-$ complex may be formed at a large quantity of chlorine in the acid solution at an oxidation-reduction potential over 0.8 V. Practically, such conditions in the zone of oxidation of ore deposits are rare. In the pH range from 5 to 10 and the Eh value from −0.470 V to −0.170 V gold exists in solution in the form of an AuS^- complex, and at an Eh value from +0.470 to +0.760 V — in the form of a thiosulfate complex.

Also depicted in the diagram is the field of stability of gold-organic complexes with which the gold is transferred in ground waters. A comparison of thermodynamic characteristics indicates that, compared to thiosulfate complexes, gold-organic ones are stable in a wider range of oxidation-reduction potentials (Fig., Table 11). In this connection there takes place an expansion of the gold dispersion halo, connected with the leaching of gold-bearing ores by waters enriched with organic compounds.

Table 11

Comparison of thermodynamic characteristics of gold complexes formed in microbiological and chemical oxidation processes

Type of oxidation	Complex	Complex stability			
		Upper limit		Lower limit	
		Eh, V	pH	Eh, V	pH
Chemical and microbiological	$[Au(S_2O_3)_2]^{3-}$	0.8—0.6	0—9	0.8—0.4	0—10
Microbiological (products of biosynthesis and oxidation of organic substances)	gold-organic	0.7	5—9	—0.2—0.5	5—10

In the weathering crust of gold sulfide deposits mobile gold in the ionic form makes up to 77 % of the gross gold content of rocks [49], while in the soils of the Kuranakh fields considerable quantities of gold enrich the ground and surface waters in the non-mineral getterion form and in the form of gold-organic complexes, and are evacuated from the places of primary concentration. Chibisov [50] calculated that from 55 to 88 % of gold is evacuated during processes of soil formation. The reduction of the ionic form of gold may take place in water flows with the formation of colloidal particles whose migration over long distances is carried out in the presence of protective colloids — silica, ferric hydroxide, organic acids and microorganisms. Correlations of the different forms of gold and of the distances over which its increased concentrations are capable of being preserved, have been studied poorly. Calculations based on the gross gold content of waters indicate that rivers transport considerable quantities of gold (Table 1). Deposition of dissolved and colloidal gold from surface water flows may take place on solid biochemical barriers whose role may be played also by organic substances. If one takes into account that the total amount of organic matter in the sedimentary rocks is

3.8×10^{15} tons, this means that it is bound up with at least 3.8×10^{6} tons of gold, which have been deposited from true solutions.

It is even more difficult to assess the hypergene migration of gold with account of the history of the Earth's development.

The calculation of gold mining from deposits of different origin and formed at different geological epochs in the development of the Earth points to the two major stages in the accumulation of gold (Table 12). The first stage goes back in time to the Middle and Lower Proterozoic when deposits of gold-bearing conglomerates took shape, and also numerous stratiform deposits for which one may assume a sedimentary origin. They coincide with marine deposits formed under littoral, delta, beach, gulf-lagoon and marine conditions. As a rule, they are enriched with coaly matter. Gold is concentrated mainly by

Table 12

Distribution of gold reserves in endogenous gold ore deposits of different origin and age [51]

Time of deposit formation	Quantity of gold reserves, % of the total
Archean	20
Lower and Middle Proterozoic	40
Upper Proterozoic-Neogaea	25
Neogaea-Quarternary period	15

chemical and biochemical means, as testified by the predominance of exceptionally fine gold associated with sulfides. The formation of sulfides points to the intensive development of sulfate reduction processes in the medium where the formation of gold deposits took place. The gold brought into a body of water in the dissolved state was precipitated by sulfides and a coaly substance.

Characteristic of the second stage is a predominance of continental placers, with pre-eminently mechanical accumulation of gold taking place in them. This can be explained by the climatic features of the period when the placer deposits were formed. All the placer deposits dating back to the Neogaea-Quarternary period are situated on the continents of the Northern hemisphere which in the Quarternary period underwent continental glaciation. The drop of temperatures and development of perennial congelation on these continents resulted in a lower biological activity and slower oxidation processes so that physical factors of gold accumulation came to be dominant in the formation of placers. The processes of redistribution of gold in the placer deposits of Siberia under the effect of biological and chemical agents were, apparently, insignificant. The processes of gold migra-

tion in these regions are weakly developed apparently also at present. Thus, the quantity of newly formed gold in old dumps, according to Kozhevnikov [52], over a period of 30 years was only 2.5 %. More significant effects of organic substances on the redistribution of gold in placers have been described by Freise in Brazil, where the climatic conditions were conducive to the active transfer of gold in the zone of hypergenesis in the form of gold-organic complexes [53].

CONCLUSION

Data available in the literature indicate that gold is unstable in nature. Bacterial activity results in its dissolution, migration, and deposition under certain conditions. Microorganisms are capable of carrying out the complete cycle of gold in nature. It is worth noting that the limits of the migration of gold in the sphere of influence of biogenic factors are considerably expanded. This is indicative not only of the important role of microorganisms in the geological history of gold deposits, their formation and weathering but also of the possibility to use them for leaching or concentrating gold.

REFERENCES

1. Vinogradov, A.P., *Geokhimiya*, No. 7, 555 (1962).
2. De Gracia, A.R., and Haskin, L., *Geochim. Cosmochim. Acta*, *28*, 4, 559 (1964).
3. Phan, K.D., *Chronique des mines et de la recherche minière*, No. 343, 175 (1965).
4. Shcherbakov, Yu.G., Distribution and Conditions of Concentration of Gold in Mining Provinces, F.N. Shakhov (Ed.), Nauka, Moscow (1967) (in Russian).
5. Voskresenskaya, N.T., and Zvereva, N.F., *Geokhimiya*, No. 4, 422 (1968).
6. Anoshin, G.N., Yemel'yanov, E.M., and Perezhogin, G.A., *Geokhimiya*, No. 9, 1120 (1969).
7. Jenney, W.P., *Min. Eng. Trans.*, *33*, 445 (1903).
8. Du Bois, G.C., *Tschermaks mineralog. u. petrog. Mitt.*, *22*, 1 (1903).
9. Stone, R.W., Coal near the Black Hills, Wyoming-South Dakota, U.S. Geol. Surv. Bull., 499 (1912).
10. Goldschmidt, V.M., and Peters, C.I., *Gesellsch. Wiss. Göttingen. Nachricht. Math.-Phys. KI.*, No. 4, 371 (1933).
11. Fuchs, W., *Indust. Eng. Chemistry*, *27*, 1099 (1935).
12. Leutwein, F., *Hallesches Jahrb. mitteldeutsche Erdgeschicht*, *1*, 83 (1951).
13. Razin, L.V., and Rozhkov, I.S., Geochemistry of Gold in the Crust of Weathering and the Biosphere of Gold Ore Deposits of the Kuranakh Type, A.V. Astrov (Ed.), Nauka, Moscow (1966) (in Russian).
14. Yurovsky, A.Z., Mineral Components of Solid Fuels, Nedra, Moscow (1968) (in Russian).

15. Volosikova, R.N., Lyakhova, I.G., and Zavorotnykh, I.R., In: Geochemical Methods of Prospecting for Gold Ore Deposits by Secondary Halos, V.V. Polikarpochkin (Ed.), Papers of the Transbaikal Branch, Geographical Society, Chita, Issue 83, 46 (1973) (in Russian).
16. Otakuziyev, E.N., and Baskakov, G.P., *Uzb. geol. zhurn.*, No. 6, 34 (1978).
17. Vinogradov, A.P., Geochemistry of Rare and Scattered Elements in Soils, USSR Academy of Sciences, Moscow (1957) (in Russian).
18. Noddack, I., and Noddack, W., *Zeitschr. Phys. Chemie, 154 A*, 207 (1931).
19. Lezhneva, N.D., *Zapiski Uzbekskogo otd. VMO AN Uzb. SSR*, No. 31, 207 (1978).
20. Kropachev, G.P., *Sovetskaya zolotopromyshlennost'*, No. 8, 46 (1935).
21. Zvyagintsev, O.E., In: Geochemistry of Gold, A.E. Fersman (Ed.), USSR Academy of Sciences, Moscow — Leningrad, 3 (1941) (in Russian).
22. Pogrebnyak, Yu.F., Pustel'nikov, O.S., and Trimonis, E.S., *Geokhimiya*, No. 1 (1980).
23. Krauskopf, K.B., *Econom. Geol., 46*, 6, 858 (1951).
24. Peshchevitsky, B.I., Anoshin, G.N., and Erenburg, A.M., *Dokl. AN SSSR, 162*, 4, 915 (1965).
25. Vaganov, P.A., and Shtangeyeva, I.V., *Uchen. zapiski*, Leningrad University, No. 392, 163 (1978).
26. Vaganov, P.A., Kulikov, R.D., and Shtangeyeva, I.V., *Geokhimiya*, No. 11, 1740 (1978).
27. Kovalevsky, L.A., In: Geochemical Methods of Prospecting for Gold Ore Deposits by Secondary Halos, V.V. Polikarpochkin (Ed.), Papers of the Transbaikal Branch of the Geographical Society, Chita, Issue 83, 61 (1973) (in Russian).
28. Kakovsky, I.A., *Izv. AN SSSR*, OTN, No. 7, 29 (1957).
29. Lyalikova, N.N., and Mokeicheva, L.Ya., *Mikrobiologiya, 38*, 5, 805 (1969).
30. Korobushkina, E.D., and Gukasyan, A.B., In: Biology of Heterotrophic Microorganisms, A.B. Gukasyan (Ed.), Krasnoyarsk, 77 (1971) (in Russian).
31. Pares, Y., *Ann. Inst. Pasteur, 107*, 7, 141 (1964).
32. Pares, Y., *Ann. Inst. Pasteur, 107*, 4, 573 (1964).
33. Pares, Y., and Cuper, J., *Ann. Inst. Pasteur, 107*, 4, 568 (1964).
34. Pares, Y., In: Proceedings of the 8th International Congress on Mineral Processing, Leningrad, 2, 53 (1969).
35. Korobushkina, E.D., Transactions of the 5th Congress of USSR Mineralogical Society, Section: Ecology of Microorganisms, Yerevan, 36 (1975) (in Russian).
36. Mineyev, G.G., *Geokhimiya*, No. 4, 577 (1976).
37. Korobushkina, E.D., Chernyak, A.S., and Mineyev, G.G., *Mikrobiologiya, 43*, 1, 49 (1974).
38. Korobushkina, E.D., Zav'yalova, L.L., and Korobushkin, I.M., *Izv. AN SSSR, Ser. Biol.*, No. 6, 936 (1977).
39. Korobushkina, E.D., Mineyev, G.G., and Praded, G.P., *Mikrobiologiya, 45*, 3, 535 (1976).
40. Mineyev, G.G., Korobushkina, E.D., Chernyak, A.S., Semenova, L.P., and Syrtlanova, T.S., In: All-Union Conference on the Ecology and Geochemistry of Microorganisms, Microbiological Oxidation of Sulfides and the Leaching of Various Metals, Abstracts of papers, M.V. Ivanov (Ed.), Pushchino (1974) (in Russian).
41. Chernyak, A.S., and Shestopalova, L.F., *Kolloidny zhurnal, 37*, 1, 202 (1975).

42. Mineyev, G.G., and Semenova, L.P., In: Proceedings of the 1st Regional Conference "Young Researchers and Engineers for Eastern Siberia", Section of Metallurgy and Engineering, N.F. Losev (Ed.), Irkutsk, 7 (1969) (in Russian).
43. Mineyev, G.G., Chernyak, A.S., and Semenova, L.P., *Prikladnaya biokhimiya i mikrobiologiya, 8*, 3, 358 (1972).
44. Tissen, S., In: Geochemical Methods of Prospecting for Ore Deposits, V.I. Smirnov (Ed.), Inostrannaya literatura, Moscow, 325 (1954) (in Russian).
45. Williams, D., Vital Metals, M.E. Vol'pin (Ed.), Mir, Moscow (1975) (in Russian).
46. Shamolina, E.P., Udodov, P.A., and Bernatonis, V.K., In: Geochemical Methods of Prospecting for Gold Ore Deposits by Secondary Halos, V.V. Polikarpochkin (Ed.), Transactions of the Transbaikal Branch of Geographical Society, Chita, Issue 83, 77 (1973) (in Russian).
47. Al'bov, M.N., In: Secondary Zonality of Gold Ore Deposits of the Urals, V.I. Smirnov (Ed.), Gosgeoltekhizdat, Moscow (1960) (in Russian).
48. Chukhrov, F.V., In: Oxidation Zone of Sulfide Deposits in the Steppes of Kazakhstan, M.I. Varentsov (Ed.), USSR Academy of Sciences, Moscow (1950) (in Russian).
49. Roslyakov, N.A., In: Geochemistry and Conditions of Gold and Rare Metals Ore Formation, A.S. Mitropol'sky (Ed.), Transactions of the Institute of Geology and Geochemistry, Siberian Branch of the USSR Academy of Sciences, Novosibirsk, *149*, 125 (1972).
50. Chibisov, N.P., M.Sc. Thesis, Geology and Mineralogy, Yakutsk (1969) (in Russian).
51. Sher, S.D., Metallogeny of Gold, *2*, Nedra, Moscow (1972) (in Russian).
52. Kozhevnikov, M.G., Transactions of "Zolotorazvedka" Trust, M.S. Bazzhina (Ed.), *1*, Department of Scientific and Technical Information, People's Commissariat of Heavy Industry, Moscow (1935) (in Russian).
53. Freise, F.W., *Econom. Geol., 26*, 4, 421 (1931).

MICROORGANISMS INVOLVED IN TURNOVER OF IRON AND MANGANESE: THEIR APPLICATION IN HYDROMETALLURGY

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INTRODUCTION

Iron and manganese are essential elements in the Earth's crust. By their contents in the Earth's crust, iron and manganese rank fourth and seventh, respectively. Both are metals of variable valence, which makes them extremely important for geochemical transformations in the biosphere.

In magmatic rocks iron and manganese occur in the bivalent form. Their penetration to the daily surface in the zone of hypergenesis is followed by their oxidation and concentration as sedimentary deposits.

Sedimentary deposits of iron in the crust of weathering largely consist of oxides in the form of minerals: goethite ($Fe(OH)O$), ferrihydrite ($2.5Fe_2O_3 \cdot 4.5H_2O$), hydrogoethite, hematite (Fe_2O_3), magnetite (Fe_3O_4) and limonite ($2Fe_2O_3 \cdot H_2O$). Reduced iron occurs in deposits in the form of siderite ($FeCO_3$) and silicates, chamosite ($4FeO \cdot Al_2O_3 \cdot 3SiO_2 \cdot 3H_2O$) as well as in the form of sulfides (FeS_2, FeS). Similar to iron, manganese forms ore deposits in the crust of weathering either as oxide ores, mainly as minerals, pyrolusite (MnO_2), manganite ($MnO_2 \cdot Mn(OH)_2$), or as reduced Mn ores, like rhodochrosite ($MnCO_3$), rhodonite ($MnSiO_3$) and some others.

Both elements are geochemically mobile. Reduced Fe and Mn in the form of hydrocarbonates and sulphates are readily soluble and, with low O_2 content, they show considerable concentrations reaching up to hundreds of mg/l. Mn is considerably more resistant to chemical oxidation by oxygen, hence its higher geochemical mobility. It belongs, together with K^+, Na^+, Ca^{2+} and Mg^{2+}, to group I of mobile elements, whereas Fe, together with Al and Ti, belongs to group IV.

According to thermodynamical calculations and experimental data, Mn can be oxidized by oxygen at a marked rate only at $pH > 8.5$ [1, 2, 3]. In fact, chemical oxidation of Mn in natural waters is extremely low [4].

In the biosphere, there is a constant circulation of iron and manganese in which 'live' organic matter is active. Getting into an oxidizing environment, their reduced forms undergo chemical and biological oxidation yielding insoluble oxide compounds. Under the action of certain factors, primarily of microorganisms, oxides are converted back to a reduced form, thereby their mobility and migration in the Earth's crust with subsequent redeposition under oxidizing conditions, are provided for. Therefore, being active in the oxidation and reduction processes microorganisms can influence geochemistry of iron and manganese.

There are many microorganisms which are able to deposit Fe and Mn oxides at the surface of cells or into the medium. They are known as iron bacteria. There is great disagreement on the question which microorganisms should be grouped with iron bacteria. And the term itself needs refinement, which will be discussed below.

Not infrequently, when microbiological oxidation of Mn is dealt with, another term, Mn-oxidizing bacteria, is used. The latter are in all cases capable of oxidizing, besides Mn, reduced Fe compounds. However not all the iron bacteria are able to oxidize Mn^{2+}, among them are, for example, *Gallionella* or *Sphaerotilus*. The reasons for this can be understood further in the narration, as mechanisms of oxidizing reactions are considered.

Due to the basic differences in the functional significance of Fe oxidation in metabolism, the so-called iron bacteria distinctly fall into two groups. The first one, including a rather limited number of species, consists of obligately acidophilic iron bacteria utilizing bivalent Fe as energy substrate. The other group comprises microorganisms developing in neutral, weakly acidic or alkaline media. All recent studies of well-known species of this group in pure cultures show that they are heterotrophic in their metabolism and that oxidation of reduced forms of Fe and Mn is not their energy source for chemolithoautotrophic or lithoheterotrophic growth. Moreover, according to the thermodynamical calculations of Lees et al. [36], free energy yield from a Fe^{2+} oxidation reaction per mole decreases rapidly with pH increasing to over 3.5, reaching the value lower than necessary for ATP formation. Consequently, biological use of the energy of Fe^{2+} oxidation by microorganisms in neutral medium is ruled out.

Obligately acidophilic iron bacteria. Winogradsky's idea (1888) of oxidation of Fe by microorganisms being a source of energy was confirmed by studies of obligately acidophilic iron bacteria. For them, it was significantly shown that the energy of reduced Fe oxidation was used in constructive metabolism for CO_2 assimilation in chemolithotrophs or assimilation of organic substances in lithoheterotrophic microorganisms. They form the first group of "true" iron bacteria. The group comprises a limited number of species, namely *Thiobacillus ferrooxidans, Leptospirillum ferrooxidans* [5, 6], *Sulfolobus acidocaldarius* [7] and an organism related to it and described by C. Brierley and J. Brierley [8]. Seemingly, acid-resistant '*Metallogenium*' can be ascribed to this group [9, 10]. It is noteworthy that *S. acidocaldarius* developing at a wide range of pH (from 2 to 7.0), is able to use Fe^{2+} oxidation as a source of energy for its growth only at pH < 3.0 [8, 11].

The enzymatic mechanism of reactions of Fe oxidation in acidic medium is sufficiently well-known, the evidence available being reviewed by many authors [11—13].

They occur most commonly in underground waters of sulphide deposits and occasionally in acidic waters of ferric springs in pyritized

peat bogs. Fe oxidation in acidic waters with the participation of microorganisms should be regarded as a special case of total turnover of iron in the zone of hypergenesis.

Thus, obligate acidophilic iron bacteria can develop under extreme conditions of acidic medium, where Fe resistable to chemical oxidation at pH < 4.5, can serve as an available source of energy.

The practical importance of bacteria in question in leaching metals from ores cannot be overestimated. It has been discussed extensively in literature [14].

Microorganisms oxidizing Fe and Mn in neutral and alkaline media. This group is heterogenous in physiological and taxonomic terms. It includes 'filamentous' bacteria having a sheath, flexibacteria, unicellular bacteria of taxonomically different groups (budding, corynebacteria, pseudomonadas and others), mycoplasmas, phototrophic green, and also cyanobacteria, etc.

In the past, several attempts were made to classify iron bacteria as a physiologically single group [15—18]. With a lack of physiological evidence and studies of pure cultures, the classification was based on an ecologico-morphological approach. The outward morphological features, such as specific deposition of metallic oxides, size and form of cells, structure of cellular aggregates microcolonies, were predominantly taken into consideration as their diagnostic features.

Somewhat later it was shown that the morphology depended, to some extent, on environmental conditions, and consequently one can more reasonably distinguish between morphologically different types of iron bacteria, than treat them as different species [19—21].

The taxonomy had to be revised in view of the results of studies of some of the best known iron bacteria in pure cultures. It was concluded that iron bacteria could not be regarded as a physiologically homogeneous group, as its members possessed different types of metabolism. The ability to oxidize Fe and Mn was only manifested under definite conditions. Recently it was shown that bacteria of *Metallogenium*, *Gallionella* and *Siderococcus* gen. *Metallogeniaceae* family, had no cell wall and were saprophytic mycoplasmas [22—24]. Placed into this group are *Siderococcus* gen. earlier classed with autotrophs, *Siderocapsaceae* family, [23, 25] and acid-resistant *Metallogenium* sp. [10] that is much closer to *Gallionella* gen. by its morphology and ability to oxidize Fe but not Mn.

All these microorganisms make up a new group of saprophytic mycoplasmas commonly occurring in nature.

It was established that different species of *Siderocapsa* gen. taxonomically belonged to corynebacteria of *Arthrobacter* genus [21]. Morphological forms of growth characteristic of other members of *Siderocapsaceae* family, such as *Siderobacter*, *Sideronema*, *Naumaniella*, *Ferribacterium*, developed in pure cultures of *Arthrobacter* spp. under varied cultural conditions. These genera, like the corresponding family on the whole, have no taxonomic value of their own.

Then, *Sphaerotilus natans*, when developing in ferric oligotrophic waters, is indistinguishable from species of *Leptothrix* genus [19]. The question of the taxonomical value of the latter two genera is still open to debate [26, 27].

In some 'filamentous' bacteria, including certain phototrophs, such as green and cyanobacteria, flexibacteria, actinomycetes and others, ferric oxides precipitate on their sheaths, and morphological structures characteristic of *Leptothrix* are formed as a result.

A wide range of procaryotic and also eucaryotic microorganisms are able to oxidize Fe and Mn, to a greater or lesser extent. Their enumeration would give a long list of members of almost all taxonomic groups of aerobic and facultatively anaerobic microorganisms.

In view of the tremendous role of these microorganisms in geochemical processes of Fe and Mn oxidation, in ore formation as well as in man's economic activities, they should be looked upon as an ecologically important group.

In addition to commonly known microorganisms, there is a considerable number of algae species able to precipitate Fe and Mn oxides on their cell surface. These 'iron organisms', as called by Pringsheim, belong to different taxonomic groups: *Cyanophyceae*, *Chrysophyceae*, *Volvocales*, and others. Their comprehensive list was made by Pringsheim [28, 29].

None of these species occurs in nature in large amounts, and their geological activity is negligible compared to that of iron bacteria. Whether Fe oxidation is significant for them remains unclear. It cannot be excluded that it has a peroxide mechanism identical with that of heterotrophic 'iron bacteria'. It looks still more probable if one considers the significant amounts of hydrogen peroxide released into the medium by a number of cyanobacteria [30—33].

The ability to oxidize Fe and Mn extensively manifested by microorganisms of different types of metabolism suggests that they must have a common non-specific mechanism of the oxidative reactions in question. Indeed, it was shown by recent studies that it was based on the effect of metabolically liberated hydrogen peroxide [34, 35]. The mechanism is described at length further in the narration.

Pathways of microbiological oxidation of Mn and Fe. Accumulation of oxides by iron bacteria in the presence of dissolved forms of Fe or Mn in the medium may be due to many biological or purely chemical reasons which are sometimes difficult to distinguish between.

The main pathways of microbial oxidation or accumulation of Fe oxides are as follows:

(1) oxidation of ferrous and manganous oxides by metabolic products;

(2) deposition of Fe as a result of utilization of the organic portion of its complex or chelate compounds;

(3) chemisorptional phenomena on the cell surface.

1. Oxidation of Fe^{2+} and Mn^{2+} compounds by metabolic products. As it has lately been established, oxidation of Mn and Fe by iron

bacteria typical and abundant in nature is due to the effect of metabolically released hydrogen peroxide from oxidation of organic compounds [34]. Oxidation of Mn by this mechanism seems to be the principal, if not the only, pathway of its oxidation by microorganisms.

Possessing both oxidative and reductive properties, hydrogen peroxide may give a number of chemical or biological reactions with Fe and Mn compounds.

1) At pH > 8.5 $Mn^{2+} \xrightarrow{H_2O_2} Mn^{4+}$ chemical

2) At pH ⩽ 7 $Mn^{4+} \xrightarrow{H_2O_2} Mn^{2+}$ —"—

$Fe^{2+} \xrightarrow{H_2O_2} Fe^{3+}$ —"—

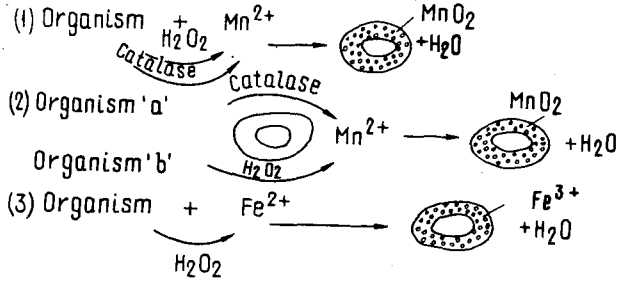
3) At pH ⩽ 7 $Mn^{2+} \xrightarrow[H_2O_2]{H_2O_2} Mn^{4+}$ + catalase biological

$Fe^{2+} \xrightarrow{H_2O_2} Fe^{3+}$ —"—

As seen from the equation, at neutral pH microorganisms metabolically releasing considerable amounts of H_2O_2 may participate in oxidation of Fe.

As follows from the first and third equations, biological oxidation of Mn is an enzymatic process requiring the presence of both H_2O_2 and catalase. As seen from (2) above, excess of H_2O_2 in the medium may reduce Mn^{4+}. Consequently, the direction and the product of reaction depend upon the rate of H_2O_2 production controlled, in its turn, by the concentration of organic substrate in the medium. Enzymatic oxidation of Mn^{2+} with H_2O_2 together with catalase is possible when cells continuously release H_2O_2, catalase performing the function of peroxidase. Cell-free culture medium would not oxidize Mn. A significant role in oxidation of Mn and Fe and accumulation of oxides seems to be played by the surface cell structures (glycocalix), as reacting substances: catalase, H_2O_2, reduced forms of metals, are accumulated in them. Unlike Mn, oxidation of Fe and accumulation of oxides occurs not only at the cell surface in capsules, but also in the environment, hence glycocalix is not obligatory for the reactions.

Possible pathways of microbiological oxidation of Fe and Mn with the participation of hydrogen peroxide can be schematically presented as follows:



1) The first pathway is typical of the majority of Mn-oxidizing microorganisms and has been proven for typical members of the main groups of iron bacteria releasing H_2O_2 and producing catalase, i.e., *Leptothrix, Arthrobacter (Siderocapsa), Metallogenium.*

2) The development connected with Mn oxidation, of an organism releasing H_2O_2 but possessing low, if any, catalase activity, is possible in an association with another or several other organisms extracellularly accumulating catalase. This case illustrates the so-called 'symbiotic' oxidation of Mn widely occurring in microorganisms, although virtually this is more likely to be the case of syntrophic relations in a microbial association. It was investigated by us in *Metallogenium.*

It should be noted here that in symbiotic cultures oxidation of Mn was subject to joint culturing of symbionts, neither could be replaced by cultural medium or suspension of cells grown independently.

3) H_2O_2 released by bacteria in the absence of catalase can be easily disrupted by ferrous ions, which is followed by deposition of insoluble oxides near the cells or in the medium. In fact, any microorganisms accumulating H_2O_2 extracellularly, can mediate this reaction.

The above three reactions are characteristic of iron bacteria and are physiologically significant.

There are some other pathways of microbiological oxidation of Fe and Mn, but they are of minor interest in terms of ecology and geochemistry.

2. **Precipitation of Fe due to utilization of organic matter of complex organic compounds of iron.** Under natural conditions bi- and trivalent iron may occur in a solution as complex organic or chelate compounds with a number of other substances: humic acids, polyphenols, organic acids (sideramins) and some others, and also as positively charged colloid solutions. Early ecological studies revealed correlations between the occurrence of individual species of iron bacteria and the presence in natural waters of humates of Fe, so it was concluded that accumulation of Fe oxides in capsules of unicellular iron bacteria was due to the utilization by them of the organic portion of the complex as a source of organic matter [37]. Later on, the ability of many water and soil heterotrophic bacteria to deposit Fe from a number of such compounds, including humic complexes, was proved experimentally [38, 39].

Occurrence of complex organic compounds of Mn under natural conditions is rather limited [40] and can hardly be of any importance for geochemistry of Mn.

When bacteria utilize complex organic compounds of Fe^{2+} or Fe^{3+}, the latter is accumulated in the capsules of the organism in the form of oxides irrespective of H_2O_2 formation, though the latter would promote oxidation of Fe^{2+}.

3. **Adsorption of Fe and Mn on the surface of cells and cellular structures.** The accumulation of Fe oxides in the capsules (glycocalix)

of a wide range of bacteria may be due to adsorption processes. The presence on the surface of cells of mucopolysaccharides, polysaccharides, phospholipids occasionally of a high negative charge, promotes the adsorption of positively charged colloid forms of Fe^{3+}. The rate of these processes depends greatly upon the concentration and pH of solutions, sharply increasing in highly alkaline or highly acidic medium [40, 41]. Thus, for example, binding of iron in glycocalix of a mucoid composition by the Heil reaction can only occur at pH 0.2—5.0 and consequently cannot take place in microorganisms developing in a neutral medium. In many bacterial species, adsorption of iron occurs at a low rate at the cell wall surface or in glycocalix [39]. Neither of these cases results in more or less distinctly expressed structures characteristic of known forms of iron bacteria, the rate of Fe adsorption being insignificant.

The adsorption phenomena and autocatalytic oxidation of Mn with the participation of MnO_2 accumulated at the cell surface of iron bacteria may be of some significance for the formation of biogenic structures encrusted with manganese dioxide, but these processes develop a sufficiently high rate at pH > 8.5 [3].

It is important that chemical interaction of MnO_2 and Fe^{2+} results in oxidation of the latter in anaerobic medium and concentration of its oxides at the cells of microorganisms:

$$MnO_2 + Fe^{2+} \rightarrow Fe^{3+} + Mn^{2+}$$

Physiological significance of oxidation of Fe and Mn by microorganisms. It is a long-established fact that growth of filamentous iron bacteria in the presence of Fe^{2+} and Mn^{2+} is markedly stimulated on media with negligible organic compounds content. As mentioned above, known filamentous and unicellular iron bacteria (*Leptothrix*, *Metallogenium*, *Siderocapsa-Arthrobacter)* utilize organic carbon yielding hydrogen peroxide. The latter is accumulated intracellularly affecting a number of enzymatic systems and other cellular components. The high toxicity of hydrogen peroxide and of its decomposition products is due to an extremely high reactivity. It is known to possess well-pronounced oxidative properties and to be a much stronger oxidant than O_2, I_2, Cl_2. This owes to the fact that, whereas activation energy required for ionization of a mole of O_2 with breaking of the oxygen bond in the oxygen molecule (O = O) is 117 kcal, that in the H_2O_2 (-O-O-) molecule is only 30—40 kcal [42].

Generation of H_2O_2 in *Leptothrix* results in the inhibited cell growth on media with organic compounds, and the latters' concentrations exceeding 2—5 g/l, and consequently, increased H_2O_2 synthesis, lead to lysis of cells. The addition of ferrous or manganous compounds to the medium improves markedly bacterial growth, and it was shown that no accumulation of H_2O_2 in the medium took place. MnO_2 (Fig. 1) as well as catalase degrading H_2O_2 added to the nutritional medium has a similar effect [34, 35].

Thus, the presence in the medium of Fe^{2+}, Mn^{2+} and Mn^{4+} compounds markedly improves bacterial growth by degrading the metabolically released H_2O_2.

To recapitulate, biological processes of oxidation of Fe and Mn by a wide range of microorganisms are based on a common peroxide mechanism. It consists of a set of reactions between hydrogen peroxide and reduced compounds of metals of variable valence. The reversibility of reaction between H_2O_2 and Mn^{2+} and Mn^{4+} stipulates the conditions of accumulation of Mn oxides, that is oligotrophic conditions, which is in a fair agreement with ecological observations.

The physiological significance of these reactions is in the defence against a harmful metabolic product, H_2O_2. It is a determining factor of development of 'iron bacteria' in specific ecological niches, where iron and manganese are present.

It must be emphasized that the mechanism of interaction of exogenous reduced compounds of iron and manganese with hydrogen peroxide discussed above underlies a new type of defence reactions of microorganisms against a toxic effect of partially reduced species of oxygen.

It follows from the foregoing that the traditional term, iron bacteria, cannot be applied indiscriminately to a wide range of microorganisms, whose metabolism is differently dependent upon the functional significance of iron. It seems reasonable to use the term 'iron bacteria' in reference to a group of obligately acidophilic chemolithotrophic microorganisms able to utilize Fe^{2+} as energy substrate.

For an abundant group of heterotrophic microorganisms displaying a non-specific function of peroxide oxidation of Fe and Mn at neutral and weakly alkaline pH, the term 'iron precipitating' and 'manganese precipitating' microorganisms, originally proposed by Naumann, should be used, since it fully reflects the essence of the processes. To iron-precipitating microorganisms belong some phototrophic microorganisms (green and purple sulphur bacteria, cyanobacteria) that accumulate metallic oxides at the cell surface. In this case, too, the most plausible mechanism is the peroxide pathway of oxide formation described above.

Quite obviously, the participation in oxidative reactions of a metabolic product, H_2O_2, released in a number of metabolic reactions, must account for a wide occurrence in different biotopes of microorganisms able to oxidize manganese and iron. According to numerous reports, in fresh waters, under favourable conditions iron bacteria can amount to 70—90 % of total bacterioplankton, while on the average they make 7—12 %; in soils, they amount to 5—15 % of microflora [43]; in iron sediments of seas and oceans, they make an average of 13 % [44, 45].

It suggests enormous geochemical activities of microorganisms in turnover of iron and manganese. The role of microorganisms in

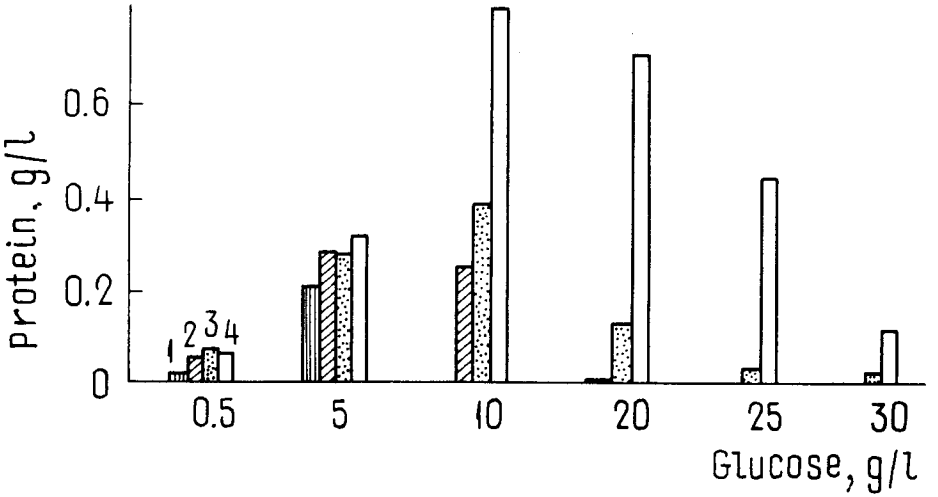


Fig. 1. Dependence of the stimulating effect of Mn^{2+} and Mn^{4+} on the growth of *Leptothrix pseudoochraceae* upon glucose content of the medium:

1 — control; 2 — +$MnSO_4$; 3 — +MnO_2; 4 — +catalase (DCP, 2,4-dichlorphenol, catalase inhibitor)

circulation of Fe and Mn in water basins and in ore formation was dealt with in a number of reviews and in pertinent sections of books [23, 44, 46—52].

REDUCTION OF IRON AND MANGANESE BY MICROORGANISMS

In the geochemical cycle of Fe and Mn microorganisms reducing their oxidized compounds, are involved.

Reduction of Fe. As discussed above, Fe II ion is unstable in the zone of hypergenesis. In acidic medium (occurring rather rarely under terrestrial conditions) ferrous iron is stable even when the medium is saturated with oxygen. At pH values close to neutral, in the presence of oxygen reduced Fe is stable in complex compounds. Fe II is also stable at negative Eh values, hence reduction of Fe may predominantly proceed in anaerobic conditions. It was shown that diverse microflora, e.g. anaerobic, facultatively anaerobic and aerobic microorganisms, participated in reduction of Fe.

Non-specific reduction of iron by microorganisms. For a number of microorganisms metabolically releasing into the medium strong reductants, such as H_2S, some organic acids, muca, complexing agents, reduction of iron is a minor process. A non-specific bacterial reduction of Fe has been under investigation since long ago [55—62] and it has been shown that it may be caused by anaerobic members of *Bacillus*, *Clostridium*, *Desulfovibrio*, *Desulfotomaculum* genera and others. It has also been shown that oxidized iron can be reduced by aerobic

heterotrophic microorganisms, such as bacteria *(Arthrobacter aurescens)*, actinomycetes (*Actinomucor repens*), fungi *(Fusarium* sp., *Aspergillus niger)* [65, 66], seemingly, with the aid of complexing agents.

Specific, iron-reducing microflora. It was found that in specific microflora reduction of Fe was an enzymatic process. According to Ottow et al. [66—68], some heterotrophic facultatively anaerobic microorganisms (e.g., representatives of *Enterobacteriaceae* and anaerobic *Pseudomonas*) having nitrate-reductase, are capable not only of anaerobic respiration with nitrate, but also of reduction of Fe oxides. When both Fe III and nitrate are present in the medium, microorganisms reduce nitrate as energetically more beneficial acceptor of electron [69] and reduction of Fe ceases [70, 62, 89]. After Ottow obtained 'nit-' mutants from a number of organisms having nitrate-reductase, namely, *Aerobacter aerogenes, B. cereus, B. circulans, B. polymyxa, Serratia marcescens* and *P. aeruginosa*, he discovered that some nitrate-reductase deficient organisms continued to reduce oxidized Fe. So it was concluded [67, 68] that besides nitrate-reductase, there were some other reductases in the electron transfer chain, participating in the reduction of Fe, they were tentatively called ferri-reductases [71]. Galstyan [45] observed that the amount of reduced iron bound to reducing bacterial systems increased with $NAD \cdot H_2$ and $NADP \cdot H_2$ added to the bacterial suspension. Galstyan et al. also postulated the existence of Fe_2O_3-reductase, but they failed to isolate it. It was also demonstrated that $NAD \cdot H_2$ and $NADP \cdot H_2$ influenced reduction of Fe_2O_3 by organisms of *Bacillus, Pseudomonas, Clostridium* genera, by some representatives of actinomycetes and fungi, under anaerobic and aerobic conditions. That bacterial reductases were involved in reduction of Fe was also reported by Japanese workers [72—74] who succeeded in blocking the process by inhibitors of the electron transfer chain. In the presence of inhibitors of dehydrogenase no bacterial reduction of Fe occurred [61, 75]. The role of dehydrogenase in the reduction of oxidized iron and other inorganic compounds in *Micrococcus lactolyticus* was studied by Woolfolk [76]. Reduction of Fe III under hydrogen was observed in a microorganism strain isolated on Schlegel's medium [77] with 0.02 % yeast extract and Fe III in atmosphere of H_2 and CO_2 [78]. This facultative anaerobe capable of hydrogen nitrate reduction and possessing potent nitrate-reductase develops small biomass due to the growth inhibition by the final product (reduced iron or nitrite), therefore it is impossible to follow further the fate of the lithotrophic organism. The ability to reduce Fe was also demonstrated in aerobic lithoautotrophic organisms: *T. thiooxidans, T. ferrooxidans, S. acidocaldarius* [79] able to utilize Fe III as an acceptor of electron and reduce it in oxygen deficiency.

Microorganisms can reduce not only dissolved oxidized Fe (e.g., $FeCl_3$), but also its insoluble compounds, such as $Fe(OH)_3$, minerals.

Bacillus 29 and *Bacillus* 29A strains reduced limonite, goethite and hematite on a glucose-peptone medium [80]. *Pseudomonas* sp. reducing Fe III, was able to reduce ferrihydrite [78, 81]. Reduction of Fe III was not always preceded by acid formation, i.e. dissolution of the mineral, and reduction may occur at weakly alkaline pH [65, 67, 68]. Thus, irrespective of the site of reduction of Fe (inside or outside the cell), the major role in these processes may seemingly be played by complexing agents-transmitters [82]. Fe II has a low migration ability, at low concentrations it can migrate in the form of humates and fulvates [57, 83, 84].

Reduction of Fe permanently proceeding under natural conditions is vitally important for all organisms, Fe II being part of cytochromes, catalase and other metallic enzymes involved in essential biochemical functions of organisms. For certain autotrophic microorganisms Fe II is energy substrate.

Reduction of manganese. Mn II is stable in almost all natural waters up to pH 8 [1, 2]. Oxidized Mn is reduced at lower Eh and it is readily involved in exchange redox reactions with polyphenols, H_2O_2, Fe II.

Reduction of Mn IV as a minor process in microorganisms. Microorganisms generating large amounts of H_2O_2, while growing on organic substrate, can reduce Mn IV to Mn II [34, 85].

When microorganisms with deposition of MnO_2 in the capsule are submerged into an anaerobic zone containing Fe II, they participate indirectly in reduction of Mn, as the following reaction goes at their surface: Mn IV + Fe II → Mn II + Fe III [86, 87]. An important part in the reduction and migration of Mn II is played by organic acids, chelating agents, humus and fulvoacids [84].

The ability of microorganisms to reduce Mn IV was investigated by many workers. A number of heterotrophic microorganisms involved were described by Grodzinskaya [88] and Troshanov [89]. On the basis of the accumulated evidence about manganese-reducing microorganisms, they can be classified into three groups.

The first group comprises facultative anaerobes able of nitrate reduction. A representative of the group, *Bacillus polymyxa*, can reduce NO_3, Mn IV and Fe III. Nitrate blocks the reduction of both Mn IV and Fe III. It is supposed that reduction occurs by a nitrate-reductase pathway [90—92].

The second group consists of microorganisms reducing Mn IV and Fe III, but not NO_3^-. Nitrate does not block reduction of Mn IV or Fe III. For example, *Clostridium butyricum* mutant deficient in nitrate-reductase, reduces Mn IV and Fe III. MnO_2 blocked reduction of Fe, the presence of NO_3 in the medium had no effect on the reduction [90]. Castro and Ehrlich discovered on *Bacillus* sp. 29A reducing only Fe III and Mn IV, that a stable product of non-enzymic origin served as a reductant [80].

The third group includes microorganisms reducing only Mn IV [93, 94]. A possible pathway of reduction of Mn IV is the peroxide one.

The participation of microorganisms and the mechanism of reduction of Mn remain largely unexplored. Presumably, an important role is played by microorganisms that are non-specific reductants of Mn IV.

Manganese is an essential element of biogenic structures, an activator of enzymes, an element involved in photosynthesis. Deficiency of reduced manganese in substrates results in heavy metabolic abnormalities. Therefore, reduction of Mn is a link in its cycle which is important for life on earth.

Leaching of Fe and Mn from ores by microorganisms. Fe and Mn of ore components can be washed out with organic and mineral acids produced by microorganisms (e.g., decomposition of ore components with sulphuric acid produced by *T. thiooxidans)*, and also during bacterial reduction of Fe and Mn. It should be noted that research work on the problem of leaching of Fe and Mn from ores is yet at its initial stage, both as regards isolation of microorganisms involved and the knowledge of its mechanism.

The ability of microorganisms to reduce Fe III and Mn IV can be employed by industrial microbiology for extraction of Fe and Mn from poor ores, since reduced Fe and Mn are relatively mobile, as opposed to their oxides that are insoluble at neutral pH. Fe and Mn can be extracted from ores by known laboratory microorganisms, as well as by newly isolated ones. Isolation of microorganisms reducing Fe and Mn is usually carried out on liquid and agarized medium of Bromfield [61, 75] containing KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $(NH_4)_2SO_4$; 1.0 g; sucrose, 10.0 g; $CaCO_3$, 5.0 g; yeast extract, 0.3 g; agar, 20.0 g; distilled water, 1 liter; pH 7.0. Similar media were used by Ottow [62] and Troshanov [93]. Ore was crushed, sterilized and added to the medium at 50—100 g/l. It was shown by several investigators that the reduction processes depend upon ore particle size, the ratio of a solid and liquid phases of the medium, time of culturing, removal and addition of sterile medium, continuous-flow conditions (percolators). Microflora reducing Fe and Mn and resistant to their ions can be isolated from ore deposits. By this approach Gvilava and Sakhvadze [95] succeeded in isolating active manganese-reducing microorganisms *Pseudomonas herbicola* and *Aeromonas* from the Chiatura Mn deposit. It was observed that isolated microorganisms produced organic acids that were complexing agents. Mn-reducing ability of microorganisms was proportional to malate dehydrogenase activity. For leaching of Mn ores, Babenko and Grigoryev [96] isolated *Achromobacter delicatulus* reducing 50—60 % of Mn IV in the medium. American workers [97] isolated microflora from dumps of mines on a medium with addition of leaves, humus, stagnant water, that leached 98 % of Mn in 60 days. Agate et al. [98] isolated Mn-reducing *Pseudomonas*, *Bacillus* and *Arthrobacter* from mineral sediments of water-supply pipes of different areas in India. The isolated

microorganisms extracted 90 % Mn from ores (*Pseudomonas* and *Bacillus* during 90 day, and *Arthrobacter* during 40-day culturing).

As it was mentioned in the foregoing, laboratory cultures of microorganisms can be used for leaching Fe III and Mn IV. A method of leaching Mn by means of *T. thiooxidans* was proposed by Japanese authors [99]. After culturing *T. thiooxidans* on a medium with sulphur, Mn ore was added to the medium, H_2S or SO_2 were blown through the cultivator; by the end of experiment accumulation of $MnSO_4$ occurred.

Apparently, for the purpose of leaching *B. polymyxa* and *B. circulans* can be used, as they can reduce as much as 600 mg/l Mn II and 240 mg/l Fe II in 12 days [93] and, being sporous organisms, are more resistant to heavy metal concentrations. For reduction of Mn IV in ores, museum cultures of bacteria producing large amounts of H_2O_2, can also be used. Evidently, for this reason in the work of Agate [98] *Arthrobacter* displayed a higher speed of leaching Mn from ores than *Pseudomonas* and *Bacillus* (growing on organic media, *Arthrobacter* releases considerable amounts of H_2O_2 (35).

CONCLUSION

As seen from the above review of literature, the problem of leaching of Mn and Fe by microorganisms from ores, even under laboratory conditions, remains largely unexplored, the underlying mechanisms

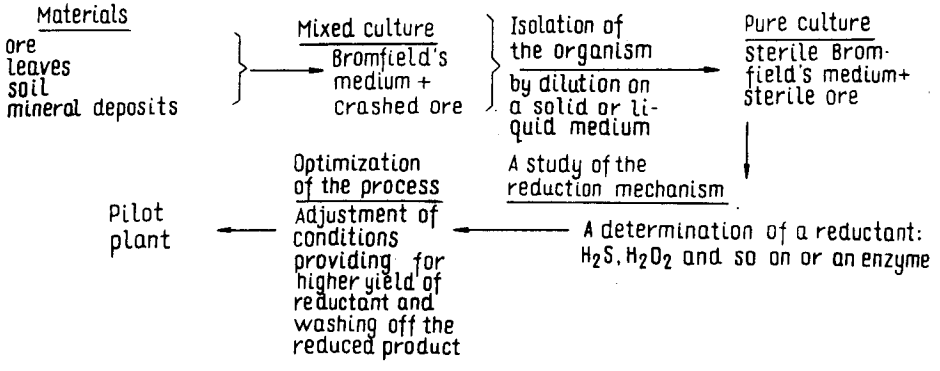


Fig. 2. A scheme of isolation and application of organisms for the purpose of leaching manganese from ores

being unclear. And then there is a long way to go from laboratory to pilot plant studies. The researcher needs stable bacterial cultures with known mechanisms of reduction of Fe and Mn, to be able to control the process and adjust technological regimes for a pilot plant (Fig. 2).

REFERENCES

1. Listova, L.P., Physico-chemical Studies of the Conditions of Oxide and Carbonate Manganese Ore Formation, USSR Academy of Sciences, Moscow (1961) (in Russian).
2. Krauskopf, K.B., *Geochim. Cosmochim. Acta, 12*, 61 (1957).
3. Hem, J.D., Survey Water Supply paper, 1667-A, Washington, 1 (1963).
4. Morgan, J.J., Principles and Applications of Water Chemistry, S.D. Faust and I.V. Hunter (Eds.), New York (1967).
5. Markosyan, G.E., *Biol. J. Armenii, 25*, 26 (1972).
6. Balashova, V.V., Vedenina, I.Ya., Markosyan, G.E., and Zavarzin, G.A., *Mikrobiologiya, 43*, 581 (1974).
7. Brock, T.D., Brock, K.M., Belly, R.T., and Weiss, R.L., *Arch. Mikrobiol., 84*, 54 (1972).
8. Brierley, C.L., and Brierley, J.A., *Can. J. Microbiol., 19*, 183 (1973).
9. Walsh, F., and Mitchell, R., *J. Gen. Microbiol., 72*, 369 (1972).
10. Walsh, F., and Mitchell, R., *Arch. Mikrobiol., 90*, 19 (1973).
11. Nielands, J.B., In: Microbiol Iron Metabolism, J.B. Nielands (Ed.), Academic, New York, 1 (1974).
12. Aleem, M.I.N., *Ann. Rev. Plant Physiol., 21*, 67 (1970).
13. Zavarzin, G.A., Lithotrophic Microorganisms, Nauka, Moscow (1972) (in Russian).
14. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.I., The Role of Microorganisms in Leaching Metals from Ores, Nauka, Moscow (1972) (in Russian).
15. Dorff, P., *Pflanzenforschung, 16*, 25 (1934).
16. Beger, H., *Ber. Dtsch. Bot. Ges., 62*, 7 (1949).
17. Beger, H., In: Bergey's Manual of Determinative Bacteriology, 7th ed., Williams and Wilkins, Baltimore (1957).
18. Kholodnyi, N.G., Iron Bacteria, USSR Academy of Sciences, Moscow, 14 (1953) (in Russian).
19. Pringsheim, E.G., *Proc. Roy. Soc. (B), 233*, 453 (1949).
20. Drake, H., *Gewässer und Abwässer*, 39/40, 41 (1965).
21. Dubinina, G.A., and Zhdanov, A.V., *Int. J. Syst. Bacteriol., 24*, No. 4, 340 (1965).
22. Balashova, V.V., *Dokl. AN SSSR, 184*, No. 6, 1429 (1969).
23. Balashova, V.V., Mycoplasmas and Iron Bacteria, Nauka, Moscow (1964) (in Russian).
24. Dubinina, G.A., *Dokl. AN SSSR, 184*, No. 6, 1433 (1969).
25. Dubinina, G.A., and Kuznetsov, S.I., *Int. Rev. Ges. Hydrobiol., 61*, 1 (1976).
26. Crombach, W.H.L., van Veen, W.L., and van der Vlies, A.W., *Antonie van Leeuwenhoek, J. Microbiol. Serol., 40*, 217 (1974).
27. Dondero, N.C., *Ann. Rev. Microbiol., 29*, 407 (1975).
28. Pringsheim, E.G., *Phil. Trans. Roy. Soc. London, Ser. B.*, No 588, *232*, 311 (1946).
29. Pringsheim, E.G., *Biol. Rev. Cambridge Phil. Soc., 24*, No. 2 (1949).
30. Patterson, C.O., (in ref. 31) (1971).
31. Patterson, C.O., and Meyer, J., *Plant Physiol., 51*, 104 (1973).
32. Tefler, A., Cammack, R., and Evans, M.C.W., *FEBS Letters, 10*, 21 (1970).
33. Telitchenko, M.M., and Ivanov, E.V., In: Topical Problems of Biology of Blue-green Algae, Nauka, Moscow, 79 (1974) (in Russian).
34. Dubinina, G.A., *Mikrobiologiya, 47*, 4, 591 (1978).
35. Dubinina, G.A., *Mikrobiologiya, 47*, 5, 783 (1978).

36. Lees, H., Kwok, S., and Suzuki, I., *Can. J. Microbiol., 15*, 43 (1969).
37. Hardman, I., and Henrici, A., *J. Bacteriol., 37*, 97 (1939).
38. Aristovskaya, T.V., *Dokl. AN SSSR, 136*, 954 (1961).
39. MacRae, I.C., Edwards, J.E., and Davis, W., *Appl. Microbiol., 25*, 991 (1973).
40. Morgan, J.J., and Stumm, W.J., *Colloid Science, 19*, No. 4, 347 (1964).
41. Delfino, I.I., and Lee, G.F., *Environ. Sci. Technol., 2*, No. 2, 1094 (1968).
42. Shamb, W., Setterfield, Ch., and Ventvors, R., Hydrogen Peroxide, Inostrannaya Literatura, Moscow (1958) (in Russian).
43. Alexander, M., Introduction to Soil Microbiology, J. Wiley and Sons Inc., New York — London (1961).
44. Ehrlich, H.L., Ghiorse, N.S., and Johnson, G.L., *Dev. Ind. Microbiol., 13*, 57 (1972).
45. Galstyan, A.Sh., and Oganesyan, N.A., *Dokl. AN Arm. SSR, 1*, 51 (1973).
46. Ehrlich, H.L., *Soil Sci., 119*, 36 (1975).
47. Kuznetsov, S.I., Microflora of Lakes and its Geochemical Activity, Nauka, Moscow (1970) (in Russian).
48. Perfiliev, B.V., and Gabe, D.R., The Role of Microorganisms in the Formation of Iron-manganese Deposits, Nauka, Moscow — Leningrad (1964) (in Russian).
49. Chukhrov, F.V., Zvyagin, B.B., Gorshkov, A.I., Ermilova, L.P., and Balashova, V.V., *Izv. AN SSSR, Ser. Geol., 4*, 23 (1973).
50. Chukhrov, F.V., In: Hypergenous Oxides of Iron, Nauka, Moscow (1975) (in Russian).
51. Gorlenko, V.M., Dubinina, G.A., and Kuznetsov, S.I., Ecology of Aqueous Microorganisms, Nauka, Moscow (1977) (in Russian).
52. Dubinina, G.A., In: Geology and Geochemistry of Manganese, *III*, Publishing House of the Hungarian Academy of Sciences, Budapest, 305 (1981).
53. Ehrlich, H., In: Microbial Life under Extremal Conditions, Nauka, Moscow (1981) (in Russian),
54. Krumbein, W.E., *Naturwissenschaften, 58*, 56 (1971).
55. Runov, E.V., *Vestn. bakteriol. stantsii*, No. 24, Moscow (1926).
56. Zavalishin, A.A., In: In Memory of Glinka, Leningrad (1928) (in Russian).
57. Aristovskaya, T.V., Microbiology of Podzol Soils, Nauka, Moscow — Leningrad (1965) (in Russian.)
58. Duda, V.I., and Kalakutsky, L.V., *Nauchn. dokl. vyssh. shkoly, Ser. Biol. Nauki*, I (1961).
59. Troshanov, E.P., *Mikrobiologiya, 37*, 5, 394 (1968).
60. Starkey, R.L., and Halvorson, H.O., *Soil Sci., 24*, No. 4 (1927).
61. Bromfield, S.M., *J. Soil Sci., 5*, 1 (1954).
62. Ottow, J.C.G., *Z. Allg. Mikrobiol., 8*, 5, 441 (1968).
63. Ottow, J.C.G., *Zbl. Bact. II Abt., 123*, 6, 585 (1969).
64. de Castro, A., and Ehrlich, H.L., *Antonie van Leeuwenhoek, J. Microbiol. Serol., 36*, 3, 356 (1970).
65. Shakhobova, B.B., *Pochvovedenie, 8*, 145 (1976).
66. Ottow, J.C.G., and Klopotek, A., *Appl. Microbiol., 18*, 1, 41 (1969).
67. Ottow, J.C.G., *Z. Allg. Mikrobiol., 10*, 1, 55 (1970).
68. Ottow, J.C.G., and Ottow, H., *Zbl. Bact. II Abt., 124*, 3/4, 314 (1970).
69. Mahler, H.R., and Cordes, E.H., *Biol. Chem.*, London, 207 (1971).
70. Motomura, S., *Soil Sci. Plant Nutr., 8*, No. 5 (1962).
71. Ottow, J.C.G., and Glathe, H., *Soil Biol. Biochem., 3*, 235 (1971).
72. Kamura, T., Takaj, V., and Ischikama, K., *Soil Sci. Plant Nutr., 9*, 5 (1963).

73. Kojama, T., *J. Geophys. Res.*, 3971 (1963).
74. Takaj, V., Kojama, T., and Kamura, T., *Soil Sci. Plant Nutr.*, *9* (1963).
75. Bromfield, S.M., *J. Soil Sci.*, *5*, No. 1 (1954).
76. Woolfolk, C.A., and Whitely, H.R., *J. Bacteriol.*, *84*, 4, 659 (1962).
77. Schlegel, H.G., Kaltwasser, H., and Gottschalk, G., *Arch. Mikrobiol.*, *38*, 209 (1961).
78. Balashova, V.V., and Zavarzin, G.A., *Mikrobiologiya*, *43*, 5, 773 (1979).
79. Brock, T.D., and Gustavson, J., *Appl. Environ. Microbiol.*, *32*, 4, 567 (1976).
80. Ehrlich, H.L., In: Soil Biochemistry, A.D. McLaren, J. Skujins (Eds), New York, 2 (1971).
81. Balashova, V.V., *Mikrobiologiya* (in press) (1984).
82. Ratledge, C., and Patel, P.V., In: The Biology of Nocardia, London (1976).
83. Aristovskaya, T.V., *Pochvovedenie*, 4, 87 (1975).
84. Aristovskaya, T.V., Microbiology of the Soil Formation Processes, Nauka, Leningrad (1980) (in Russian).
85. Tarygina, G.I., and Proskurina, R.D., *Prikladnaya biokhimiya i mikrobiologiya*, *14*, 4, 643 (1978).
86. Einsele, W., *Naturwissenschaften*, *28* (1940).
87. Dubinina, G.A., and Stravinskaya, E.A., In: Geochemical Activity of Microorganisms in Basins and Deposits, Nauka, Moscow (1973) (in Russian).
88. Grodzinskaya, K.P., In: The Use of Microelements, Polymers and Radioactive Isotopes in Agriculture, 1, Kiev (1962) (in Russian).
89. Troshanov, E.P., *Mikrobiologiya*, *38*, 4, 634 (1969).
90. Munch, J.C., and Ottow, J.C.G., *Z. Pflanzenernäher. Bodenkd.*, *140*, 549 (1977).
91. Ponnamperuma, F.N., and Castro, R.U., In: Proc. 8th Intern. Congr. Soil Sci., Bucharest, 2 (1964).
92. Ponnamperuma, F.N., Juan, W.L., and Nhung, M.T.M., *Nature*, *207*, No. 1103, 1104 (1965).
93. Troshanov, E.P., In: The Role of Microorganisms in the Formation of Iron-manganese Lacustrine Deposits, Moscow — Leningrad (1964) (in Russian).
94. Duda, V.I., Obukhov, A.I., Chernova, N.I., Chernov, N.M., and Gegamyan, I.O., In: Chemistry of Rice Field Soils, Moscow (1976) (in Russian).
95. Sakhvadze, L.I., and Gvilava, M.M., Microbiology of Environment, Nauka, Alma-Ata, Kaz. SSR (1980) (in Russian).
96. Babenko, Yu.S., Grigoriev, E.F., Tarygina, G.A., Arkhipov, A.S., and Dolgikh, L.M., Author's Certificate No. 495353, Bulleten' izobretenii No. 46 (1975).
97. Perkins, E.C., and Novielli, F., *Min. Congr. J.*, *44*, No. 8 (1958).
98. Agate, A.D., and Deshpande, H.A., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York (1977).
99. Imai, K., Tano, T., and Noro, H., US Patent No. 3433629 EM. J. March 254 (1970).

MICROBIAL LEACHING OF METALS FROM ROCKS
(Essentially from granitic rocks)

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INTRODUCTION

Interactions between microorganisms and metals have been considered under different aspects with emphasis on: solubilization or concentrations of elements of economic importance (sulfides leaching, sulfides deposits...); soils fertility or soils formation; metabolism of inorganic compounds; toxicity and pollution; removal of toxic metals or trace elements from industrial and municipal wastes [1—17].

During the last few years, microbial leaching of metals has attracted a larger interest and a better attention in many countries and several international meetings were devoted to such processes. However most of the reports concerned the leaching of sulfides minerals with *Thiobacilli* and essentially the recovery of copper from sulfides or of uranium associated to sulfide minerals. But few studies were done on leaching of metals from rocks by such bacteria.

On the other hand heterotrophic microorganisms and nitrifying bacteria that control the carbon and the nitrogen cycles were mainly considered under a pedogenetic and agronomic point of view. But such microorganisms are able by different mechanisms (acidolysis, complexolysis, alkalinolysis, uptake of elements in microbial cells, direct or indirect reduction) to mobilize mineral elements from rocks and minerals and merit to receive a larger attention.

Heterotrophic microbial solubilization of elements has been studied for a wide range of minerals (silicates, oxides, phosphates, sulfides, native elements, carbonates), but as mentioned in a recent report [18] "considerably less work has been reported on the biodegradation of rocks''.

The present report considers mainly the solubilization or leaching of elements from rocks, i.e. from any consolidated or coherent and relatively hard, naturally formed, mass of mineral matter generally formed of several minerals.

The principal elements considered here will be the major elements (Si, Al, Fe, Mn, Mg, Ca, K) and some trace elements (Ti, Cu, U...) essentially from granitic rocks.

METHODS IN ROCKS MICROBIAL LEACHING

As for minerals, microbial solubilization of rocks was studied in batch cultures, in percolation devices, and also in solid medium with plating techniques.

In batch cultures, flasks containing samples of rocks received nutrient media and are inoculated by one strain, or a mixed population of microorganisms. In some experiments, to prevent contact between minerals and microorganisms and to separate direct (enzymatic) from indirect processes (role of metabolic compounds) or to distinguish influence of large molecular weight compounds from low molecular weight compounds mineral samples were placed in dialysis bags [19, 20]. But such devices including "fermentors" are relatively closed and confined systems.

As for the studies of the microbial soil processes, most of the percolation tests, concerning minerals or rocks leaching, were performed under conditions where the effluents were recycled [21—23]. However, the continuous or semi-continuous flow method seemed also interesting and appeared to be most appropriate for modelling natural conditions because this device, which is an open system, was presumably closest to natural conditions [20, 24]. Nutrient solutions were perfused at regular intervals through columns containing rocks samples. Plant materials can be placed at the top of the column as carbon source.

Experiments in the field were based on a method, relative to the perfusion one, and used lysimetric columns containing rocks samples that received plant materials as carbon and energy source.

In all these experiments sterile or abiological controls were obtained by autoclaving or by adding antimicrobial compounds.

Plating techniques have been generally used only to study solubilization of phosphates, carbonates and silicates.

EVIDENCE OF HETEROTROPHIC MICROBIAL SOLUBILIZATION OF MAJOR AND TRACE ELEMENTS FROM ROCKS

In presence of different available sources of carbon and energy, heterotrophic microorganisms form new cell structures and new chemical compounds (fermentation end products, excess biosynthetic products...) that can act directly or indirectly on minerals. In batch culture or in percolation devices, in presence of glucose nutrient media it has been observed that heterotrophic microorganisms have utmost multiplied respectively by 14, 102, 112, 113, 12, 5, 5, 97, 8 the chemical solubilization of Si, Al, Fe, Mn, Mg, Ca, K, U and Cu from soil and granite-sand, granites and syenites [20, 25, 26].

During semi-continuous flow percolations, of columns of granite sand from an acid brown forest soil, by a glucose nutrient medium, a complex microflora from this soil (bacteria and fungi) have solubilized significantly ($P = 0.01$) Si, Al, Fe, Mn, Mg, Ca and K through the formation of acid compounds. During an eight months semi-continuous flow percolation, respectively 268, 159, 72, 163, 112, 226, 3 mg of SiO_2, Al_2O_3, Fe_2O_3, MnO, MgO, CaO and K_2O were solubi-

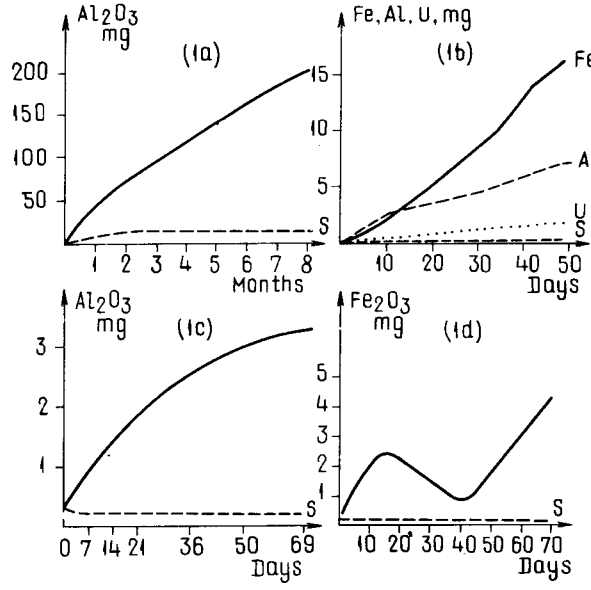

Fig. 1. General aspects of the kinetics of heterotrophic microbial solubilization of elements from rocks:

a — percolation device with a complex microflora; b — percolation device with a relatively simplified microflora (partial sterilization); c — batch culture with a pure strain or a simple mixed population; d — batch culture with a complex population (S — sterile control)

lized through the formation of 28 milliequivalents of acids by the microflora which used approximately 39 g of glucose [20]. In this experiment, attempts to determine the general aspect of the kinetics of microbial solubilization according to the cumulative curves represented by the figure 1a conducted to consider an equation of the following type $y = a + bt - ae^{-ct}$, i.e. an exponential function to which one can add a linear variation.

In batch cultures, two main different types of heterotrophic microbial solubilization curves were observed. With pure strains or simple mixed population, the kinetic aspect of microbial solubilization is similar to growth curves (i.e. exponential) (figure 1c). But with some complex microflora, one can observe successive phases of solubilization and insolubilization (figure 1d). Such phases correspond to the production then to the biodegradation of metabolic organic compounds. Considering the figure 1d, chromatographic analysis has shown that iron microbial solubilization corresponds to the production of mainly lactic, succinic, citric acids and that insolubilization was due essentially to citrate biodegradation [20, 27]. Similar curves have also been observed [28–30] for Fe, Al, U, Zn from aluminosilicates, granite sand, granitic uranium ore, zinc silicate.

Generally in all these types of experiments, the extraction of elements occurred at a high rate at the beginning, then the process either decelerates or stops, depending on the devices used and the experimental conditions. Auto-poisoning of the microflora, consumption of nutrients, blocking of rock surfaces, decrease of production of metabolic compounds... can be involved.

Copper and uranium respectively from a granite containing essentially malachite and from an aplite (figure 1b) containing uranium oxides were also solubilized by heterotrophic microflora [25]. Table 1 summarized results of microbial solubilization of elements from rocks obtained by different authors.

SOLUBILIZATION OF ROCKS ELEMENTS BY AUTOTROPHIC BACTERIA

Leaching of rocks with *Thiobacilli*, except for sulfide minerals or ores associated to sulfides minerals (like uranium) was practically not studied and nitrifying bacteria were also studied in regard of stones deterioration and soils formation or degradation [31—35]. Berthelin et al. [35] have observed that, during *in situ* litter decomposition in lysimetric columns containing granite sand from a brown acid forest soil, calcium and magnesium solubilization were significantly ($P = 0.01$ and $P = 0.05$) correlated to the formation of nitrate (Figure 2). Such processes were not observed in abiological controls. Calcium and magnesium is possibly provided by plagioclase and ferromagnesian minerals present in the rock samples.

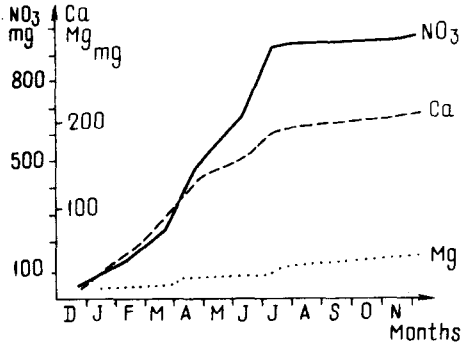

Fig. 2. Influence of nitrification, occurring during biodegradation of a litter of graminaceous plant *(Festuca sylvatica)*, on calcium and magnesium solubilization from a granite sand

MECHANISMS OF MICROBIAL ROCKS LEACHING

As leaching of elements from rocks by *Thiobacilli*, except for sulfide ores, has not been studied extensively, sulfur and iron oxidation will not be discussed here.

Table 1

Microbial solubilization of elements from different rocks materials

Rocks	Elements	Microorganisms	Processes	References
Granodiorite	Fe	Different fungi (greater ef-	Acidification Complexation	37

Rocks	Elements	Microorganisms	Processes	References
		ficiency with *Aspergillus)*		
Pegmatites	Li, Si, Al, Fe	Fungi		38
Aplites, bitumens, granite sand, granites, syenite	Si, Al, Fe, Mn, Mg, Ca, K, U	Fungi, heterotrophic bacteria, nitrifying bacteria *(Pseudomonas* appeared active)	Acidification Complexation Reduction Uptake	9, 20, 24, 27, 35, 36, 39—41, 84
Rocks containing uranium	U	Moulds, heterotrophic bacteria (but *Thiobacilli* appear most efficient)	Acidification Complexation	42
Sandstone, amphibolite	K, Mg, Fe, Al, Si	Yeasts and filamentous fungi	Acidification Complexation (oxalic and citric, gluconic)	43, 44
Greensand	K	*Aspergillus niger*	Acidification Complexation	45
Dunite	Fe, Mg	*A. niger*	Acidification Complexation	46
Granite Granitic gneiss Hard pans Serpentine rocks Soils	Si, Fe, Mn	Different fungi (*A. niger* seems more active)	Acidification Complexation (oxalic, citric, fumaric)	47
Kalitrachyte	K	Silicates bacteria		48
Basalt		Lichen mycobionts	Acidification Complexation	49
Limestone	Ca	Nitrifying bacteria	Acidification	31
Carbonate and silicate ore of copper	Cu	*A. niger, Lactobacillus*		50, 51
Serpentinized ultrabasic rocks	Ni	Nitrifying bacteria	Acidification	34
Basalt	Fe, Al	*Penicillium simplicissimum*	Acidification Complexation	52, 53
Ferrallitic soils (laterites)	Fe, Ca, Al, Mg, K	Soil microflora	Acidification Complexation	54

Table 1, continued

Rocks	Elements	Microorganisms	Processes	References
Aplite, episyenite, granite, bitumens	U, Cu, Fe, Al, Mg	Soil and rock microflora, bacteria and fungi	Acidification Complexation	25
Laterites	Au, Cu	Different heterotrophic bacteria		55, 56
Limestone	Ca	*Thiobacilli*	Acidification	32
Diabase	Fe	Different fungi (*A. niger* seems the more efficient)	Acidification Complexation	57, 58
Amphibolite	Fe, Co, Eu, Yb, Ca, Ba, Sc, Lu, Cr, Th, U	Yeast	Uptake and solubilization	59
Granite Soil Marl	Fe	Lichens	Acidification Chelation	60, 61
Basalt, granite Granodiorite Rhyolite, andesite, peridotite, quartzite	Ti, Al, Fe, Mg, Si	*P. simplicissimum*	Acidification Complexation (citric)	62, 63
Granite	Na, K, Ca, Mg, Fe, Al, Si	Heterotrophic bacteria	Acidification Complexation (lactic, glucuronic, oxalic)	23
Sandstone		Silicate bacteria, *Pseudomonas* sp.	Acidification Complexation Organic acids	64

a) **Influence of metabolic compounds.** In batch cultures, correlation between production of metabolic compounds and microbial solubilization of Si, Al, Fe, Mn, Mg, Ca and K of a granite sand, has been observed [20, 36] (Table 2). It is interesting to note, in such experiment, a positive correlation between total microbial acidity produced by microorganisms and solubilization of all the elements considered. But the production of volatile acidity (acetic, propionic, butyric, lactic) was only positively correlated with Mn, Ca and K solubilization and the production of oxalic acid only with Si, Al, Fe, Mn and Mg.

Table 2

Effect of metabolic products on the solubilization of mineral elements from a granite sand: positive correlation (P) or absence of correlation (A) (test of Spearman for P = 0.05) between microbial solubilization and metabolic compounds simultaneously formed

	Si	Al	Fe	Mn	Mg	Ca	K
Total microbial acidity	P	P	P	P	P	P	P
Total volatile microbial acidity	A	A	A	P	A	P	P
Oxalic microbial acidity	P	P	P	P	P	A	A

According to the degradation and transformation of minerals, in particular phyllosilicates, two main processes, acidolysis and complexolysis, were involved and are summarized respectively by the following general reactions (1) and (2).

(1) (Mineral) $M^+ + H^+R^- \rightarrow H^+$ (mineral) $+ M^+R$
($R = NO_3$, R_1COO^-, HCO_3, SO_4^{2-})

(2) (a) (Mineral) $M^+ + H^+L^- \rightarrow H^+$ (mineral)$^-$ $+ LM$
(b) $H^+L^- + LM \rightarrow L_2M + H^+$ (L = organic ligands)

Acidolysis of minerals is defined as a process involving proton activity and solubilizing mineral elements from rocks in ionized form. Acidification may result either from the formation of an acidic metabolite or from a preferential utilization of alkaline substrates. Microbial oxidation of inorganic (sulfide, sulfur, ammonia...) and organic compounds may produce non complexing or weak complexing acids (carbonic, nitric, sulfuric, formic, acetic, butyric, lactic, succinic, gluconic, etc.). Another way of acidification is for instance the assimilation of ammonium ions leaving acidic anions (sulfate, chloride) in solution.

Complexolysis is a process corresponding to microbial formation of complexing or chelating agents that solubilize mineral elements (iron, aluminium, copper, zinc, nickel, manganese, calcium, magnesium...). Thus metal-organic complexes or metal-organic chelates are formed. Chelation may be defined as the equilibrium reaction between a metal ion and a complexing agent characterised by the formation of more than one bond between the metal and the molecule of the complexing agent and resulting in the formation of a ring structure incorporating the metal ion. Metal ions are bound strongly to chelating agents. This increasing stability of metal ions depends on the number of rings formed by one molecule of chelating agent with the metal ion, the size of the rings and the nature of the donor atoms.

Among the chelating compounds formed by microorganisms are organic acids (citric, oxalic, 2 keto-gluconic, tartric...), phenols (salicy-

lic, 2—3 dihydroxybenzoic acids...) or more complex compounds such as for instance siderophores (i.e. trihydroxamic compounds or tridiorthophenol compounds).

Some authors [37, 40, 65, 66] have shown by different methods the formation of stable complexes with Si, Al, Fe, Mg and Ca during the heterotrophic microbial solubilization of elements from rocks and minerals.

Microflora obtained through partial sterilization, by addition of antimicrobial compounds (methyl para-hydroxybenzoate, thymol, pine resin...) to the perfusion solution or to the soil and rock samples, have promoted significantly the microbial solubilization of Si, Al, Fe, Mn, Mg, Ca, K, U, Cu from granitic rocks through the formation of larger molecular weight (ca. 2000—3000) organo-metallic compounds [24, 25, 40, 68] (Table 3).

Table 3

Cumulative net solubilization of Fe, Cu, Al, Mn (in mg), after 30 days perfusion, of 200 g of a cupriferous granite containing 2.5 % of Cu, by a glucose nutrient medium

	Fe	Cu	Al	Mn
Complex soil microflora	32	49	20	44
Partial sterilization (+ thymol)	130	160	75	76
Partial sterilization (+ lipids)	45	83	23	64
Sterile control (+ Na merthiolate)	0.04	20	0.1	2

Such compounds were formed by the association of simple organic compounds (i.e. iso-citric, citric, oxalic, lactic, succinic, parahydroxybenzoic, protocatechic, ortho and para coumaric, ferrulic acids) and mineral elements (Figure 3). Microorganisms involved in such processes seemed mainly *Pseudomonas* and eventually some associated *Bacilli* and yeasts [40, 68].

Alkalis of microbial origin (ammonia, sodium carbonate) are also involved in release of silica from nepheline, plagioclase, quartz, phytoliths and glass but alkalinolysis were not apparently considered on release of silica from rocks [69].

b) **Bacterial reduction processes possibly involved in rocks leaching.** Anaerobic bacteria (*Clostridium* sp.) and facultatively anaerobic bacteria (*Bacillus*) are able to reduce iron and manganese [70—74] by different mechanisms not well understood in particular for iron. Results from Ottow [72, 73] and Munch and Ottow [74] have suggested that the dissimilative nitrate reductase enzyme systems or a similar enzymatic system and/or an unknown enzyme system designated as dissimilative ferri-reductase is perhaps involved in ferric reduction.

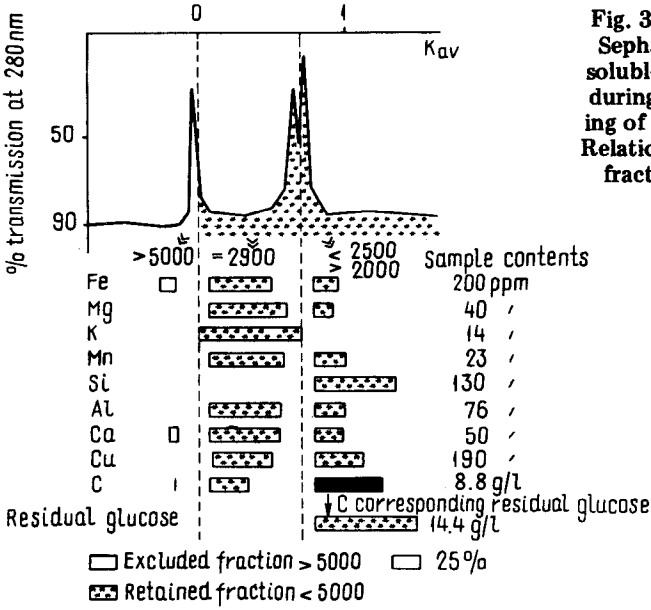

Fig. 3. Fractionation, on a Sephadex G25 gel, of the soluble compounds formed during the microbial leaching of a cupriferous granite. Relation between organic fractions and solubilized elements

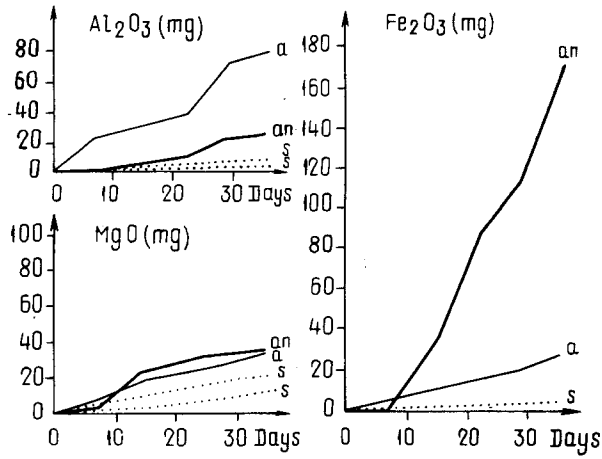

Fig. 4. Solubilization of aluminium, iron and magnesium by aerobic and anaerobic microflora during percolation of 50 g of a granite sand:

a — aerobic; an — anaerobic; S — sterile controls

Such microorganisms promote iron and manganese solubilization from granitic rocks [26, 39, 67]. As they also formed acid compounds (mainly formic, acetic, propionic, butyric, lactic acids) they are also able to solubilize simultaneously other elements (Si, Al, Mg, Ca, K, U, Cu) but without enhancing the "normal" microbial solubilization of these elements (Figure 4). Recently Ehrlich [75] has discussed the possible leaching of manganese ores by such bacteria able to attack Mn^{4+} or Mn^{3+} oxides.

c) **Uptake of elements from rocks by microbial cells.** For their mineral nutrition or in excess of their mineral nutrition, microorganisms can absorb soluble mineral elements. But it appears that they are also able to uptake on or in their cells insoluble elements from minerals and rocks. Weed et al. [19], Thiam [76] have shown respectively that fungi have uptaken potassium from a mica and copper from metal. But Rades-Rohkohl et al. [59] have recently reported uptake of Fe, Co, Eu, Yb, Ca, Ba, Sc, Lu, Cr, Th and U from amphibolite. Munier-Lamy [25] and others [84] have observed uptake of uranium from different rocks (aplite, granite, bitumens) by fungi (*Aspergillus ochraceus, Penicillium funiculosum*) (Figure 5). Deficient

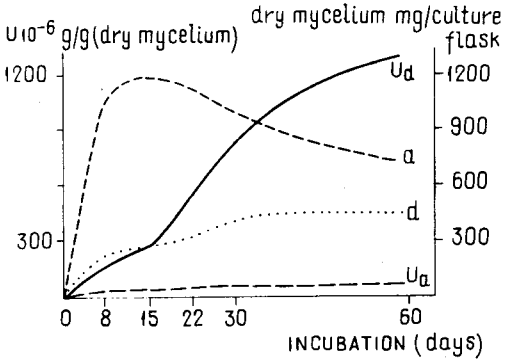

Fig. 5. Uptake of uranium from aplite by *Aspergillus ochraceus* in a deficient (Ud) and in non-deficient (Ua) medium:

a — growth in non-deficient medium; d — growth in deficient medium

nutrient media decreased mycelium growth but have promoted uranium uptake that has reached 2.8 mg U/g of dry biomass. They have also shown that this uranium preconcentration and recovery appear to depend on the cell energetic processes (Table 4).

Table 4

Influence of inhibitors on the growth of *A. ochraceus* and uranium uptake from aplite after 15 days incubation

	pH	Dry mycelium (g/culture flask)	U uptake (μg U/g mycelium)
Non-deficient nutrient medium	6.4	1.176	34
Non-deficient nutrient medium + 2—4 DNP	4.9	0.100	158
Non-deficient nutrient medium + NaN_3	5.0	0.072	58

a) **Nutrient mineral deficiency.** Some bacteria and fungi are able to produce larger amounts of chelating agents in deficient media. Peters and Warren [77], Emery [78] have reported larger production of siderophores-like substances, respectively by *Bacillus subtilis* and *Ustilago sphaerogena*, in iron deficient media. Aristovskaya [79] has observed that deficiencies in Ca, K and Mg promote microbial acid formation by bacteria and *Penicillium*. A soil yeast, *Lipomyces starkeyi*, in deficient media (glucose alone) solubilize more mineral elements from clay minerals than in non deficient media (glucose + mineral salts) because larger amounts of glucuronic and volatile acids seemed to be produced [20]. Mineral deficient media have promoted the development of microbial populations (bacteria and fungi from acid brown forest soil) that produced larger amounts of organic acids and solubilized much more mineral elements from a granitic sand (Table 5). Mineral deficient media can also promote element uptake [84] (Figure 5).

Table 5

Influence of deficiency on the microbial solubilization of elements from a granite sand after 69 days incubation in batch cultures. (Acidity in milliequivalents by culture flask)

	Non-deficient medium		Deficient medium (glucose alone)
Microbial solubilization (μg/culture flask)	SiO_2	6703	9133
	Al_2O_3	3383	7329
	Fe_2O_3	580	8037
	MnO	1239	1955
	MgO	1764	1101
	CaO	3134	5819
	K_2O	135	71
pH (non sterile)		3.0	2.9
pH (sterile controls)		4.4	4.5
Total microbial acidity		1.277	2.660
Total volatile microbial acidity		0.165	0.955
Fumaric microbial acidity		0	0
Lactic and succ. micr. acidity		0.094	0.282
Oxalic microbial acidity		0.054	0.275
Citric microbial acidity		0	0

b) **Antimicrobial compounds.** Partial sterilizations by some antimicrobial compounds (methyl parahydroxybenzoate, thymol, pine resin, lipids) have promoted the development of species that synthesized larger amounts of complexing agents. So that, they promote the solubilization of mineral elements (Si, Al, Fe, Mn, Mg, Ca, K, Cu, U) from granitic rocks (Table 3) [24, 25, 40, 68]. Berthelin et al. [82] have observed that microbial solubilization of uranium from syenite is multiplied by 8 when partial sterilization by thymol occurred.

c) **Particles size of rocks.** Solubilization of mineral elements is a surface phenomenon but, there are few studies concerning the influence of particles size on microbial leaching. Henderson and Duff [47], Muller and Forster [83], Berthelin [20, 27] have observed a highest release of Si, Al, Fe, Mn, Mg, Ca, K from fine material than from coarse material in the following decreasing order "clay > silt > sand" in agreement with the geochemical considerations.

d) **Crystalline structure and nature of the minerals constituting the rocks.** Among the mineral fractions of a diabase rocks (magnetite, pyroxene-amphibole, plagioclases), an *A. niger* solubilized much more iron from magnetite than from the pyroxene-amphibole fraction and much more silica from pyroxene-amphibole than from plagioclase [58].

The fungal solubilization of iron from the minerals forming a granodiorite (augite, hornblende, biotite, magnetite, hematite) is more important from biotite than from hornblende and generally much more from silicates than oxides [37]. But with ferric reducing bacteria, iron from oxides (hematite, goethite, lepidocrosite) was much more solubilized than iron from silicates (biotite) [26].

Studies concerning microbial weathering of granites and syenites have shown that ferromagnesian phyllosilicates (biotite, chlorite) were much more attacked than other constituting minerals [39, 40].

Silverman and Munoz [63] have observed that solubilization of titanium from different rocks by *P. simplicissimum* reached 80 % of Ti content of granite and followed the decreasing order: "granite > quartzite > granodiorite > basalt".

MICROORGANISMS AND CARBON SOURCES INVOLVED

Considering the Table 1, fungi appear as the microorganisms used to a large extent in the studies concerning the leaching of elements from rocks. But the comparison of 20 strains of heterotrophic bacteria *(Flavobacterium, Pseudomonas, Bacillus, Serratia, Acetobacter, Lactobacillus)* and of 12 strains of fungi (*Aspergillus, Penicillium,*

Lipomyces) have shown that a *Pseudomonas* sp. isolated after partial sterilization of an acid brown forest soil, solubilized actively aluminium, iron and magnesium from a granite [20].

Most of the leaching studies of rocks were performed with nutrient media in which glucose or sucrose were the carbon and energetic sources. But starch and cellulose were used with success [20]. Micro-

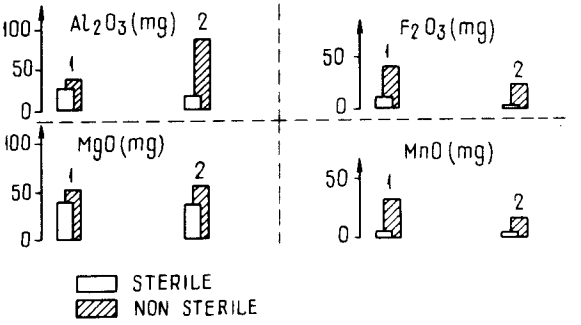

Fig. 6. Comparison of two carbon sources (glucose and starch) on the microbial solubilization of Al, Fe, Mn, Mg from a granite sand (236 g) associated to a brown forest soil (85 g) during 3 months of percolation (1 — starch, 2 — glucose)

bial solubilization of aluminium, iron, manganese and magnesium from columns of brown forest soil and granite sand was, in presence of starch, as efficient as in presence of glucose (Fig. 6).

Raw plant materials were also used as carbon and energetic sources. Anaerobic decomposition of cocksfoot (*Dactylis glomerata)* or lucerne (*Medicago sativa)* has increased the solubilization of elements from oxides (Fe, Mn, Zn, Co, Ni, Mn, Pb, Mo) [80]. Iron from oxides, silicates and ferrallitic soils was solubilized during decomposition of aspen, birch, teak and niaouli leaves [54, 66, 81].

In perfusion devices, microbial decomposition of forest litters has promoted solubilization of elements from granite sand mainly in anaerobic conditions and essentially for iron, manganese, magnesium, calcium and silica [20, 26].

Attempts to compare the global energetic cost of microbial solubilization in different conditions of aeration and carbon sources, have shown, in presence of a granite sand, that the decomposition of a litter of graminaceous plant (*Festuca sylvatica*) has led to the lower global energetic cost but in anaerobic conditions. In aerobic conditions glucose has given the best results (Table 6).

Table 6

Energetic global cost of the microbial leaching of a granite sand (50 g) associated to a brown forest soil (15 g) in presence of different carbon sources (glucose, forest litters) and different conditions of aeration

	Microorganisms + 1 kcal (plant materials or glucose) Solubilized mineral elements, mg				
	Aerobic conditions			Anaerobic conditions	
	Glucose	Festuca	Calluna	Festuca	Calluna
SiO_2	3.2	0	0	1.7	1.8
Al_2O_3	5.0	0	0	0	0
Fe_2O_3	4.3	0	0	11.5	3.7
MnO	1.4	0.6	0	10.8	8.9
MgO	1.1	0.3	0	1.1	2.0
CaO	1.4	1.9	0	11.5	0
K_2O	0.1	1.2	0	14.9	0

One considers that 1 kg of dry organic matter corresponds to 4,800 kcal and to 700 litres of CO_2.

CONCLUSION

According to the literature, different studies have concerned the microbial leaching of metals from rocks (Table 1). A relatively more important number of references have considered the biodegradation of minerals forming the rocks but have not been discussed here, except for some comparisons and discussion with the microbial leaching of rocks.

Most of these works, concerning rocks leaching, were performed in order to determine in soil ecosystems the pedogenetic, metallogenic and agronomic incidences of the microbial activity. *Thiobacilli* which are the autotrophic bacteria of economic importance for treating certain sulfide ores have not been extensively studied in leaching of elements from rocks. Some data concerned the implications of nitrifying bacteria and have shown that they can play a relatively important role in the biogeochemical cycles of certain elements (Ca, Mg) in some ecosystems such as brown acid forest soil [35].

Microorganisms involved in these studies of leaching of rocks were essentially heterotrophs. Fungi (Table 1) in particular *Aspergillus* sp. and *Penicillium* sp. appear as efficient agents of solubilization of elements but some bacteria (e.g. *Pseudomonas* sp.) are also very active.

Extent of microbial solubilization is often much more important in presence of mixed cultures or complex associations than in presence of pure strains involved in such processes [82]. So that microbial association must be considered with interest for mutualistic or commensalistic effect.

Different elements and essentially Si, Al, Fe, Mn, Mg, Ca, K, Ti, Li, U, Cu, Au, were significantly solubilized from different rocks. However most of these works have been done on granitic rocks.

The main process actually studied in the leaching is the formation of acid compounds. Among them the most efficient will be the complexing or chelating compounds that form strong and stable organometallic complexes or chelates. Other mechanisms as the formation of alkalis, the reduction of iron and manganese, the uptake of elements on or in the microbial cells were also studied but at a less extent. However they appear as interesting processes for the mobilization of some elements.

Factors such as antimicrobial compounds (partial sterilization), mineral and growth factors deficiency, seem able to promote the solubilization of some elements by modifying the development of the microbial population and by enhancing the formation of efficient metabolic compounds. Crystalline structure, nature of minerals, size of rocks particles must be also considered.

Starch, cellulose, plant materials appear as possible and less expensive sources of carbon and energy than glucose and sucrose. Perhaps microbial transformations of agricultural wastes could be used in order to produce strong chelating agents that could act separately or directly on rocks and thus leach elements with a high yield and at a moderate cost.

It is also possible to consider that in the future some specific microbial processes (production of specific chelating agents or specific metal uptake) will be used for mineral purification or metal concentrations particularly if the supplying in these metals becomes more difficult. But a better knowledge of these processes will be necessary to improve metal extraction.

Actually, under an economic point of view, that considers mainly the cost of energetic sources, heterotrophic microorganisms cannot be used in leaching processes. But they must be considered with a great attention for their occurrence in soil formation and soil degradation processes, in soil fertility and plant nutrition, in the cycling of some elements and finally, under a geological point of view, in the superficial mobilization and concentration of elements of economic importance.

REFERENCES

1. Kuznetsov, S.I., Ivanov, M.V., and Lyalikova, N.N., Introduction to Geological Microbiology, McGraw Hill, New York (1963).
2. Silverman, M.P., and Ehrlich, L., *Adv. Appl. Microbiol., 6,* 153 (1964).
3. Zajic, J.E., Microbial Biogeochemistry, Academic Press, New York — London (1969).
4. Dommergues, Y., and Mangenot, F., Ecologie microbienne du sol, Masson, Paris (1970).
5. Aristovskaya, T.V., and Zavarzin, G.A., In: Soil Biochemistry, A.D. McLaren and J. Skujins (Eds.), Marcel Dekker Inc., New York, *2,* 385 (1971).
6. Krumbein, W.E., *Rev. Ecol. Biol. Sol, 9* (3), 283 (1972).
7. Bertrand, D., *Rev. Ecol. Biol. Sol, 9* (3), 349 (1972).
8. Alexander, M., Introduction to Soil Microbiology, 2nd ed., John Wiley and Sons Inc., New York (1977).
9. Berthelin, J., *Science du Sol, 1,* 13 (1977).
10. Brierley, C.L., *Crit. Rev. Microbiol., 6* (3), 207 (1978).
11. Iverson, W.P., and Brinckman, F.E., In: Water Pollution Microbiology, Ralph Mitchell (Ed.), John Wiley and Sons, New York, *2,* 201 (1978).
12. Summers, A.O., and Silver, S., *Ann. Rev. Microbiol., 32,* 637 (1978).
13. Berthelin, J., and Toutain, F., In: Pédologie, Constituants et Proprietes du Sol, M. Bonneau and B. Souchier (Eds.), Masson, Paris, *2,* 123 (1979).
14. Brock, T.D., Biology of Microorganisms, Prentice Hall Inc., Englewood Cliffs, New Jersey (1979).
15. Lundgren, D.G., and Silver, M., *Ann. Rev. Microbiol., 34,* 263 (1980).
16. Murr, L.E., *Minerals Sci. Engng., 12* (3), 121 (1980).
17. Ehrlich, H.L., Geomicrobiology, Marcel Dekker Inc. (1981).
18. Dacey, P.W., Wakerley, D.S., and Le Roux, N.W., The Biodegradation of Rocks and Minerals with particular reference to Silicate Minerals: a Literature Survey, Warren Spring Laboratory, Department of Industry, Stevenage, Hertfordshire, United Kingdom (1981).
19. Weed, S.B., Davey, C.B., and Cook, M.G., *Soil Sci. Soc. Proc., 33,* 702 (1969).
20. Berthelin, J., Thèse de Doctorat d'Etat, Université de Nancy I (1976).
21. Bryner, L., Beck, J., Davis, D., and Wilson, D., *Ind. and Eng. Chem., 46,* 2587 (1954).
22. Fisher, J.R., In: Annual Western Meeting, Winnipeg, October 1965, *Transactions, LXIX,* 164 (1966).
23. Wagner, M., and Schwartz, W., *Z. Allg. Mikrobiol., 7,* 33 (1967).
24. Berthelin, J., and Kogblevi, A., *Rev. Ecol. Biol. Sol., 9,* 3, 407 (1972).
25. Munier-Lamy, C., Thèse de Doctorat de Spécialité, Université de Nancy I (1980).
26. Cheikhzadeh-Mossadegh, D., Thèse de Doctorat d'Etat, Université de Nancy I (1981).
27. Berthelin, J., *Science du Sol, 1,* 11 (1971).
28. Aristovskaya, T.V., Daragan, A.Yu., Zykina, L.V., and Kutuzova, R.S., *Soviet Soil Science, 5,* 538 (1969).
29. Magne, R., Berthelin, J., and Dommergues, Y., In: Formation of Uranium Ore Deposits, IAEA, Vienne (Autriche), 183/18, 73 (1974).
30. Agbim, N.N., and Doxtader, K.G., *Soil Biol. Biochem., 7,* 275 (1975).
31. Kauffman, J., *Corrosion et anticorrosion, 8,* 3, 87 (1960).
32. Pochon, J., and Jaton, C., *Chem. Indust.,* 1587 (1967).

33. Ivarson, K.C., Ross, G.J., and Miles, N.M., *Soil Sci. Soc. Am. J., 42,* 518 (1978).
34. Lebedeva, E.V., Lyalikova, N.N., and Bugel'sky, Yu.Yu., *Mikrobiologiya, 47,* 6, 1101 (1978).
35. Berthelin, J., Belgy, G., and Wedraogo, F.X., Colloque "Humus et Azote", Reims, France, 112–117, Commissions I et II (1981).
36. Berthelin, J., and Dommergues, Y., *Rev. Ecol. Biol. Sol., 9,* 3, 397 (1972).
37. Arrieta, L., and Grez, R., *Appl. Microbiol., 22,* 4, 487 (1971).
38. Avakyan, Z.A., Karavaiko, G.I., Mel'nikova, E.O., Krutsko, V.S., and Ostroushko, Yu.I., *Mikrobiologiya, 50,* 1, 156 (1981).
39. Berthelin, J., Boymond, D., In: Environmental Biogeochemistry and Geomicrobiology, W.E. Krumbein (Ed.), Ann Arbor Sciences Publ., *2,* 659 (1978).
40. Berthelin, J., and Belgy, G., *Geoderma, 21,* 297 (1979).
41. Magne, R., Berthelin, J., and Dommergues, Y., *C.R. Acad. Sci.,* Paris, D, *276,* 2625 (1973).
42. Ebner, H.G., and Schwartz, W., *Z. Allg. Mikrobiol. morphol. physiol. Okol. Mikroorg., 14,* 2, 93 (1974).
43. Eckhardt, F.E.W., In: Environmental Biogeochemistry and Geomicrobiology, W.E. Krumbein (Ed.), Ann Arbor Science Publ., *2,* 675 (1978).
44. Eckhardt, F.E.W., *Zeitschr. Pflanz. und Boden, 142,* 3, 434 (1979).
45. Eno, C.F., and Reuszer, H.W., *Soil Science, 80,* 199 (1955).
46. Goni, J., Leleu, M., and Sarcia, C., *Bull. du B.R.G.M.,* Section II, No. 5, 35 (1971).
47. Henderson, M.E.K., Duff, R.B., *J. Soil Sci., 14,* 236 (1963).
48. Holobrady, K., Bujdos, G., and Dobis, A., *Biologia* (Bratislava), *25,* 1, 39 (1970).
49. Jones, D., and Wilson, M.J., Abstracts of the Second International Symposium on Microbial Ecology, University of Warwick, Coventry, England, 87 (1980).
50. Kiel, H., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, Weinheim, New York, 261 (1977).
51. Kiel, H., and Schwartz, W., *Z. Allg. Mikrobiol., 20,* 10, 627 (1980).
52. Mehta, A.P., Torma, A.E., and Murr, L.E., *I.R.C.S. Med. Sci., 6,* 416 (1978).
53. Mehta, A.P., Torma, A.E., and Murr, L.E., *Biotechnol. Bioengin., 21,* 875 (1979).
54. Moureaux, C., *Rev. Ecol. Biol. Sol., 9,* 539 (1972).
55. Pares, Y., *Ann. Institut Pasteur, 107,* 132 (1964).
56. Pares, Y., and Cupper, J., *Ann. Institut Pasteur, 107,* 568 (1964).
57. Ribeiro, R.M., Moureaux, C., and Mussisantos, A., *Cah. ORSTOM, série Pédologie, XI,* 1, 57 (1973).
58. Ribeiro, R.M., Moureaux, C., and Novikoff, A., *Cah. ORSTOM, série Pédologie, XIV,* 2, 161 (1976).
59. Rades-Rohkohl, E., Hirsch, P., and Franzle, O., *Appl. Environ. Microbiol., 38,* 6, 1061 (1979).
60. Schatz, A., *Naturwis., 22,* 518 (1962).
61. Schatz, A., *Agricul. Food Chem., 11,* 2, 112 (1963).
62. Silverman, M.P., and Munoz, E.F., *Science, 169,* 985 (1970).
63. Silverman, M.P., and Munoz, E.F., *Appl. Microbiol., 22,* 5, 923 (1971).

64. Wood, P.A., and McRae, I.C., *Int. Biod. Bull.*, *8*, 1, 25 (1972).
65. Tsyurupa, I.G., *Soviet Soil Science*, *3*, 46 (1964).
66. Daragan, A.Yu., *Soviet Soil Science*, *3*, 5, 567 (1971).
67. Berthelin, J., and Kogblevi, A., *Rev. Ecol. Biol. Sol.*, *11*, 499 (1974).
68. Berthelin, J., Kogblevi, A., and Dommergues, Y., *Soil Biol. Biochem.*, *6*, 6, 393 (1974).
69. Kutuzova, R.S., *Soviet Soil Sci.*, *5*, 111 (1973).
70. Bromfield, S.M., *J. Soil Science*, *5*, 1, 129 (1954).
71. Bromfield, S.M., and David, D.J., *Soil Biol. Biochem.*, *8*, 1, 37 (1976).
72. Ottow, J.C.G., *Zbl. Bakt. Parasitk. Infekt. Hyg.*, *123* (6), 600 (1969).
73. Ottow, J.C.G., In: Pseudogley and Gley, E. Schlichting and U. Schwertmann (Eds.), Verlag Chemie, 29 (1972).
74. Munch, J.C., and Ottow, J.C.G., *Z. Pfl. Bodenk.*, *140*, 549 (1977).
75. Ehrlich, H.L., In: Biogeochemistry of Ancient and Modern Environments, Trudinger, P.A., Walter, M.R., and Ralph, B.J., (Eds.), Springer, New York, 604 (1980).
76. Thiam, M.L., *Ann. Institut Pasteur*, Paris, *114*, 468 (1968).
77. Peters, W., and Warren, R.A.J., *Can. J. Microbiol.*, *16*, 1179 (1970).
78. Emery, T., In: Microbial Iron Metabolism, J.B. Nielands (Ed.), Academic Press, New York, 107 (1974).
79. Aristovskaya, T.V., 6th Congr. Sc. Sol., Paris, Vol. *C*, Com. III, *43*, 263 (1956).
80. Kee, N.S., and Bloomfield, D., *Geochim. Cosmochim. Acta*, *24*, 206 (1961).
81. Boquel, G., and Suavin, L., *Rev. Ecol. Biol. Sol.*, *11*, 2, 187 (1974).
82. Berthelin, J., Belgy, G., and Magne, R., In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie. Weinheim, New York, 251 (1977).
83. Müller, G., and Förster, I., *Zbl. Bakt.*, *116*, 2, 372 (1963).
84. Berthelin, J., Munier-Lamy, C., Environmental Biogeochemistry, *Ecol. Bull.* (Stockholm), *35*, 395 (1983).

MICROFLORA OF ROCK AND ITS ROLE IN THE LEACHING OF SILICATE MINERALS

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INTRODUCTION

Rock composed of silicate and aluminosilicate minerals makes up the main mass (some 95 %) of the earth's crust. Rock weathering occurs in the hypergenesis zone under the influence of physical, chemical, and biological factors. An essential role in the dissolution, migration, and redeposition of various elements making up the rock minerals, as well as in the formation of crusts of weathering, ore deposits and soils resultant from these processes is played by microorganisms.

This paper considers the ecology and geochemical activity of microorganisms of rock, the action mechanism of the effect of microorganisms on silicate minerals and the possibility of using microorganisms in the processing and dressing of silicate mineral raw material.

THE MICROFLORA OF ROCK

The studies of the spread of microorganisms in rock and of their geochemical activity have been boosted by the concept of S.N. Vinogradsky about microorganisms as being the main agents in the circulation of matter in nature and by the theory of V.I. Wernadsky of the geological role of organisms [1, 2].

Microbiological studies have been performed on freshly erupted magmatic rock, on massive-crystalline rock in connection with the primary processes of soil formation, on modern and ancient crusts of weathering and in some ore deposits. The results of the studies are summed up in Table 1.

The first inhabitants on the lava of the Krakatoa volcano five years after the eruption were discovered to be cyanobacteria [5]. Lichen and cyanobacteria are considered by a number of authors as the most widespread organisms developing volcanic magmatic rock in various climatic zones of the world [6—8]. At the same time, the studies of microflora of rock before its colonization by lichen, carried out mainly by Soviet scientists, made it possible to discover various groups of autotrophic and heterotrophic microorganisms.

N. Krasil'nikov studied the crusts of weathering of basalts, granite and tuffs in Armenia and discovered up to 2.5×10^6 cells per gramme of rock of oligonitrophilic microorganisms, chiefly asporogenic

bacteria and mycobacteria [9—11]. D. Novogrudsky came to the conclusion that in the earliest stages of weathering rock has a heterotrophic bacterial flora, mainly the *Bacterium* and *Mycobacterium* species [12].

A diverse microflora including nitrifying bacteria, cyanobacteria, green algae, heterotrophic bacteria and fungi has been discovered on rock in the Pamirs and the Tien Shan [13—19].

An analysis of the microflora taking part in the colonization of pyroclastic material of the Tyatya and the Tolbachik volcanoes has shown that the most widespread are chromogenic bacteria representing the genera *Micrococcus*, *Mycobacterium*, *Flavobacterium*, *Arthrobacter*, etc, which are marked by halotolerance, acid-resistance and an oligotrophic character of alimentation [3, 4].

Fungi and heterotrophic bacteria were isolated out of weathered granite, gneiss and diorite and the dominant forms among them were those that do not produce spores. It has been shown that the more weathered the rock is, the more bacteria it has [18].

These studies have shown that microorganisms are present in different types of rock. But it doesn't seem possible to make any conclusions about the geochemical activity of the discovered microorganisms, about the character of coenoses developing in various physical and chemical conditions at different stages of weathering and about the regularity of the change of the forms of microorganisms, since the quantitative account of different groups of microorganisms was incomplete and the determination of their specific composition has not yet been carried out in full.

Table 1

Microorganisms of rock

Rock	Microorganisms	Reference
Magmatic volcanic rock:		
volcanic ashes (Kunashir Island)	Chromogenic nonsporulating bacteria, corynebacteria	3, 4
cold lava (Hawaiian Islands)	Cyanobacteria, lichen	5, 6
zones of weathering of basalt, tuff and granite (Armenia)	Oligotrophic bacteria, mycobacteria, lichen	9—11
weatherable rock of nival, mountain-meadow and mountain-forest belts of the Pamirs and Tien Shan prior to lichen colonization	*Bacterium* sp., *Mycobacterium* sp.	12

Table 1, continued

Rock	Microorganisms	Reference
after lichen colonization	Cyanobacteria, nitrifying bacteria, green algae, sporulating and nonsporulating heterotrophic bacteria, mycobacteria, fungi	13—19
zones of weathering of nepheline syenites and granite (Khibiny)	Algae, oligonitrophilic and ammonifying heterotrophic bacteria, fungi, actinomycetes	20
granite, diorite and gneiss of different stages of weathering (Northern Scotland)	Heterotrophic bacteria, fungi, actinomycetes	21
zones of desert weathering: crusts of desert varnish on granite, basalt and quartz gravel	Cyanobacteria, fungi, *Metallogenium symbioticum*	22
crusts of desert varnish on magmatic rock (California)	Fungi, *Metallogenium symbioticum*, *Pedomicrobium* sp.	23
crusts of desert varnish on magmatic rock	Fungi, heterotrophic bacteria oxidizing iron and manganese, *Arthrobacter* sp., *Pedomicrobium* sp.	24
Crusts of weathering and ore deposits:		
crusts of weathering of ultrabasites of the Central Urals and Cuba	Nitrifying bacteria of 1-st and 2-nd stages, thionic bacteria, heterotrophic bacteria	25
crust of weathering of Mesozoic-Cenozoic coaly shales (East-Siberian platform, Tomsk)	*T. ferrooxidans*, *T. thiooxidans*, heterotrophic bacteria, nonsporulating species predominate	26
zone of weathering of pegmatite, coaly-aleurolite shales and spodumene on a spodumene deposit (Eastern Siberia)	Nitrifying bacteria, thionic bacteria, fungi, nitrogen-fixing and oligonitrophilic bacteria, sulfate-reducing bacteria	27
laterite bauxites (Hawaiian Islands)	*Hyphomicrobium* sp., *Metallogenium* sp., *Siderocapsa* sp., *Penicillium* sp.	28
zone of weathering of nepheline-syenites (Africa)	Heterotrophic bacteria, yeast	29

Table 1, continued

Rock	Microorganisms	Reference
Sedimentary rock:		
silificated limestones (Central and Lower Tatras, Alps)	Nitrifying bacteria, thionic bacteria, spore-forming and asporogenic heterotrophic bacteria, *Arthrobacter* sp., oligonitrophiles, fungi	30, 31

Complex research, involving the study of the microorganisms' composition along with the determination in model experiments of the geochemical activity of the isolated cultures, has been carried out on a spodumene deposit, on the crusts of weathering of ultrabasites and while determining the mechanism of the development of the desert varnish.

The analysis of the microflora on a spodumene deposit was conducted on areas exposed as a result of mining. The results of the microbiological research are given in Table 2. An analysis of the results enables us to make the following conclusions. Destroyed samples of spodumene and enclosed rock represent a wide spectrum of autotrophic and heterotrophic microorganisms. The diversity and number of microorganisms rise with the extent of destruction of the samples. Preserved spodumene and mine water contain nitrifying bacteria *Nitrobacter* sp. and *Nitrosomonas* sp. Thionic bacteria of the *T. thioparus* and *T. thiooxidans* type are found in samples which had undergone degradation and in mine water. *T. ferrooxidans* develops in areas with pyritized shales with pH 3.0 and lower. Sulphur-reducing bacteria are chiefly found in underground water. In the case of solid rock only a few samples of destroyed spodumene indicated the presence of this group of bacteria.

A quantitative account of different groups of microorganisms has shown the dominating role of heterotrophic nonsporulating bacteria, whose number in ores and rock is 10^5--10^7 cells per gramme of sample. The number of autotrophic bacteria normally did not exceed 10^3--10^4 cells per gramme of sample.

The most widespread in the deposit are heterotrophic bacteria of the genera *Arthrobacter* (*A. globiformys*, *A. pascens*, *A. simplex*, as well as *Nocardia* sp. and *Pseudomonas* sp.). Fungi and yeast were isolated from samples of destroyed rock with a low pH value. A study of the physiology of heterotrophic microorganisms isolated from the deposit has shown that the dominating species are capable of developing in media with a low concentration of organic matter and that they make use a wide range of organic compounds: low-molecular

Table 2

The microflora of a spodumene deposit

Characteristics of samples*	pH of samples	Number of samples with microorganisms in % of the total number of samples						
		thionic bacteria	nitrifying bacteria	denitrifying bacteria	nitrogen fixatiors and oligonitrophiles	heterotrophes		fungi
						aerobes	anaerobes	
Preserved dry spodumene	6.0—6.5	—	10	—	—	80	—	10
Preserved moist spodumene	6.5	—	—	100	100	100	—	—
Destroyed moist spodumene	5.6—7.0	30	10	100	90	100	—	—
Destroyed moist pegmatite; spodumene leached	2.4—8.5	37	30	75	62	100	12	25
Destroyed moist shale	3.9—6.5	28	28	14	57	100	14	42
Fracture downpours in shales	4.5—5.0	100	—	—	—	60	—	50
Water from under rock	6.8—7.8	43	—	100	100	100	—	—

* — In each case, from 10 to 20 samples were analyzed.
Note. Symbol "—" means that microorganisms of this group have not been detected.

organic alcohols and acids, humic acids and hydrocarbons and phenol. A specific feature of the isolated microorganisms is their ability to grow on media with a low nitrogen concentration. These data speak for the fact that in conditions of the examined deposit the activity of heterotrophic microflora is supported chiefly at the cost of organic matter of enclosed rock and mine water. A certain role is also played by autotrophic and heterotrophic fixation of CO_2.

Experiments involving carbon-labelled phenol, acetate, and bicarbonate (Table 3) have made it possible to determine microorganisms' activity directly in various samples. The most active is the flora of partly destroyed spodumene and of shales.

When inoculating samples selected at the deposit onto mineral media containing quantities of spodumene, pegmatite or shale (S : L = 1 : 20) the development was observed of *Arthrobacter* sp. and *Nocardia* sp. which, as the medium grew increasingly acid, were replaced by fungi. Thus, it was confirmed that arthrobacteria and nocardiabacteria on a spodumene deposit are not some incidental forms and that they are capable of developing in oligotrophic conditions.

Table 3

Radioactivity of cells of microorganisms in rock during incubation with carbon-labelled compounds

Samples	pH	Radioactivity of cells on different substrata, imp/min/1 g of sample		
		phenol	acetate	CO_2
Preserved spodumene	5.4	0	550	—
			300	—
Partly destroyed spodumene	6.1	14000	0	—
		20800	0	—
Destroyed spodumene	6.4	790	200	—
		1100	200	—
Destroyed pegmatite	6.8	700	8000	1600
		800	—	—
Shale with pegmatite	4.9	2300	6400	650
		1600	9700	500
Destroyed shale	7.0	800	10300	—
		—	8000	1600

Note. Symbol "—" means that measurements were not carried out.

A peculiar coenosis of microorganisms has been found in crusts of desert varnish. Desert varnish is a fine film of products of weathering, covering rock in arid regions. The film consists of hydrous manganous and ferric oxides as well as argillaceous minerals which are products of weathering.

A number of researchers have studied the microflora of the crusts of desert varnish and with the help of the isolated microorganisms have experimentally proved the biogenic origin of these formations.

Krumbein and Jens [22] isolated from the crusts of desert varnish, developed on the surface of various rocks, cyanobacteria, microorganisms oxidizing iron and manganese, in particular *Metallogenium symbioticum*, and fungi.

Dorn and Oberlander [23] have suggested that the crusts of desert varnish developing on rocks in arid regions are of the same origin as the crusts of weathering on the rock surface in other climatic zones, and that they are of one and the same biogenic origin. In samples of weather varnish they found cultures of bacteria similar to *Metallogenium* sp. and *Pedomicrobium* sp.

The specific composition of the coenosis typical of crusts of weathering, can vary. Taylor-George et al. [24] studying the microflora of desert varnish in the same regions as the previous authors, have shown that the coenosis consists of fungi developing in the form of microcolonies on rock surface and of bacteria oxidizing iron and manganese. They failed to find a culture of the *Metallogenium* type. Using the isolated cultures, these authors have experimentally succeeded in making a desert varnish on the surface of unweathered rock, by inoculating it with a mixture of fungi and bacteria.

The studies carried out indicate that the microflora of the crusts of weathering is an example of a microbe ecosystem adapting to extreme conditions of survival in conditions of low water activity, low concentrations of organic matter and high solar radiation.

The qualitative and quantitative composition of the rock's microflora and its activity is governed by a number of physical and chemical factors, among them the humidity and jointing of the rock, the pH and Eh of rock and pore solutions, the chemical content of rock, temperature and the availability of sources of energy and constructive metabolism. Microorganisms developing in rock receive elements of mineral nutrition from this rock. That is why it is necessary to estimate the availability of substrates for constructive and energy metabolism.

The wide incidence of nitrifying bacteria in the crusts of weathering of ultrabasites in different climatic zones indicates that the development of a group of microorganisms in rock is determined by the presence of favourable physical and chemical conditions and substrates for constructive and energy metabolism. Bacteria have been found in nickeliferous crusts of weathering, performing the first and second stages of nitrification. Their population amounts to 2.5×10^6 cells per

gramme of sample. The presence in rock of substantial concentrations of calcium and magnesium, which neutralize the nitrous and nitric acids formed by microorganisms, is conducive to the activity of nitrificators in the rock of the given type [25].

As an energy substrate nitrifying bacteria use an ion of ammonium, oxidizing it to nitrous acid (the first stage of nitrification) and then nitrous to nitric acid (the second stage of nitrification). Substantial amounts of nitrogen are found in rocks of various types, mostly in the form of an ion of ammonium which is part of a number of widespread aluminosilicate minerals, where it replaces the K^+ ion. It has now been experimentally shown that nitrifying bacteria can use the ammonium of silicate minerals [32].

There are published data on the use of rock nitrogen in constructive metabolism by oligonitrophilic microorganisms [33, 34]. The suppliers of nitrogen in coenoses developing on rock are nitrogen-fixing cyanobacteria and heterotrophic bacteria discovered in various types of rock (Table 1).

For the development of thionic bacteria reduced compounds of sulphur are necessary as an energy substrate. According to some researchers [35, 36], sulphide minerals, chiefly pyrite, are found in rock of different types and their concentration varies from 2 to 40 kg per t. The presence of sulphide mineralization determines the development of thionic bacteria in coaly-aleurolite shales and pegmatites contacting with them [27]. The development of thionic bacteria is also observed in the crusts of weathering of ultrabasic rock with an embedded sulphide mineralization [25], as well as in sedimentary rock characterized by a karst structure [30, 31].

As it has already been shown, heterotrophic microorganisms using organic compounds as an energy and constructive substrate, are a constant component of rock coenoses. This requires the solution to the problem of the resources for their existence in the oligotrophic conditions of the initial stage of rock destruction. However, allowance should be made for the ability of the bacteria to reproduce on media with low concentrations of organic compounds [37]. Investigation disclosed that bacteria of oligotrophic water bodies possess such ability. The ability of heterotrophic bacteria and fungi isolated from rock to develop on media without a source of carbon in the presence of ground rock has been shown by a number of authors [13, 23, 27].

According to geochemical data, dispersed organic matter is present in volcanic ash [38], and in rock subjected to thermal metamorphism [39, 40].

The suppliers of organic matter can be autotrophic bacteria and microscopic algae, developing on rock.

Microorganisms develop on rock and minerals in close contact with hard surface. The character of the interaction of microorganisms with rock and minerals has yet been studied poorly. Basalts have been

observed to be selectively covered with lichen and no growth was discovered on tuff [9, 10]. Lichen was found on minerals and areas of rock characterized by an increased content of Ca and Mg [47].

It has been determined that species of microflora developing on rappakivi granite are more diversified than those growing on other rocks [41]. As to mineral media without organic compounds, rod-shaped bacteria develop on the surface of feldspars, while fungi grow on syderite and muscovite [42].

On the surface of quartz sand particles in the littoral region of the Baltic Sea microorganisms are found in separate colonies consisting of 5—150 cells. Most microorganisms live in hollows and tiny cracks [43]. The character of the colonization of minerals by microorganisms depends not only on the surface's topography but also on the nature of a mineral. Thus, the number of microorganisms on the surface of hornblende particles is greater than in deeper layers; with the distribution of microorganisms on biotite particles it is the other way round [44].

Bacteria isolated from sandstone retained their ability to be adsorbed on the surface of crystals of synthetic quartz [45].

THE GEOCHEMICAL ACTIVITY OF THE MICROFLORA OF ROCK

The geochemical activity of microorganisms in silicate rock was studied chiefly in connection with the process of soil formation on massive-crystalline rock. The behaviour of microorganisms in crusts of weathering and ore deposits has been studied inadequately. The data available are summarized in Table 4.

Nitrifying and thionic bacteria are considered by a number of authors as agents enhancing the development of the processes of karst formation [25, 30, 31].

Nitrifying bacteria are actively involved in the weathering of serpentinous ultrabasites in various climatic zones. The nitrous and nitric acids which they form through the oxidization of ammonium of rock, are conducive to the release of calcium, magnesium and silica resulting in the enrichment of the mentioned zones of weathering with nickel and the formation of nickeliferous crusts of weathering [25].

Sulphide mineralization of rock and an appropriate oxidative — reducing environment is a major factor in the development in rock of thionic bacteria [25, 26, 27, 30, 31], which is accompanied by the production of sulphuric acid. Sulphuric acid is one of the factors affecting rock minerals and quickening their weathering.

Spodumene weathering in the hypergenesis zone is accompanied by the release of alkaline elements and the formation of the argilla-

ceous minerals, montmorillonite and kaolinite [27]. In alkaline and neutral media, the spodumene disintegrates with the formation of montmorillonite:

$$2LiAlSi_2O_6 + 2H_2O \rightarrow Al_2[Si_4O_{10}](OH)_2 + 2LiOH$$

In acid medium the latter is converted to kaolinite:

$$2Al_2[Si_4O_{10}](OH)_2 + 2LiOH + 5H_2O + CO_2 \rightarrow$$
$$Al_4[Si_4O_{10}](OH)_2 + Li_2CO_3 + 4H_2SiO_3$$

In the process of sulphate weathering the spodumene is converted to kaolinite:

$$4LiAlSi_2O_6 + 6H_2O + 2H_2SO_4 \rightarrow 2Li_2SO_4 + Al_4[Si_4O_{10}](OH)_8 + H_2SiO_3$$

The microorganisms of the pegmatite deposit in Western Siberia apparently enhance the process of weathering of spodumene and enclosing rock, accelerating the release of cations and the formation of argillaceous minerals [46]. It has been experimentally determined that cultures of fungi (isolated from a spodumene deposit) increase the release of lithium, iron, aluminium and silicium from spodumene, pegmatite and shales [47]. The development of thionic bacteria in pyritization zones leads to a decrease in rock and mine water pH and creates conditions favourable for the formation of kaolinite as a final product of weathering of spodumene [27].

In the process of rock and mineral destruction new minerals are formed and microorganisms can participate in the process. A graphic example of the close link between the processes of weathering and the processes of the formation of new minerals are biogenic crusts of weathering and crusts of desert varnish. The coenosis of microorganisms, which includes species of fungi and bacteria oxidizing iron and manganese, degrades minerals of the mother rock. The process is accompanied by the formation of hydrous ferric and manganous oxides and of argillaceous minerals [22—24].

Aristovskaya showed the ability of fungi and *Metallogenium* sp., isolated from samples of Hawaiian bauxite, to accumulate on the surface of cells aluminium, which they extract from aluminoorganic compounds during the mineralization of the latter. Thus, certain microorganisms, whose fossilized remains are found in samples of bauxites of different origin [28], apparently participate in the formation of minerals of aluminium hydroxide and in their concentration.

THE MECHANISMS OF MINERALS DESTRUCTION BY MICROORGANISMS

Specific features of the destruction of silicate minerals and the mechanism of these processes are largely determined by the nature of the minerals and the nature of the microorganisms and their metabolites affecting these minerals.

Table 4

The geochemical behaviour of bacteria in rock

Rock	Biogeochemical processes	Reference
Crust of weathering of Mesozoic-Cenozoic shales (East-Siberian platform)	Pyrite oxidization, formation of sulphuric acid, shale degradation, formation of argillaceous minerals	26
Crusts of weathering of ultrabasites (Central Urals, Cuba)	Ammonium oxidization, formation of nitric acid, leaching of calcium, magnesium, silicium, serpentinite degradation, formation of nontronite and ochres	25
Contact-karst crusts of weathering (Central Urals)	Pyrite oxidization, formation of sulphuric acid, leaching of calcium, magnesium, karst formation	25
Zone of weathering of coaly-aleurolite shales (Eastern Siberia)	Pyrite oxidization, formation of sulphuric acid, shale degradation	27
Zone of weathering of spodumene and pegmatites (Eastern Siberia)	Oxidization of ammonium and pyrite, formation of nitric and sulphuric acids, oxidization of organic compounds, complexation, leaching of lithium, aluminium, iron and silicium, formation of argillaceous minerals	27
Sedimentary rock (Tatra mts., Alps)	Ammonium and pyrite oxidization, formation of nitric and sulphuric acids, oxidization of organic compounds, degradation of carbonates, aluminosilicates, karst formation	30, 31

The data obtained during experimental investigations by various researchers of the degradation of silicate and aluminosilicate minerals under the effect of microorganisms, are summarized in Table 5.

Table 5

The destruction of silicate minerals by microorganisms

Minerals	Microorganisms	Active metabolites	References
Nesosilicates			
Datolite	*Pseudomonas* sp.	2-ketogluconic acid	48
Olivine	Fungi	Organic acids	49
Inosilicates			
Augite, bustamite, diopside, enstatite, hypersthene, pectolite, rhodonite, wollastonite	Heterotrophic bacteria	2-ketogluconic acid	48
Spodumene	Fungi	Organic acids	47
	B. mucilaginosus	Organic acids, exopolysaccharides	46
Phyllosilicates			
Apophyllite	Fungi	Organic acids	49
Biotite	Heterotrophic bacteria	Organic acids	41, 51, 52
	T. thiooxidans	Sulphuric acid	
	B. mucilaginosus	Organic acids	50, 53—55
	B. mucilaginosus, *B. megaterium*	Organic acids	56
	Az. chroococcum, *Bacterium* sp.	Mucous matter	57
Kaolinite	*B. mucilagenosus*	Exopolysaccharides	58
Nacrite	Diatom algae	Exopolysaccharides	59
Bentonite	*Mycobacterium* sp.	Metabolite complex	55
Muscovite	*B. mucilaginosus*	Organic acids, exopolysaccharides	54, 60, 61
	Heterotrophic bacteria	2-ketogluconic acid	48
	Fungi	Organic acids	49, 53, 62
Phlogopite	Heterotrophic bacteria	2-ketogluconic acid	48
	Fungi	Organic acids	49, 62
	B. mucilaginosus	Organic acids, exopolysaccharides	54

Table 5, continued

Minerals	Microorganisms	Active metabolites	References
Saponite	Heterotrophic bacteria	2-ketogluconic acid	48
	Fungi	Organic acids	49
Serpentine	Heterotrophic bacteria	Organic acids	25, 48
	Nitrosomonas sp., *Nitrobacter* sp.	HNO_2 HNO_3	25
Talc, vermiculite	*Erwinia* sp., *Bacillus* sp., *Pseudomonas* sp.	2-ketogluconic acid	48
	Fungi	Organic acids	49
Tectosilicates			
Albite	*B. mucilaginosus*, *B. oligonitrophilus*, *B. salivarius*	Organic acids	61
Feldspar	Heterotrophic bacteria		52, 63
Heulandite	Fungi	Organic acids	49
Leucite	Thionic bacteria	H_2SO_4	51
	Heterotrophic bacteria	Organic acids	41, 52
	Scopulariopsis breyicaule, *Penicillium expansum*, *Aspergillus niger*	Organic acids	64
Microcline	*A. niger*,	Organic acids	51, 65
	Bacillus sp.		57
	Mycobacterium sp. 120		55
Natrolite	Heterotrophic bacteria	Organic acids	48
	Fungi	Organic acids	49
Nepheline	*Mycobacterium* sp.	Alkalifying of medium	66
	Penicillium notatum	Organic acids	67
	Fungi	Organic acids	49
	Heterotrophic bacteria	2-ketogluconic acid	48
	Thionic bacteria	H_2SO_4	41
Oligoclase	*Aspergillus* sp. *Penicillium* sp.	Organic acids	65

Table 5, continued

Minerals	Microorganisms	Active metabolites	References
Orthoclase	Heterotrophic bacteria	2-ketogluconic acid	48
	Aspergillus niger	Oxalic acid	69
Plagioclase	*Pseudomonas* sp.	Organic acids, mucous matter	67
Quartz	*Sarcina ureae*	Alkalifying of medium	68
	Photosynthesizing microorganisms	Change in pH of medium	70
	Proteus mirabilis	Exempted from test	71
	B. mucilaginosus	Exempted from test	72

There are known to exist about 500 species of silicate and aluminosilicate minerals.

According to the type of crystalline structure, which forms the basis of silicate minerals, they fall into the following main groups: nesosilicates (separate silico-oxygenous tetrahedrons are linked together through cations of metals), inosilicates (formed by single or double chains of silico-oxygenous tetrahedrons linked together in the chain through a siloxane bond), phyllosilicates (two-dimensional structures of silico-oxygenous tetrahedrons linked with each other), tectosilicates (three-dimensional structures formed by silico-oxygenous tetrahedrons linked together through atoms of oxygen). In aluminosilicate minerals part of the silico-oxygenous tetrahedrons in the crystalline structure are replaced by alumino-oxygenous tetrahedrons. Hence, in aluminosilicates along with a siloxane bond (Si-O-Si) there is an alumino-oxygenous bond (Al-O-Si), which is weaker than a siloxane bond [72, 73].

According to state of the art concept, chemical weathering of silicate minerals leads to the destruction of their crystalline structure through the reaction of hydrolysis, which involves the ions H^+, OH^+, H_3O^+. In its general form the process of the destruction of heterogeneous (in composition) silicate and aluminosilicate minerals is described in Equation 1.

$$M^+ \text{ mineral}^- + H^+OH^- \rightarrow H^+ \text{ mineral}^- + M^+OH \qquad (1)$$

where: M^+ mineral$^-$ is the original mineral, H^+ mineral$^-$ is the weathered mineral, and M^+ are cations of metals that are present in the mineral [74].

All the processes affecting the concentration and state of reacting compounds and products of reaction will affect the rate of the weathering processes.

The action mechanisms of chemical weathering of silicate rock and minerals under the influence of various chemical compounds, among them organic and mineral acids, are considered in a number of reviews and monographs [73—78].

The data presented in Table 5 indicate that various microorganisms relating to different systematic groups participate in the degradation of the basic types of silicate minerals, thus stimulating the release of metal cations and the destruction of the siloxane and alumino-oxygenous bonds which constitute the basis of the crystalline structure of these minerals.

Microorganisms-producers of mineral and organic acids affect minerals, thus increasing the concentration of ions H^+ in the medium, which, according to Equation 1, activates the reaction of hydrolysis and the release of metal cations [32, 46, 47, 52, 67, 68 et al.].

A siloxane bond is broken under the impact of biogenic processes of alkali formation. Aristovskaya et al. observed an increased release of silicon from quartz as a result of the alkalification of the medium brought about through the development of *Sarcina ureae* [68]. The activity of photosynthesizing microorganisms can also accelerate the degradation of quartz, since the pH of the medium rises with the absorption by cells of CO_2 [70]. In the cultures of these organisms in the absence of illumination, the photosynthetic assimilation of CO_2 ceases and the pH starts to change towards acid values unfavourable for the degradation of a siloxane bond.

Metabolites of microorganisms — amino acids, di- and tricarboxilic acids, oxyacids and other compounds — participate in the destruction of silicate rock and minerals, forming soluble complexes with cations of metals and elements that make up the crystalline structure of the minerals. The role played by the processes of complexation in weathering was noted by Schatz and other authors [79—82].

Strong soluble complexes of the chelate type, during the weathering of silicates, form lichen acids [79—81], oxyacids of bacterial origin [48]; organic complexes with Fe, Al, Ca, Mg are formed as a result of the effect of bacteria on muscovite [55, 61]. Fungi — producers of organic acids — are active weathering agents. Organic acids formed by fungi have been discovered to form complexes with Fe 2—5 times faster than lichen acids do [83].

The release of Li, Si, Al and Fe from spodumene, pegmatites and shales increased by 1.4—1.7, 2.7—4, 5—8.7, 4—18 times respectively, under the impact of organic acids formed by a mixed culture of fungi [47].

In the process of weathering through complexation, the order of the release of elements and their concentrations in solution depend

on the nature of the mineral, the nature of the elements and the nature of the complexing agent.

Organic acids affect the order in which the elements are released from argillaceous minerals, as well as the ratio of Si solubilized to Al and Fe in the solution [84]. The weathering of argillaceous minerals grew with the increase in the complexation capacity of the organic acids used [85]. Being the most active of organic acids, citric acid releases Al and Ca from Ca-plagioclase [86].

A study of the IR-spectra of humic acids and fulvoacids after their interaction with argillaceous minerals has shown that the above mentioned compounds form strong complexes with Si and Al [87].

The formation of strong complexes of organic compounds and elements occurring in minerals stimulates the progress of hydrolysis of minerals and the migration of released elements in the form of soluble compounds. The available data on the formation of silico-organic complexes indicate that the processes of complexation may play a role in the breaking of the siloxane bond in silicates [88, 89].

Microorganisms can activate the processes of mineral degradation, accumulating in the cells and consequently releasing from reactive medium the products of hydrolysis — cations of metals, in particular those which they use as elements of mineral nutrition. It has been shown that in the course of its growth on medium containing biotite, muscovite and microcline, *A. niger* accumulated K in the mycelium [53], releasing it from the nutrient medium and shifting the balance of Reaction 1 towards the dissolution and release of K. A similar effect was produced during the action of *A. niger* on the minerals orthoclase and oligoclase [65].

The importance of the defects and imperfections in the crystalline structure of minerals and of the degree of sample crystallinity during the bacterial degradation of silicates has been noted by Yakhontova et al. [72]. Like under sterile chemical weathering [73], the destruction of minerals under the impact of microorganisms progresses more intensively when there are defects in the crystalline structure [72].

One of the possible mechanisms of microorganisms' activity is oxidation of elements with a variable valency, occurring in silicate rock and minerals [78, 90].

By degrading the silicate minerals making up the rock, microorganisms accelerate the release of uranium [91, 92] and titanium [93] from rock containing these elements.

The ability of heterotrophic bacteria *B. mucilaginosus* to degrade silicate and aluminosilicate minerals and to solubilize silicon has been used to develop a technological scheme for the dressing of low-grade bauxites [94, 95].

The resistance of silicate minerals to chemical weathering has been found to increase from nesosilicates to tectosilicates [73, 74, 96]. Since the tests were conducted under varied conditions and different organisms were used, the data obtained by various researchers (Table 5) when studying the microbiological degradation of silicate minerals,

are difficult to compare in their quantitative aspect. Nevertheless, one common feature is the intensification of the processes of mineral degradation in the presence of microorganisms, as opposed to sterile weathering in the same conditions. As to the resistance of some groups of minerals to the effect of microorganisms, the general trend persists, i.e. the resistance increases with the complication of the crystalline structure of the minerals [78].

CONCLUSION

The observed selectivity in the effect of heterotrophic microorganisms, determined both by the nature of the metabolites they form and by the chemical composition and crystalline structure of rock and minerals subjected to microbiological degradation, served as a basis in the development of technological schemes for the dressing of low-grade bauxites [94, 95]; a microbiological method has been worked out to leach gold using heterotrophic microorganisms [97, 98]; it has been shown that fungi and heterotrophic bacteria can be used to de-iron kaolin, quartz sand and to extract aluminium from clays [99].

Thus the following trends can be singled out in the utilization of heterotrophic microorganisms in the processing of silicate raw materials: dressing of low-grade ores; solubilization and accumulation of precious elements, among them rare, scattered and radioactive elements. These processes will make it possible to process low-grade ores, mining dumps, etc. Since heterotrophic microorganisms demand organic matter, the question of obtaining cheap sources of such matter is in order.

A study into the effect of various groups of microorganisms on silicate rock and minerals, the determination of the nature of the on silicate rock and minerals, the determination of the nature of the activity by different species and the metabolites they form, the development of active cultures and associations of microorganisms will all facilitate the solution of the technological aspects of the problem of microorganisms' utilization in the processing of silicate minerals and will make possible the processing of low-grade ores. It will also raise the efficiency of the traditional methods of silicate ore processing and, in the long run, will stimulate the expansion of the mineral raw material base.

REFERENCES

1. Winogradsky, S.N., On the Role of Microbes In the General Life Cycle, St. Petersburg (1896) (in Russian).
2. Vernadsky, V.I., Biogeochemical Essays, USSR Academy of Sciences Publishers, Moscow-Leningrad (1940) (in Russian).
3. Tung Khak Mun, and Rogova, O.A., Izvestia AN SSSR, ser. biol., No. 2, 261 (1980) (in Russian).
4. Tung Khak Mun, and Kondratieva, L.M., *Mikrobiologiya*, *49*, 541 (1980).

5. Treub, M., *Ann. Jardin Bot. Buitenzorg*, 7, 213 (1898), in ref. [8].
6. Jackson, T.A., *Pac. Sci.*, *25*, 22 (1971).
7. Ivashov, P.V., In: The Biochemistry of the Hypergenesis Zone, Nauka, Moscow (1978) (in Russian).
8. Brock, T.D. *Oikos*, *24*, 239 (1973).
9. Krasil'nikov, N.A., *Mikrobiologiya*, *18*, 318 (1949).
10. Krasil'nikov, N.A., *Mikrobiologiya*, *18*, 492 (1949).
11. Krasil'nikov, N.A., *Uspekhi sovremennoy biologii*, *41*, No. 2 (1956).
12. Novogrudsky, D.M., Transactions of the Institute of Geography at the USSR Academy of Sciences, 45, 125 (1950) (in Russian).
13. Glazovskaya, M.A., Izvestia AN Kaz. SSR, ser. pochven., No. 6, 79 (1950) (in Russian).
14. Glazovskaya, M.A., Transactions of the Dokuchaev Institute of soil sciences, *34*, 28 (1950) (in Russian).
15. Glazovskaya, M.A., *Priroda*, No. 12, 106 (1952).
16. Glazovskaya, M.A., Transactions of the Institute of Geography at the USSR Academy of Sciences, *49*, 70 (1952) (in Russian).
17. Kalinenko, V.O., In: Transactions of the Pamir-Tajik expedition of the USSR Academy of Sciences, Moscow-Leningrad (1932) (in Russian).
18. Sushkina, N.N., and Tsyurupa, I.G., Microflora and Primary Soil Formation, MGU Publishers, 157 p. (1973) (in Russian).
19. Tauson, V.O. The Great Deeds of Little Creatures AN SSSR Publishers, Moscow (1948) (in Russian).
20. Gromov, V.V., *Mikrobiologiya*, *26*, 52 (1957) (in Russian).
21. Webley, D.M., Henderson, M.E.K., and Taylor, I.F., *J. Soil Sci.*, *14*, 1 (1963).
22. Krumbein, W.E., and Jens, K., *Oecologia*, *50*, 25 (1981).
23. Dorn, R.I., and Oberlander, T.M., *Science*, *213*, 1245 (1981).
24. Taylor-George, S., Palmer, F., Staley, J.T., Borns, D.J., Curtiss, B., and Adams, J.B., *Microb. Ecol.*, *9*, 227 (1983).
25. Bugel'sky, Y.Y., Lebedeva, E.V., and Lyalikova, N.N., In: Crust of Weathering, *16*, 9 (1978) (in Russian).
26. Udodov, P.A., Shamolina, E.P., Korobeinikova, E.P., and Korovina, P.A. In: Problems of the Geochemistry of Underground Waters in the Context of Ore Deposit Prospecting, Tomsk, 102 p. (1984) (in Russian).
27. Karavaiko, G.I., Avakyan, Z.A., Krutsko, V.S., Mel'nikova, E.O., Zhdanov, A.V., and Piskunov, V.P., *Mikrobiologiya*, *48*, 502 (1979).
28. Aristovskaya, T.V., The Microbiology of the Processes of Soil Formation, Nauka, Leningrad, 187 p. (1980) (in Russian).
29. Volkova, L.P., Bulakh, G.A., Danilova, L.A., and Litovchenko, G.N., Izvestia AN SSSR, ser. biol., No. 6 (1975) (in Russian).
30. Smyk, B., and Drzal, M., *Geographia polonica*, *2*, 57 (1964).
31. Smyk, B., and Drzal, M., *Ann. Inst. Pasteur*, *105* 341 (1963).
32. Lyalikova, N.N., and Lebedeva, E.V., In: Recent Progress in Biohydrometallurgy, G. Rossi and A.E. Torma (Eds.), 727 (1983).
33. Beijerinck, M., *Zbl. Bact.*, *2*, (7), 561 (1961).
34. Kleczkowska, J., Norman, A., and Sniesko, S., *Soil Sci.*, *49*, 185 (1940).
35. Nadezhdina, E.D., Auxiliary Minerals of the Traps of the Podkamennaya Tunguska River, USSR Academy of Sciences Publishers, Moscow (1961) (in Russian).
36. Andreyuk, E.I., Rubenchik, L.I., Kozlova, I.A., and Antonovskaya, N.S., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (Ed.), Pushchino-on-Oka, 115 p. (1976) (in Russian).

37. Romanenko, V.I., and Kuznetsov, S.I., *Mikrobiologiya, 45*, 166 (1976).
38. Markhinin, E.K., and Podkletov, N.E., *Geologia i geofizika*, So AN SSSR, No. 12, 21 (1978).
39. Sokolova, N.T., Karyakin, A.V., Efimova, N.F., and Kremneva, M.A., *Geokhimiya*, No. 11, 1687 (1979).
40. Arnold, M., Guillon, J.J., and Zimmermann, J.L., *C. r. Acad. Sci., D 288*, 1111 (1979).
41. Wagner, M., and Schwartz, W., *Z. Allgem. Mikrobiol., 7*, H2 (1967).
42. Savostin, P., *Z. für Pflanzenernährung und Bodenkunde, 132*, 1, 37 (1972).
43. Meadows, P.S., and Anderson, J.D., *J. Mar. Biol. Ass. UK, 48*, 161 (1968).
44. Frankel, L., *J. Sed. Petrol., 47*, 849 (1977).
45. Rades-Rohkohl, E., Fränzle, O., and Hirsch, P., *Microbiol. Ecol., 4*, 189 (1978).
46. Karavaiko, G.I., Krutsko, V.S., Mel'nikova, E.O., Avakyan, Z.A., and Ostroushko, Y.I., *Mikrobiologiya, 49*, 547 (1980).
47. Avakyan, Z.A., Karavaiko, G.I., Mel'nikova, E.O., Krutsko, V.S., and Ostroushko, Y.I., *Mikrobiologiya, 50*, 156 (1981).
48. Duff, R.B., Webley, D.M., and Scott, R.O., *Soil Sci., 95*, 105 (1963).
49. Henderson, M.E.K., and Duff, R.B., *J. Soil Sci., 14*, 236 (1963).
50. Aleksandrov, V.G., Silicate Bacteria, Selkhozgiz, Moscow (1953) (in Russian).
51. Wagner, M., *Z. Allg. Mikrobiol., 6*, 197 (1966).
52. Wagner, M., and Schwartz, W., *Z. Allg. Mikrobiol., 7*, 33 (1967).
53. Eno, C.F., and Reuser, H.W., *Soil Sci., 15*, 155 (1956).
54. Tesic, Z.P., and Todorovic, M.S., *Zemljiste a. Biljka, 8*, 233 (1958).
55. Tsyurupa, I.G., In: Crust of Weathering, Nauka, Moscow, *13*, 3 (1973) (in Russian).
56. Surman, K.I., On Soil Bacteria Similar to the Species *B. mucilaginosus* n.sp. (on "silicate bacteria"), A dissertation for the scientific degree of Candidate of Biological Sciences, Leningrad (1962) [in ref. 55].
57. Novorossova, L.E., Remezov, N.P., and Sushkina, N.N., DAN SSSR, *58*, 655 (1947) (in Russian).
58. Lubarskaya, G.A., Kirikilitsa, S.I., Andreyev, P.I., and Mochalov, A.Ya., *Geologicheskiy zhurnal, 41*, 90 (1981).
59. Vinogradov, A.P., and Boychenko, E.A., DAN SSSR, *34*, No. 4 (1942) (in Russian).
60. Aleksandrov, V.G., Ternovskaya, M.I., and Blagodir, R.N., Vestnik s.-kh. nauki, *12*, 39 (1967) (in Russian).
61. Antipov-Karataev, I.N., Tsyurupa, I.G., and Alferova, V.A., In: Crust of Weathering, Nauka, Moscow, 7, 53 (1966) (in Russian).
62. Weed, S.B., Davey, C.B., and Cook, M.G., *Soil Sci., 33*, 702 (1969).
63. Oberlies, F., and Pohlman, G., *Naturwissenschaften, 45*, 513 (1958).
64. Rossi, G., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York-San Francisco-London, 297 (1978).
65. Müller, G., and Forster, I., *Zentralbl. Bakteriol. Parasitenknd., Infeksionskr. Hyg. II Abt., 166*, 372 (1963).
66. Kutuzova, R.S., *Pochvovedeniye*, No. 2, 135 (1973).
67. Aristovskaya, T.V., and Kutuzova, R.S., *Pochvovedeniye, 12*, 1653 (1968).
68. Aristovskaya, T.V., Daragan, A.Yu., Zykina, L.V., and Kutuzova, R.S., *Pochvovedeniye, 9*, 95 (1969).
69. Leleu, M., Sarcia, C., and Goni, J., In: Actes de 6e Congres International de Geochimie Organique, Reuil-Malmaison, France, *905* (1973).

70. Oppenheimer, C.H., and Master, M., *Z. Allg. Microbiol.*, *5*, 48 (1965).
71. Lauwers, A.M., and Heinen, W., *Arch. Microbiol.*, *95*, 67 (1974).
72. Yakhontova, L.K., Nesterovitch, L.G., Lubarskaya, G.A., Andreev, P.I., Pyzhov, V.Kh., and Blinova, G.K., *Mineralogicheskiy zhurnal*, *5*, 28 (1983).
73. Matveyeva, L.A., In: Crust of Weathering, Nauka, Moscow, *14*, 227 (1974) (in Russian).
74. Loughnan, F.C., Chemical Weathering of the Silicate Minerals, Elsevier, New York, 154 (1969).
75. Keller, W.D., In: The Geochemistry of Lithogenesis, Inostrannaya Literatura, 85 p. (1963) (in Russian).
76. Pedro, G., Experimental Studies into the Geochemical Weathering of Crystalline Rock, Mir, Moscow, 252 p. (1971) (in Russian).
77. Matveyeva, L.A., Sokolova, E.I., and Rozhdestvenskaya, Z.S., Experimental Study of Aluminium Release in the Hypergenesis Zone, Nauka, Moscow, 167 p. (1974) (in Russian).
78. Silverman, M.P., In: Biogeochemical Cycling of Mineral-forming Elements, P.A. Trudinger and D.J. Swaine, (Eds.), Elsevier, Amsterdam — Oxford — New York, 445 (1979).
79. Schatz, A., Cheronis, N.D., Schatz, V., and Trelawney, G.S., *Pa. Acad. Sci. Proc.*, *28*, 44 (1954).
80. Schatz, A., Schatz, V., and Martin, J.J., *Geol. Soc. Am. Bull.*, *68*, 1792 (1957).
81. Schatz, A., Schatz, V., Trelawney, G.S., and Bath, K., *Pa. Acad. Sci. Proc.*, *30*, 62 (1956).
82. Berthelin, J., and Dommerges, Y., *Rev. Ecol. Biol. Sol.*, *9*, 397 (1972).
83. Williams, M.E., and Rudolph, E.D., *Mycologia*, *66*, 648 (1974).
84. Huang, W.H., and Keller, W.D., *Am. Minerol.*, *55*, 2076 (1970).
85. Huang, W.H., and Keller, W.D., *Am. Minerol.*, *56*, 1082 (1971).
86. Huang, W.H., and Keller, W.D., *Clays Clay Miner.*, *20*, 69 (1972).
87. Tan, K.H., *Soil Sci.*, *120*, 188 (1975).
88. Kononova, M.M., Aleksandrova, I.V., and Titova, N.A., *Pochvovedeniye*, No. 10, 1 (1964).
89. Bartels, H., and Erlenmeyer, H., *Helvetica Chem. Acta*, *47*, 7 (1964).
90. Ehrlich, H.L., In: Soil Biochem., A.D. MacLaren and J. Scujins (Eds.), *2*, 361 (1971).
91. Magne, R., Berthelin, J., and Dommerges, Y., *C.r. Acad. Sci.*, Paris, *D276*, 2625 (1973).
92. Ebner, H.G., and Schwartz, W., *Z. Allg. Mikrobiol.*, *14*, 93 (1974).
93. Mehta, A.P., Torma, A.E., and Murr, L.E., *Biotechnol. Bioeng.*, *21*, 875 (1979).
94. Andreyev, P.I., Shavlo, P.A., and Lyubarskaya, G.A., *Obogascheniye rud*, No. 2, 11 (1977).
95. Andreyev, P.I., Lycheva, L.V., and Segodina, V.Y., Izvestia vuzov, ser. Tsvetnaya metallurgiya, No. 5, 7 (1979).
96. Goldich, S.S.A., *J. Geol.*, *46*, 17 (1938).
97. Pares, Y., and Cuper, J., *Ann. Inst. Pasteur*, *107*, 568 (1964).
98. Korobushkina, E.D., Chernyak, A.S., and Mineev, G.G., *Mikrobiologiya*, *43*, 49 (1974).
99. Grudev, S., Genchev, F., Mochev, D., and Gaidarzhiev, S., In: 30 Years of the Higher Mining-Geological Inst., Sofia, 71 (1983) (in Bulgarian).

PART III

PHYSICOCHEMICAL PRINCIPLES AND MECHANISM OF BACTERIAL OXIDATION OF SULFIDE MINERALS

MECHANISM AND KINETICS OF BACTERIAL OXIDATION OF SULPHIDE MINERALS

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INTRODUCTION

The mechanism of bacterial oxidation of sulphide minerals is rather complicated and until now has not been studied at sufficient length. Although there exist different views on the matter, they fail to encompass the entire diversity of the processes going on when living organisms interact with a solid inorganic substrate. Nevertheless, interaction between bacterial cells and mineral particles constitutes a fundamental issue in the theory and technology of sulphide mineral leaching. A study of the interaction between microorganisms and the surface of minerals is essential for the understanding of the mechanism of their oxidation and for the intensification of the leaching process.

DESTRUCTION OF THE CRYSTALLINE STRUCTURE OF SULPHIDE MINERALS

T. ferrooxidans are known to oxidize sulphide minerals to water-soluble sulphates either directly of indirectly [1, 2]. In the case of direct oxidation, the destruction of sulphide mineral lattice is aided by enzyme systems of bacteria. Indirect oxidation of sulphide minerals is assisted by ferric iron, a product of ferrous iron and iron-containing sulphide minerals oxidized by bacteria.

For example, let us consider the chemistry of pyrite biological oxidation proceeding via the most probable reaction:

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4 \qquad (1)$$

The above reaction illustrates the direct bacterial oxidation of pyrite. The resultant ferric sulphate, in turn, oxidizes pyrite, forming ferrous sulphate and elemental sulphur:

$$FeS_2 + 7Fe_2(SO_4)_3 + 8H_2O \rightarrow 15FeSO_4 + 8H_2SO_4 \quad (2)$$

$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2S \quad (3)$$

Ferrous iron and sulphur undergo bacterial oxidation:

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \quad (4)$$

$$2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad (5)$$

This way of pyrite oxidation is regarded as the indirect one. The direct mechanism of sulphide mineral oxidation by bacteria, when the minerals contain no iron, has been proved in the case of chalcocite (Cu_2S) oxidation [3]. Here, Cu^{1+} and S^{2-} are oxidized by enzymes to Cu^{2+}, and S^0 and SO_4^{2-}, respectively. What is not clear, however, is the mechanism underlying the phenomena occurring when bacterial cells and the surface of a sulphide mineral interact, nor the mechanism of sulphide mineral lattice destruction.

The first stage of *T. ferrooxidans*' and other bacteria's interaction with a sulphide mineral consists in their adsorption on the surface, whereupon the substrate being oxidized is attacked biochemically.

Microorganisms are adsorbed on the surface of minerals owing to such properties of cells as stickiness, adhesiveness, etc. The true background of this phenomenon is not clear yet. Krishnamurti and Sowan [4] investigated the problem and found the adsorption of microorganisms to be similar to that of colloids. They obtained an S-shaped isotherm of microorganism adsorption which does not contradict the adsorption equation of Krishnamurti deduced for the adsorption of molecules and ions. The adsorptive character of this phenomenon is further proved by the growing number of adsorbed microorganisms as the temperature decreases.

Several hypotheses have been advanced regarding the origins of adsorption forces. Some researchers [5, 6] tend to emphasize electrostatic forces, claiming that adsorption is an interaction of two surfaces carrying opposite charges. However, in the case of microorganism adsorption, it is mostly negatively charged surfaces that interact. Notwithstanding the aggregate negative charge, some areas of the cells may carry a positive charge in view of the high heterogeneity of their surface, which also features complex formations of proteins, lipids and polysaccharides, along with hydrophilic and hydrophobic areas and diverse functional groups. These are important not only for bacterial adhesion to the substrate being oxidized but also for the formation of complexes with the lattice ions.

Thus, bacterial leaching of metals from sulphide minerals may be said to involve a process of bacterial adsorption on the mineral surface conditioned both physically, by the forces of molecular interaction, and chemically, by the formation of chemical bond between the cell, and its surface formations, and the elements of the mineral's lattice.

A study of the mineral surface and of the chemistry of oxidation processes helps to better understand the mechanism of bacterial-chemical oxidation of sulphide minerals.

We conducted an X-ray study of an arsenopyrite sample before and after bacterial oxidation. The sample's lattice parameters were found to be in full conformity with standard values. Chemical oxidation of arsenopyrite with ferric sulphate leads to the formation of goethite or hydro-goethite ($HFeO_2$; $HFeO_2 \cdot nH_2O$) accounting for 30—40 % of all surface compounds. After chemical oxidation the surface of arsenopyrite features up to 10 % elemental sulphur of rhombic syngony. Goethite is a product of ferric hydrate conversion to a more stable condition.

In the case of arsenopyrite bacterial oxidation, its surface also contains goethite (appr. 30 %), but the quantity of elemental sulphur is much greater, reaching 50 %.

X-ray diffraction patterns of solid products formed after bacterial oxidation of arsenopyrite and sphalerite, and isolated from the solution, show that their lattices are absolutely identical, although they differ from the standard modifications of elemental sulphur. The lattice parameters of such sulphur are similar to those of the sulphur taken from a polished section of arsenopyrite specimen which had been subjected to bacterial oxidation.

The results of chemical analysis of arsenopyrite oxidation products also indicate that they essentially consist of elemental sulphur.

The fact that such reactions do take place when chalcopyrite and chalcocite are being oxidized in an iron chloride solution, is mentioned by a number of researchers [7, 8], all of them noting the formation of elemental sulphur on the mineral surface. Thus, the interaction of microorganisms with the mineral surface, when in direct contact, is above all due to the interaction with solid products of a sulphide surface oxidation. As long as elemental sulphur is the only solid product of oxidation of all sulphide minerals without exception, the interaction of microorganisms with the mineral surface largely seems to take the form of their interaction with elemental sulphur.

Corrans [9], in particular, underlines the possibility of bacterial effect on a sulphide (covellite) through dissolution on its surface of elemental sulphur, produced as a result of this mineral's oxidation in a bacterial solution, and a depolarized cathode reaction.

In view of the foregoing, we suppose that in studying the mechanism of sulphide mineral bacterial oxidation, it is important to analyse the basic thermodynamic regularities peculiar to the process of sulphide mineral oxidation. Pourbet diagrams can be used for the purpose

because they describe the "element-water" condition with different values of pH and redox potential. On the basis of the diagrams plotted for sulphide minerals, it is possible to judge the condition of the mineral surface at different values of pH, of the medium redox potential and of the mineral's electrochemical potential. Therefore, a galvanic cell may serve as an experimental model of a sulphide oxidation process: a sulphide immersed in the electrolyte functions as an electrode, while the oxidation processes are measured by the electromotive force, produced at the interface "mineral-solution". The φ value, then, can be determined as the value of the sulphide steady state electrode potential in a particular solution. The sign and magnitude of the electrochemical potential reflect the redox processes going on in the near-electrode layer and indicate the kind of chemical reactions that occur on the mineral surface as a result of its contact with the bacterial culture.

We made thermodynamic calculations and plotted φ-pH diagrams for the systems "iron-water", "sulphur-water", "arsenic-water" as well as thermodynamic stability diagrams for chalcopyrite, sphalerite and arsenopyrite, and for the products of their oxidation in water. Also, the system "sulphide mineral — bacterial culture" was subjected to electrochemical measurements which made it possible to assess as a whole the electrochemical phenomena taking place in the course of sulphide mineral bacterial oxidation (Figs 1, 2, 3).

In this connection, electrode potentials of arsenopyrite, chalcopyrite and sphalerite were determined in a 0.1 N solution of potassium chloride depending on the pH value, as well as in 9K medium with and without bacteria.

Analysis of the diagrams shows that *T. ferrooxidans* develop in the region where stable products of sulphide mineral oxidation exist and sulphides display thermodynamic instability. However, since in the absence of oxidants, the sulphides of nonferrous metals are insoluble in the water, their free oxidation energy is used by *T. ferrooxidans* and some other bacteria.

The findings of the thermodynamics analysis were further verified when we studied the behaviour of iron, arsenic, and sulphur in the course of arsenopyrite bacterial oxidation.

The structural formula of arsenopyrite is $Fe^{3+}[As^{2-}S^{-}]^{3-}$. When arsenopyrite is oxidized in the presence of *T. ferrooxidans*, ferric iron is continually accumulated in the solution and in the deposit; conversely, the absence of bacteria prompts the accumulation of ferrous iron in the solution. Increased ferric iron content of the solution is due to the oxidation of ferrous to ferric iron by bacteria, accompanied by the alkalization of the medium:

$$2FeSO_4 + H_2SO_4 + 0.5O_2 \rightarrow Fe_2(SO_4)_3 + H_2O \qquad (6)$$

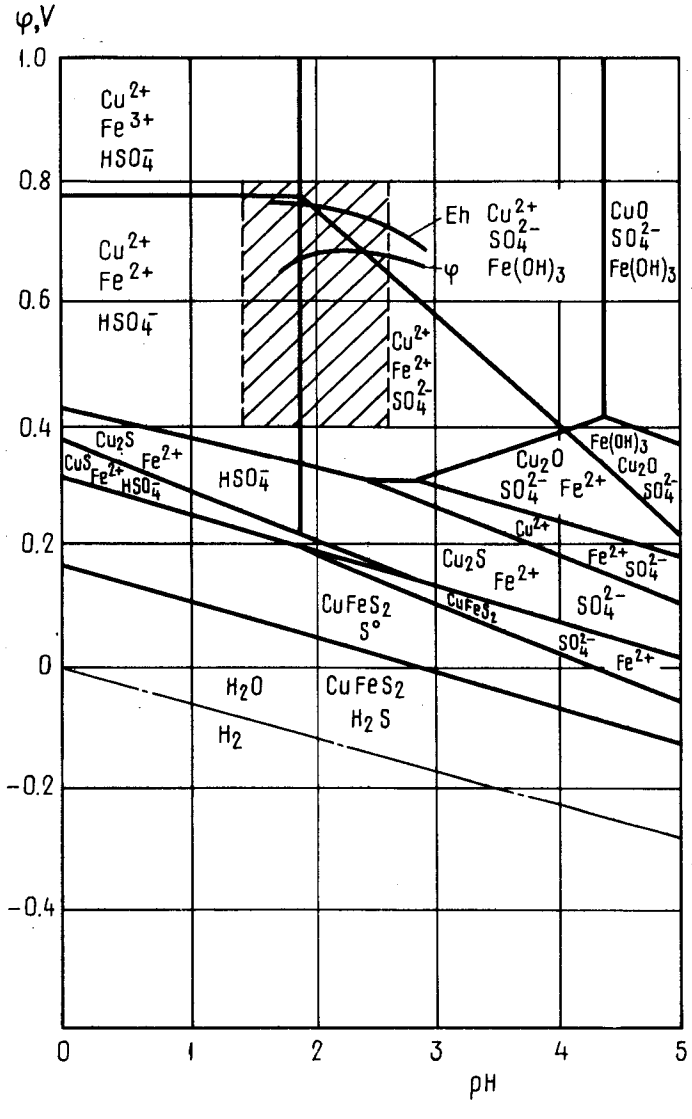

Fig. 1. Diagrams of thermodynamic stability of chalcopyrite and of its oxidation products in water.

Eh — redox potential of the bacterial solution; φ — change in the electrode potential of chalcopyrite relative to normal hydrogen electrode

Upon reaching a certain concentration, ferric sulphate hydrolyzes into ferric hydroxide which precipitates and acidifies the medium:

$$Fe_2(SO_4)_3 + 6H_2O \rightleftarrows 2Fe(OH)_3 + H_2SO_4 \quad (7)$$

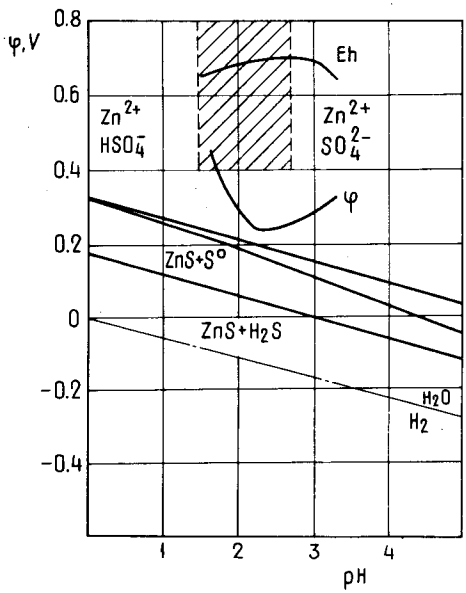

Fig. 2. Diagrams of thermodynamic stability of sphalerite and of its oxidation products in water.

Eh — redox potential of the bacterial solution; φ — change in the electrode potential of sphalerite relative to normal hydrogen electrode

Normally, towards the end of the experiments it accounts for 54 % of the total Fe in the solution and in the precipitate.

Arsenic contained in arsenopyrite is solubilized as a result of leaching in the tri- and pentavalent state in the ratio $As^{3+} : As^{5+} = 5.1 : 1$. In the solution, arsenic is present in the form of arsenious acid, because arsenic exists in the form of a cation only in highly acidified solutions:

$$As^{3+} + 3H_2O = [AsO_3]^{3-} + 6H^+ = H_3AsO_3 + 3H^+ \quad (8)$$

Arsenious acid is oxidized by oxygen to arsenic acid:

$$H_3AsO_3 + 0.5O_2 \rightarrow H_3AsO_4 \quad (9)$$

In the presence of ferric iron in the solution, iron arsenate is formed, which precipitates:

$$Fe^{3+} + H_2AsO_4^- \rightarrow FeAsO_4^- + 2H^+ \quad (10)$$

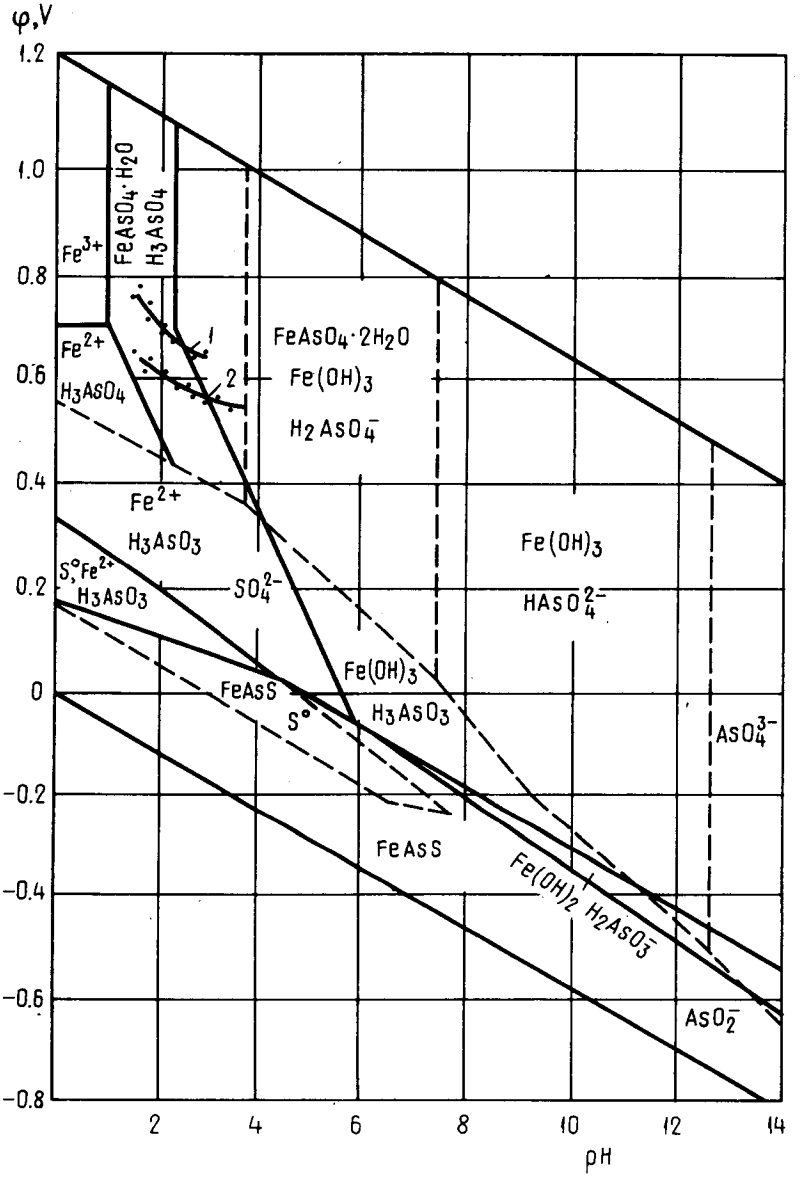

Fig. 3. Diagrams of thermodynamic stability of arsenopyrite and of its oxidation products in water.

Eh — redox potential of the bacterial solution; φ — change in the electrode potential of arsenopyrite relative to normal hydrogen electrode

Precipitation of iron simultaneously with arsenic begins at lower pH values than in the case of Fe^{3+} alone.

As arsenopyrite is being oxidized by bacteria, elemental sulphur accumulates gradually to make up to 60 % of the total sulphur leached by the end of the experiment. Intermediate sulphur compounds are also accumulated (up to 20 %), although towards the end of the experiment their content sharply declines due to oxidation to sulphate sulphur.

The accumulation of elemental sulphur and of intermediate valency sulphur compounds results from chemical and electrochemical oxidation of arsenopyrite, whereas the oxidation of these compounds and of elemental sulphur to the higher valency state is performed by bacteria.

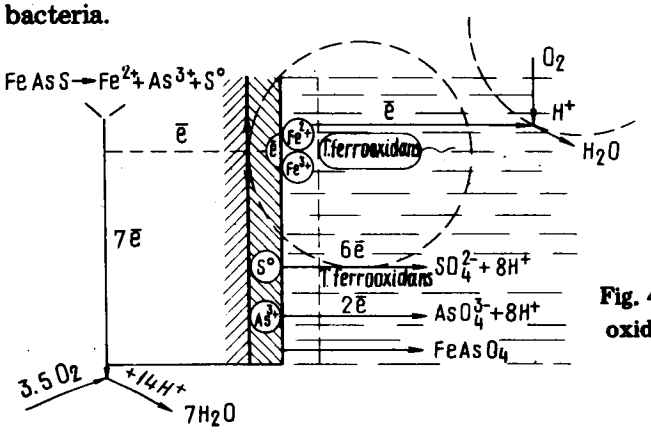

Fig. 4. Model of biological oxidation of arsenopyrite

Thus, the mechanism underlying the process of sulphide mineral oxidation and of their lattice destruction, in the case of arsenopyrite, can be represented as follows (Fig. 4).

Oxidation of arsenopyrite by bacteria takes place concurrent with arsenopyrite interacting with ferric iron which is present in the case of both bacterial and chemical oxidation processes and may be described by the following electrochemical reaction:

$$FeAsS + Fe_2(SO_4)_3 + 1.5H_2O + 0.75O_2 = 3FeSO_4 + S^0 + H_3AsO_3 \quad (11)$$

Ferrous ion, solubilized from the mineral, remains in the diffusion layer, where it is oxidized by microorganisms to the trivalent state.

The reaction of ferrous to ferric iron oxidation, where oxygen functions as an acceptor, may be thermodynamically described by the following equation:

$$4Fe^{2+} = 4Fe^{3+} + 4\bar{e}; \qquad E_0 = 0.771 + 0.091 \log \frac{(Fe^{3+})}{(Fe^{2+})}$$

$$\frac{O_2 + 4H^+ + 4\bar{e} = 2H_2O}{4Fe^{2+} + 4H^+ + O_2 = 4Fe^{3+} + 2H_2O} \quad (12)$$

Those enzymes that serve as biocatalysts of ferrous iron oxidation reactions also catalyze sulphide mineral oxidation reactions.

Oxidation of Fe^{2+} in the diffusion layer, producing Fe^{3+}, a strong sulphide oxidant, in the boundary layer itself, ensures its fast interaction with the mineral. The resultant ferrous iron is re-oxidized by bacteria to the ferric iron and the cycle is repeated.

Sulphide sulphur (S^{2-}) apparently is oxidized bacterially to sulphuric acid. However, most ions (S^{2-}) in the electrochemical cell under study appear to be converted to S^0 in the course of electrochemical destruction of sulphide lattice.

Elemental sulphur, a product of arsenopyrite electrochemical oxidation, is oxidized by microorganisms to sulphuric acid in accordance with this reaction:

$$S^0_{\text{rhombic}} \rightarrow S^0_p \rightarrow SO_3^{2-} \rightarrow SO_4^{2-} \qquad (13)$$

The resultant crystalline phase of arsenopyrite and sphalerite oxidation product is identified by us as elemental sulphur of a very rare type simulating the β-modification of selenium. A similar modification of elemental sulphur was isolated in Portugal from hot sulphur springs where sulphur-oxidizing thermophilic microorganisms may be present [10].

The structure of elemental sulphur was also determined for comparative analysis before and after its treatment by bacteria. The initial elemental sulphur had a pronounced standard orthorhombic structure; however, following bacterial treatment, its structure is identical with that of sulphur after bacterial oxidation of arsenopyrite and sphalerite.

Thus, it should be born in mind that bacterial oxidation of elemental sulphur occurs when its standard rhombic modification is transformed into the β-selenium modification which is oxidized by microorganism to SO_3^{2-} ion, and further to SO_4^{2-} ion. Oxygen serves as an acceptor of electrons during sulphur oxidation and is reduced to H_2O in an acid medium.

Our investigations have shown that the "direct" mechanism of bacterial attack on the crystal lattice of sulphide minerals is based on the electrochemical (corrosion) process intensified by bacteria. The mechanism of electrochemical process intensification on the surface of sulphide minerals has not been fully studied yet. It has been proved, though, that while in contact with a mineral, the bacteria tend to change its electrode potential [11], to depolarize the mineral surface through oxidation of S^0 and Fe^{2+}, and to change Eh of the medium (electrolyte) producing highly oxidizing conditions.

BIOCHEMISTRY OF LATTICE IONS OXIDATION IN THE CASE OF SULPHIDE MINERALS AND ELEMENTAL SULPHUR

At present, the mechanism of oxidation of Fe^{2+}, S^{2-} and S^0 is being studied at all levels of bacterial cell organization. Lundgren and Tano [12] point out that in the course of iron and sulphur ions oxida-

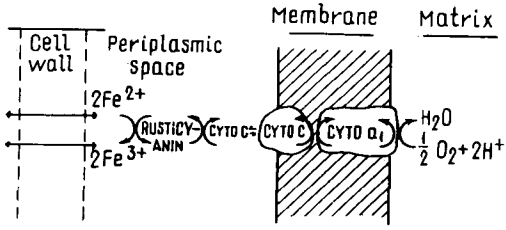

Fig. 5. The proposed pathway of electron transfer in *T. ferrooxidans*

tion by *T. ferrooxidans* the whole of the cell envelope is important, i.e. both the cytoplasmic membrane and the cell wall.

Oxidation of Fe^{2+}. When considering the mechanisms of Fe^{2+} bacterial oxidation we shall deal only with the chemosmotic hypothesis advanced by Ingledew *et al.* [13], which, in our view, provides the most satisfactory explanation of the mechanism of energy production by *T. ferrooxidans.* The earlier hypotheses were discussed in a number of books and reviews [1, 14].

The respiration chain of *T. ferrooxidans* consists of *c*- and *a*-type cytochromes, one of which functions as the final oxidase of a copper-containing protein and a Fe^{3+}-chelating protein. Fig. 5 shows the path of electron transport in *T. ferrooxidans* in the oxidation of Fe^{2+}. At the first stage, Fe^{2+} is transported through the membrane of the cell wall into the periplasmic space where it is accepted by a copper-containing protein, rusticyanin. Cox *et al.* [15] found rusticyanin to be stable at pH 2.0 and to interact with Fe^{2+}. Thus, the copper-containing protein serves as a primary acceptor of Fe^{2+}. The electron then follows the cytochrome chain to reach oxygen. Reduction of O_2 takes place on the interior face of the cytoplasmic membrane.

Ferric iron is removed from the cell with organic compounds, in particular, with protein forming chelate-like complexes [16].

The second reaction relating to energy generation ($2\bar{e} + \frac{1}{2}O_2 + 2H^+ \rightarrow H_2O$) is localized on the interior face of the cytoplasmic membrane.

Fig. 6 shows the diagram of oxidative phosphorylation in *T. ferrooxidans.*

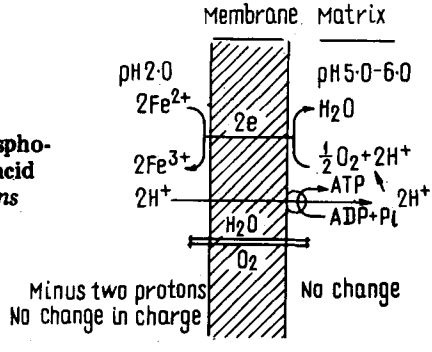

Fig. 6. Scheme for oxidative phosphorylation, at the expense of the acid environment, in *T. ferrooxidans*

The basic concept of energy generation is derived from a thermodynamic analysis of Fe^{2+} oxidation process. It is sufficient to have Eh = 330 mV to oxidize Fe^{2+} and to synthesize one ATP for two transferred electrons. On the interior face of the membrane, where O_2 is reduced, Eh of the $\frac{1}{2}O_2/H_2O$ couple is 890 mV (pH = 5.0—6.0). However, outside the cell (pH = 2.0), Eh of the $\frac{1}{2}O_2/H_2O$ couple equals 1.1 V, i.e. the difference in Eh values is 210 mV. It should be stressed that this membrane potential is essentially due to pH gradient, only 120 mV being provided by the respiration chain.

Now, we have seen that most of the energy in the course of Fe^{2+} oxidation is generated at the level of H^+-ion flow as a result of a chemosmotic ATPase reaction that matches proton input in pH transmembrane gradient prior to ATP synthesis.

According to Apel and Dugan [17], the cells of *T. ferrooxidans* demand H^+ in proportion to the number of electrons formed by Fe^{2+} oxidation. The function of the respiration chain is essentially reduced to providing the flow of electrons for intracellular H^+ and for making possible the reaction of water formation.

Oxidation of S^{2-}. Oxidation of sulphide sulphur (S^{2-}), according to Lundgren and Tano [12], is described as follows:

$$SH^- \xrightarrow{\text{sulphide oxidase}} S + H^+ + 2\bar{e} \qquad (14)$$

$$2S \longrightarrow S-S$$

$$S-S + SH^- \longrightarrow S-S-SH$$

$$S-S-SH + X \longrightarrow X-S-S-SH$$

$$2\,X-S-S-SH \xrightarrow{\text{polysulphide oxidase}} X-S_6-X$$

The effect of sulphide oxidase, in the first place, is manifested in the loss of two electrons and in the sulphur atoms being polymerized. Then, short chains of polysulphide are oxidized to sulphur polymer compounds, the short chains apparently being connected with the membrane. "X" in this system corresponds to the hypothetic group of polysulphide chains bound with the membrane fraction of the cell envelope. Polysulphide oxidase catalyzes the oxidation of polysulphides.

Oxidation of S^0. As noted above, in the course of sulphide mineral oxidation, elemental sulphur is also formed. It can be oxidized practically by all known thionic bacteria in a wide range of the medium pH

values. According to Suzuki [14], the mechanism of sulphur-oxidizing enzyme action may be expressed by the following equations:

$$S_n + GSH \rightarrow GSS_nH \quad \text{and}$$

$$GSS_nH + O_2 + H_2O \xrightarrow{\text{enzymes}} GSS_{n-1}H + SO_3^{2-} + 2H^+, \qquad (15)$$

where GSH — reduced glutathione, S_n — elemental sulphur (S_8).

The sulphur-oxidizing enzyme was identified as oxygenase. The sulphite is further oxidized to sulphate, either through sulphite: cytochrome-*c*-oxidoreductase, or through adenosine-phosphosulphate-reductase.

Just like in the case of S^{2-}, oxidation of S^0 is accompanied with ATP generation. We know little about the early stages of bacterial effect on sulphur and about the mechanism of sulphur transport within the cell. It is established that elemental sulphur is oxidized when it comes in direct contact with the cells of bacteria.

Takakuwa *et al.* [18] studied some aspects of *T. ferrooxidans* cell adhesion to sulphur crystals and showed that the process is determined by pH and may depend on the availability of energy. It has also been shown that thiol groups are essential to the process of adhesion. It may be noted that as early as 1957 Vishnyak and Santer postulated that thiol groups were important for bacterial oxidation of sulphur. Some authors take the view that lipids and phospholipids contribute to the dissolution of sulphur and to its transport to the cells in a colloidal state.

Karavaiko *et al.* [19] used a mass-spectrometry technique to show that in the course of sulphur oxidation it was the fractions of the resultant colloid sulphur and of sulphates that acquired the light sulphur isotope S^{32}. Bacteria prefer to use this isotope as being more active energetically. Simulation experiments also showed that phospholipids, both synthesized by *T. ferrooxidans* and chemically pure reagents (lecithin and kephalin), were capable of dissolving sulphur to form S^{32}-enriched products. It is possible that these species of sulphur are similar to those found in the process of sulphide mineral oxidation that simulate the β-modification of selenium.

Thus, the process of sulphur oxidation by thionic bacteria is a multistage one. At the first stage, lipids and phospholipids produced as a result of microbial synthesis, dissolve sulphur to colloidal state. The sulphur dissolved in lipids is transported to the periplasmic space of the cell. Oxidation of sulphur apparently takes place on the exterior surface of the cytoplasmic membrane or in its invaginates.

KINETICS OF SULPHIDE MINERAL BACTERIAL OXIDATION

As shown above, bacterial enzymes take part in the oxidation of sulphide minerals, sulphur and ferrous iron.

Kinetic equations describing the rate of chemical reactions, are deduced on the strength of the mass action law. This law stipulates that the rate of an elementary process is proportional to the reagent concentration raised to the power equal to the stoichiometric coefficient in the chemical reaction equation. This postulate is applicable both to homogeneous/heterogeneous chemical reactions and to enzyme catalytic reactions, including the process of bacterial leaching.

The rate of matter consumption in a reaction of the n-th order is described as follows:

$$-\frac{ds}{dt} = k \cdot S^n \tag{16}$$

It is obvious that the order of the reaction can be determined from the kinetic curve by taking logs of equation (16):

$$\lg V = \lg k + n \lg S \tag{17}$$

It appears from the latter equation that in the case of simple reactions, the relationship in the coordinates (lgV, lgS) must be a straight line, with the slope tangent being equal to n. By differetiating graphically the kinetic curve of arsenic leaching (Fig. 7) plotted on the results of upscaled tests [20] we can find the values of the reaction rates corresponding to particular arsenic contents of the solid phase S_s. The order of the reaction, determined in terms of coordinates ($-\lg(\mathrm{tg}\ \varphi)$, $-\lg(\theta)$, where tg φ is the rate of arsenic content decline in each Pachuca tank and θ is the fraction of non-oxidized arsenopyrite, is approximately equal to 1. The differential equation of arsenic consumption (S) in the sulphide will be:

$$-\frac{ds}{dt} = kS \tag{18}$$

The integration of this equation on initial condition of $S = S_0$, where $t = 0$, results in this relationship:

$$S = S_0 e^{-kt} \tag{19}$$

$$\ln S = \ln S_0 - kt \tag{20}$$

Using the kinetic curve of the first-order reaction, the reaction rate constant can be determined graphically from the following expressions:

$$\ln([P_\infty] - [P]) = \ln[P_\infty] - kt \tag{21}$$

$$\ln \frac{[P_\infty]}{[P_\infty] - [P]} = kt \tag{22}$$

where [P] is the concentration of leached arsenic at the moment t, P_∞ — concentration of leached arsenic after the reaction is over.

.The kinetic curve of leaching (Fig. 7) shows that the depth of arsenopyrite oxidation reaction and arsenic leaching is limited by the 2.01 g/l value, never dropping to zero in the leached residues. For this reason, in order to calculate the reaction rate constant it is convenient to make use of expression (22) and to linearize the data in the coordinates ($\ln \frac{P_\infty}{P_\infty - P}$, t). Fig. 8 shows that the rate constant (k) of arsenic leaching from the concentrate is equal to the tangent of the angle (slope), at which the straight line is inclined to the X-axis:

$$k = \operatorname{tg} \varphi = 0.0215 \ h^{-1}$$

A simplest scheme used to describe the kinetics of enzymatic reactions is the so-called two-stage scheme:

$$E + S \underset{}{\overset{k_S}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (23)$$

where E — enzyme, S — substrate, k_S — dissociation constant of the enzyme-substrate complex: $k_S = \frac{[E] \cdot [S]}{[ES]}$, k_2 — first-order rate constant of the enzyme-substrate dissociation, accompanied by the formation of reaction products P and enzyme re-generation. In case when the substrate concentration by far exceeds the enzyme concentration which is a normal condition for the study of kinetics of enzymatic reactions, the equation of material balance by enzyme can be of the following form:

$$[E_0] = [E] + [ES] \qquad (24)$$

Combining these equations with the rate of the enzyme-substrate complex dissociation:

$$V = k_2 \cdot [ES] \qquad (25)$$

we can obtain the equation for the enzymatic reaction rate:

$$V = \frac{k_2 \cdot [E_0] \cdot [S]}{k_S + [S]} \qquad (26)$$

In studying the initial rates of the reactions, when the consumption of substrate can be ignored $S = S_0$, we can obtain the basic equation:

$$V = \frac{k_2 \cdot [E_0] \cdot [S_0]}{k_S + [S_0]} \qquad (27)$$

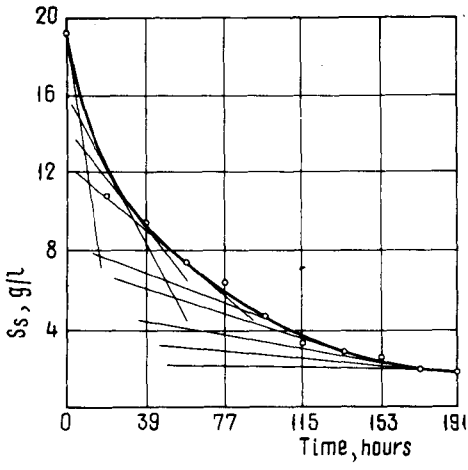

Fig. 7. Graphic differentiation of the kinetic curve representing bacterial leaching of sulphide arsenic

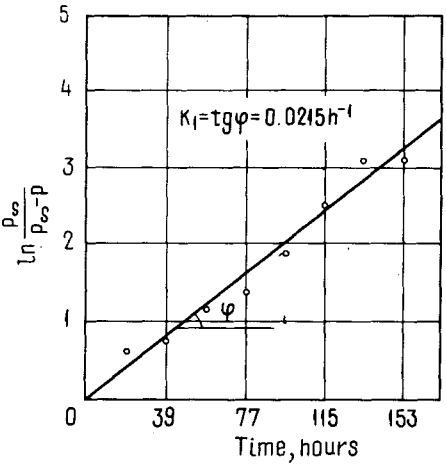

Fig. 8. Determining first-order constant rate of sulphide arsenic bacterial oxidation

Equation (27) describes the relationship between the rate of the enzymatic reaction, according to scheme (23), and the initial substrate concentration, and makes it possible to determine from the experimental data the values of k_2 and k_s constants, that are the most important characteristics of the enzymatic reactions.

Constants k_2 and k_s, normally determined from the experiment, are effective values (they depend on pH of the medium, presence of inhibitors in the system as well as of activators, additional reactions, etc.), otherwise referred to as "catalytic constant" k_{cat} and "apparent Michaelis constant" $k_{m(app)}$, respectively, of a particular enzymatic reaction:

$$V = \frac{k_{cat} \cdot [E_0] \cdot [S_0]}{k_{m(app)} + [S_0]} \qquad (28)$$

This equation is applicable to both homogeneous and heterogeneous catalytic reactions. In the case of enzymatic reactions, it is referred to as the Michaelis-Menten equation. The product $k_{cat} \cdot [E_0]$ is called maximum reaction rate, and is designated as V_m:

$$V_m = k_{cat} \cdot [E_0] \qquad (29)$$

Here, the Michaelis constant in enzymatic reactions in the general case is kinetic and not equilibrium, therefore, k_{cat} and $k_{m(app)}$ are regarded as kinetic parameters of enzymatic reactions.

To determine the kinetic parameters, various techniques of linearizing the enzymatic reaction rate depending on the substrate concentration are employed. Because we only have a complete kinetic curve of reaction product (arsenic) accumulation at our disposal, the kinetic data are processed using an integral form of the kinetic equation of the reaction rate (22, 23).

The Michaelis-Menten equation in a differential form may have the following form:

$$-\frac{d[S]}{dt} = \frac{V_m[S]}{k_{m(app)} + [S]} \tag{30}$$

The kinetic parameters V_m and $k_{m(app)}$ can be determined as follows. The integration of equation (30) with the initial conditions $[S] = [S_0]$ at $t = 0$ produces an equation establishing the relationship between the concentration of the reaction product P and the time:

$$[P] = V_m \cdot t - k_{m(app)} \cdot \ln \frac{[S_0]}{[S_0] - [P]}$$

The equation can be linearized in the Walker-Schmidt coordinates:

$$([P]/t; \frac{1}{t} \ln \frac{[S_0]}{[S_0] - [P]}).$$

The equation

$$\frac{[P]}{t} = V_m - \frac{k_{m(app)}}{t} \ln \frac{[S_0]}{[S_0] - [P]} \tag{31}$$

shows that the straight line has the slope tangent equal to $k_{m(app)}$ and cuts off a section equal to V_m on the ordinate axis.

For purposes of an integral analysis of the kinetics of an enzymatic reaction, of particular interest is the case of competitive inhibition by the reaction product:

$$\begin{gathered} E + S \underset{}{\overset{k_{m(app)}}{\rightleftharpoons}} ES \xrightarrow{k_{(cat)}} E + P \\ E + P \xrightarrow{k_p} EP \end{gathered} \tag{32}$$

where E — enzyme, S — substrate, P — reaction product, k_p — constant of inhibition by reaction product.

Normally, three types of inhibitors are distinguished: competitive, non-competitive, and mixed. If inhibitor I is a chemical analogue of substrate S, incapable of undergoing catalytic transformations, but

occupying an active place in the catalyst molecule (K), two results are possible: either an inactive product KI, or an intermediate compound KS is formed. The overall reaction rate will continue to be determined by the same factors, and, where the formation of KI complex constitutes a reversible process, the introduction of the inhibitor does not affect the maximum reaction rate, although it is of consequence for the effective value of the Michaelis constant. Noncompetitive inhibitors are all those that bring down the maximum rate of reaction without changing the Michaelis constant value. Mixed inhibitors encourage changes in both kinetic constants of the Michaelis-Menten equation.

The kinetic treatment of scheme (32) makes the concentration of the enzymatic reaction product dependent on time:

$$\frac{[P]}{t} = \frac{V_m}{1 - \frac{k_{m(app)}}{k_p}} - \frac{k_{m(app)}\left(1 + \frac{[S_0]}{k_p}\right)}{t\left(1 - \frac{k_{m(app)}}{k_p}\right)} \cdot \ln \frac{[S_0]}{[S_0] - [P]} \qquad (33)$$

The inhibition constant value k_p can be determined, for example, by finding the V_m and $k_{m(app)}$ values of the enzymatic reaction from an independent experiment (i.e. using the initial reaction rates), and by comparing these with the values of effective kinetic parameters obtained from the complete kinetic curve plotted with the inhibiting action of the reaction product [21, 22].

The analysis of the complete kinetic curve in the coordinates (P/t; $\frac{1}{t} \cdot \ln \frac{S_0}{S_0 - P}$) shows (Fig. 9) that the effective value of the maximum reaction rate

$$V_m^e = \frac{V_m}{1 - \frac{k_{m(app)}}{k_p}} \qquad (34)$$

is equal to 0.15 g/l · h, while the effective value of the Michaelis constant $k^e_{m(app)} = -0.25 \times 10^2$ g/l:

$$k_m^e = k_{m(app)} \cdot \frac{1 + \frac{S_0}{k_p}}{1 - \frac{k_{m(app)}}{k_p}}$$

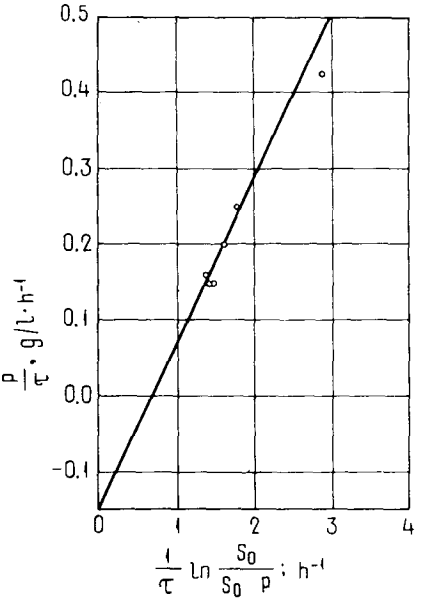

Fig. 9. Determining kinetic parameters of arsenic bacterial leaching process using an integral form of the Michaelis-Menten equation

The effective negative values of the maximum rate V_m^e and of the Michaelis constant k_m^e correspond to the case where $k_p < k_{m(app)}$, i.e. the reaction product has a closer affinity to the enzyme compared with the original substrate, there being a considerable competitive inhibition by the reaction product. The conclusion, that arsenic released into the solution as a result of arsenopyrite oxidation constitutes a competitive inhibitor, is fundamental for the process of bacterial leaching.

CONCLUSION

The study of the mechanism of bacterial oxidation of sulphide minerals is crucial for the solution of technological problems. For example, the problem of selective oxidation of sulphide minerals in tin- and gold-bearing, and copper-zinc concentrates is being successfully resolved now. It has been also shown that, using the technique of enzymatic kinetics in accordance with the above-described methodology, it is possible to obtain basically new data with regard to both the mechanism of the bacterial leaching process and the search for specific ways of process optimization, in particular, by creating conditions favourable for the maximum growth rate of bacteria, or, for example, by creating conditions that would allow changing the kinetic constants k_p, $k_{m(app)}^e$ to the values to ensure the maximum rate of sulphide mineral enzymatic oxidation in the leach concentrate.

We have considered the possibility of applying the methodology of enzymatic kinetics to the description of the process of arsenopyrite

(arsenic sulphide) oxidation. Similarly, it is possible to study the oxidation of sulphides of iron, copper, zinc and other minerals based on enzymatic catalysis.

REFERENCES

1. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.M., Role of Microorganisms in Leaching Metals from Ores, Nauka, Moscow (1972) (in Russian).
2. Adamov, E.V., Panin, V.V., and Pol'kin, S.I., *Science Results*, Dressing of Mineral Materials, VINITI, USSR Academy of Sciences, Moscow, *8*, 5 (1974) (in Russian).
3. Imai, K., Sakaguchi, H., Sugio, T., and Tano, T., *J. Ferment. Technol.*, *51*, 12, 865 (1973).
4. Krishnamurti, K., and Sowan, S.V., *Proc. Indian Academy Sci.*, *34*, B, 2, 81 (1951).
5. Zviagintsev, D.P., Interaction of Microorganisms with Solid Surfaces, Moscow State University, Moscow (1973) (in Russian).
6. Damaskin, B.B., Petrii, O.A., and Batrakov, V.V., Adsorption of Organic Substances on Electrodes, Nauka, Moscow (1968) (in Russian).
7. Yermilov, V.V., Tkachenko, O.B., and Tseft, A.L., In: Proc. Inst. of Metallurgy and Dressing Kaz. SSR Acad. Sci., Alma-Ata, *3* (1963) (in Russian).
8. Tkachenko, O.B., and Tseft, A.L., In: Proc. Inst. of Metallurgy and Dressing Kaz. SSR Acad. Sci., Alma-Ata, *30*, 15 (1969) (in Russian).
9. Corrans, J., Harris, B., and Ralph, B., *J.S. Afr. Inst. Min. Met.*, *72*, 8 (1972).
10. Ctarrido, J., and Assuncao, C., Tables dans la determination des mineraux au moyen des rayons X., Lisabonne (1953).
11. Yakhontova, L.K., Sergeev, V.M., Karavaiko, G.I., and Soukhantseva, V.S., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (Ed.), Pushchino-na-Oke, 99 (1976) (in Russian).
12. Lundgren, D.G., and Tano, T., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York, 152 (1978).
13. Ingledew, W.J., Cox, J.C., and Halling, P.J., *FEMS Microbiol. Letters*, *2*, 193 (1977).
14. Suzuki, I., *Ann. Rev. Microbiol.*, *28*, 85 (1974).
15. Cox, J.C., Aasa, R., and Malmström, B.G., *FEMS Letters*, *93*, 157 (1978).
16. Ingledew, W.J., Cox, J.C., and Halling, P.J., *FEMS Microbiol. Letters*, *2*, 193 (1977).
17. Apel, W.A., and Dugan, P.R., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York, 46 (1978).
18. Takakuwa, S., Fujimori, T., and Iwasaki, H., *J. Gen. Appl. Microbiol.*, *25*, 21 (1979).
19. Karavaiko, G.I., Miller, Yu.M., Kapustin, O.A., and Pivovarova, T.A., *Mikrobiologiya*, *49*, 6, 849 (1980).
20. Yudina, I.N., Kostyukova, O.N., Gorenkov, N.L., Panin, V.V., and Kim, D.Kh., *Tsvetnye metally*, No. 8, 109 (1979).
21. Berezin, I.V., and Klesov, A.A., Practical Course on Chemical and Enzyme Kinetics, Moscow State University (1976) (in Russian).
22. Poltorak, S.M., and Choukhrai, E.S., Physico-Chemical Fundamentals of Enzyme Catalysis, Vysshaya Shkola, Moscow (1971) (in Russian).

THE ROLE OF THE SULPHIDE CONSTITUTION IN THE PROCESS OF THEIR BACTERIAL LEACHING

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The bacterial leaching of sulphide ores is based on the oxidation of sulphide minerals with the participation of thionic bacteria, mainly *Thiobacillus ferrooxidans*, and some thermophilic bacteria in strongly acidic solutions (pH < 3).

Sulphides are semiconducting crystals which undergo destructuring when oxidized. This destructuring is mainly associated with the break of the bonds in their crystalline lattices and the loss of electrons, i.e. with the electronic disbalancing of the lattice. Electrons of oxidation reactions usually enter the sphere of the oxidizer and are utilized for its reduction. This process in aqueous solutions — electrolytes is connected with changing their chemical behaviour and state due to the admittance into the solution of components of the oxidized mineral (cations and anions) and the hydrolysis reactions of the $A^{2+} + 2H_2O \rightarrow A(OH)_2 + 2H^+$ type, being dependent on the pH of the solution.

The oxidizer receives electrons to assume a form which corresponds to the Eh-pH state of the solution. Thus Fe^{3+} ion according to Fe diagram in H_2O at 25 °C in the solution with pH < 3 is reduced according to the following scheme: $Fe^{3+} + \bar{e} \rightarrow Fe^{2+}$. Oxygen, depending on the pH of the aqueous solution, takes part in hydrolysis-reduction reactions $O_2 + H_2O + 4\bar{e} \rightarrow O^{2-} + 2OH^-$ (in acidic environment) and $O_2 + H_2O + 4\bar{e} \rightarrow 3O^{2-} + 2H^+$ (in alkaline environment). A bacterial cell receives electrons from the oxidized substrate and utilizes them in reactions connected with constructive and energy metabolism of the cell.

Every oxidizer transporting electrons creates the oxidizing potential (OP) of the medium (solution). Bacteria possess high oxidative ability which exceeds that of the universal oxidizers occurring in natural waters — Fe^{3+} ion and oxygen. Oxidizing potentials of the media containing *T. ferrooxidans* are about 0.6—0.7 V, which is noticeably higher than the OP of similar abiogenic systems with oxygen or Fe^{3+} ion (0.4—0.5 V on the average). It is clear that bacteria may create environments with rather high oxidizing potentials.

Every sulphide mineral with its peculiar crystalline lattice possesses its own stability during oxidation. For definite conditions sulphides can be placed in a sequence in which every following sulphide is more stable than the previous one. In a sulphate solution with pH=2.5 the stability sequence of most common ore sulphides is as follows: galenite (0.30), chalcocite (0.35), sphalerite (0.35), chalcopyrite (0.40), bismuthine (0.40), stannite (0.45), pyrrhotite (0.45), tetrahedrite (0.45), arsenopyrite (0.50), pentlandite (0.55), pyrite (0.55—0.60) [1—6].

If sulphide electrodes are dipped into the same electrolyte then sharp changes of the potential at the electrode-electrolyte borderline, the so-called sulphide electrode potentials (EP), proportional to the work of electron release from the crystal lattice, are a relative measure of mineral destructuring through oxidation. The above-stated stability sequence shows in parentheses the corresponding EPs (in V) of the minerals in the sequence.

Measuring the dependence of sulphide EP on pH of the solution-electrolyte allows to draw a chemical interpretation of its oxidation, i.e. to derive an oxidation equation. Besides experimental studying of the EP-pH, dependence diagrams of the elements' state in water [1, 7, 8], up-to-date experimental data on the chemical behaviour and state of different elements in aqueous solutions, as well as the investigation of natural oxidation of minerals are employed for such an investigation the results of which are of great importance for the understanding of the chemical aspect of the bacterial destructuring of minerals. At present, oxidation equations are derived for chalcocite, chalcopyrite, bornite [3, 9], galenite and sphalerite [6], bismuthine [2], tetrahedrite [5], arsenopyrite [10, 11], pyrite [4].

The investigation of the EP-pH dependence results in the construction of a plot which is usually juxtaposed with the diagram of the corresponding elements' state (Cu and S for chalcocite; Cu, Fe, S for chalcopyrite; Bi, S for bismuthine, etc.). The EP-pH plot usually represents a broken line, the slopes of which are determined by the ratio of the H^+ ion number to the number of electrons in the products of the studied oxidation reaction. Parts of the EP-pH plot parallel to the EP (Eh) axis correspond to the reactions of non-oxidation type (hydrolysis, exchange, etc.) — electrons are absent in the products of such a reaction. Parts of the plot parallel to the pH axis are characterized by oxidation reactions without free H^+ ions (only electrons!). Finally the slopes of the broken line characterize redox processes with both protons and electrons in products of corresponding reactions. In these cases, according to the well-known Nernst equation

$$Eh(EP) = Eh_0 + \frac{0.059}{n} \ln \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

for the reaction $aA + bB = cC + dD + n\bar{e}$, where [C] or [D] can be H^+ ion activity, the slope of the plot determines the ratio of the number of hydrogen ions to the number of electrons (n) for the oxidation reaction.

Thus the type of mineral oxidation practically cannot be described by one equation: every broken-line segment of the EP-pH plot requires its own equation for the oxidation reaction of the given sulphide. Fig. 1 shows as an example an experimental EP-pH plot for chalcopyrite, as well as the data on the iron, sulphur, and copper state in an aqueous solution. In accordance with different segments of the broken line and different state of the above-mentioned components in the solu-

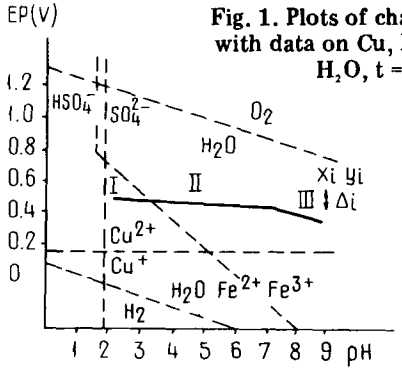

Fig. 1. Plots of chalcopyrite EP-pH with data on Cu, Fe and S state in H_2O, t = 25 °C

tion caused by the change in EP-pH conditions the following oxidation equations have been derived [3]:

$$pH < 3\ CuFeS_2 + 3.25O_2 + 7.5H_2O \rightarrow [CuHSO_4]^+ + [Fe(H_2O)_6]^{2+} + HSO_4^- + H^+ + 3\bar{e};$$

$$pH = (3—7)\ CuFeS_2 + 4O_2 + 6H_2O \rightarrow [Cu(H_2O)_6]^{2+} + SO_4^{2-} + [FeSO_4]^+ + \bar{e};$$

$$pH > 7\ CuFeS_2 + 3.9O_2 + 6.2H_2O \rightarrow [Cu(H_2O)_6]^{2+} + SO_4^{2-} + [FeSO_4]^+ + 0.4H^+ + 1.4\bar{e}$$

The knowledge of the mechanism of sulphide oxidation in acidic sulphate solutions with pH < 3 is of particular importance for the theory and practice of bacterial leaching of sulphides using *T. ferrooxidans* and other acidophilic bacteria. It should be also taken into account that bacteria may achieve more complete oxidation of mineral components than oxygen does. For instance in the solution without bacteria with pH < 3, iron, according to the diagram of Fe state in H_2O, assumes the form of bivalent cation, but in the solution with *T. ferrooxidans* it is trivalent.

High concentration of sulphate solutions (pulps) used during bacterial leaching of sulphides should be also taken into account. In such solutions the cations of the $[CuHSO_4]^+$ and $[FeHSO_4]^{2+}$ — type may be formed and cause the complexing of hydrogen; with this can be linked the pH instability of the solution with a tendency to considerable pH increase which may inhibit the growth of microorganisms (e.g. in case of chalcocite).

As for the corrosion model of bacterial leaching of sulphides, phenomena of the minerals' contact with each other are of great importance. Ores practically always represent polymineral associations.

When submerged in the solution-electrolyte the contacting minerals form galvanic couples, in which the high-potential mineral, cathode, i.e. the oxidizer, stimulates the oxidation of the low-potential (anode) mineral directly proportional to the difference in EP values of the galvanic couple components.

The use of the additional oxidizer (oxygen, Fe^{3+} ion, bacterial cell) in the system of two minerals-solution, complicates the redox processes in it. The mineral of the lowest potential will be subjected to the maximum corrosion. Experiments testify to the fact that extraction of copper with the aid of *T. ferrooxidans* from chalcocite-pyrite and chalcopyrite-pyrite couples increased as compared to monomineral chalcocite and chalcopyrite 4- and 2.5-fold, respectively [10].

As stated above, the basis of the corrosion model of oxidation, biogenic process included, is the notion that the oxidizer stimulates the electrons' release from the crystal lattice of the oxidized mineral, that electrons pass into the sphere of the oxidizer and that they are used for reduction processes. The work of the electron release from the crystal lattice is intimately linked with the peculiarities of the fine constitution of crystals — type of bonds, imperfections, degree of stoichiometry of the chemical composition, isomorphic impurities and their degree of order, i.e. the nature of their distribution in the crystal field and their electric characteristics. At present, this problem is inadequately studied. Experiments are carried out by mineralogists who study the processes of ore oxidation. Revealing the mechanism of chemical oxidation of minerals whose real constitution is well studied, allows to understand the mechanism of their bacterial corrosion as well. We posed this problem in 1974 for the first time [12].

Sulphides are semiconductors in which covalent, more exactly, donor-acceptor type of bonds prevails. These bonds are characterized by the fact that electronic pairs of one atom (sulphur, arsenic) are used in the lattice cell of another atom (iron, nickel, copper, etc.). This results in the formation of molecular orbitals. If electrons with different energy levels couple, the donor-acceptor bond is characterized by the formation of more complex hybrid molecular orbitals between atoms. This is especially characteristic of sulphides whose cations often have incomplete (e.g. up to 18 electrons) subvalent electron shells. Thus an atom of iron has 6 instead of 10 d-electrons, cobalt has 7, nickel has 8. The anion counterpart of these and other atoms in sulphides is sulphur which can be present in these compounds in the form of S^{2-} ion capable of supplying 4 electron pairs to a metallic atom or in the form of S_2^{2-} in which two sulphur atoms combined by a common pair of electrons have six electron pairs and can supply six pairs of electrons to a metallic atom. The sphalerite ZnS composition (Zn has 10 d-electrons) illustrates the first case; pyrite FeS_2 in which iron short of 6 electron pairs and in want of completing the 18-electron shell receives from S_2^{2-} the missing 12 electrons, i.e. 6 pairs, illustrates the second case. In sulphides sulphur is a donor of electrons, metals are an acceptor.

In cases when the metallic atom requires for its stability in a sulphide compound more or fewer electron pairs than sulphur can supply, metals draw together and exchange their own electrons. In the structure of such sulphides the role of metallic bonds characterized by a high collectivization of metallic atom electrons increases. This can be illustrated by the structures of pyrrhotite $Fe_{1}S$ and pentlandite $(Ni, Fe)_9S_8$ [13].

Fe^{2+} ions in the structure of pyrrhotite form an octahedron though S^{2-} ions supply only four electron pairs instead of six to the octahedron. To stabilize iron, Fe-octahedrons form columns in which they contact with their common faces and not with summits as usual. Because of that the acceptor's (sulphur) shortage of electrons is offset by the electrons of iron atoms drawn together. The electron structure of sulphide acquires specific characteristics which change its electric properties (conductivity) and greatly influence the work of electron release from its structure, i.e. oxidizability.

Nickel and part of iron in the pentlandite structure form a triangular pyramid, i.e. they are surrounded by four S^{2-} ions. But nickel for its stability requires five rather than four electron pairs (it has 8 d-electrons), while iron requires even six pairs. Motals escape from this 'uncomfortable' position through combining eight tetrahedrons in volumetric 'stars' with cluster-(converged) type relationship thereby increasing the exchange of electrons. Due to such a position of iron-nickel coordination polyhedrons in the pentlandite electronic structure, like in pyrrhotite, there appear bunches with strengthened metallic bonds and an increased number of free liberated electrons. This results in facilitating the liberation of electrons in sulphide, as in case with pyrrhotite, and thus in reducing the stability during oxidation.

The fundamental description of the electronic constitution of crystals which also determines, as stated above, the mechanism of their oxidation, is given by the band theory of crystals which together with the theory of molecular orbitals, i.e. the concept of the nature of chemical bonds in crystals, forms the basis of the physics of semiconductors, dielectrics (insulators) and metals, essential for the understanding of optical, magnetic and electric properties of crystalline solids.

The band theory in its different aspects is described in numerous special works on the solid-state physics [14, 15, etc.]. Here we mention only those statements of the theory which are necessary for the understanding and explanation of the destructuring of crystalline semiconductors through oxidation processes.

In the simplest case (two-dimensional band model) the establishing of bonds between two atoms of a metal in accordance with its electronic structure results in the formation of electron levels or bands of different energies ($1S^2$, $2S^2$, $2p^{6'}$, etc.). Energy levels of subvalent electron shells if completed up to 8, 18, etc. electron configurations, create filled levels or bands. Near them there are no vacant levels whereto electrons can move. Valence electrons usually form incomple-

tely filled energy bands — near them there are always vacant levels which form the conductivity band. In case of a three-dimensional electron structure model the described two-dimensional bands turn into energy surfaces. Electrons transferred into the conductivity band are liberated (free), i.e. they can move along the crystal field. The transition of electrons into the conductivity band involves energy consumption and crossing the space between the valency band and the conductivity band. This space, a kind of energy gap, is called a forbidden zone or gap. According to the width of the forbidden gap crystals are divided into *metals*, practically without a forbidden gap and with an overlap of the valency band and the conductivity band, *semiconductors*, with a narrow forbidden gap, and *dielectrics* (insulators), with a rather wide forbidden gap, i.e. electrons cannot jump into vacant levels under normal conditions. Sulphide minerals with a donor-acceptor type of bonds are semiconductors. There are narrow-band as well as wide-band minerals among them.

A jump of an electron from the valency band into the conductivity band causes the formation of a hole, in other words, a positive charge carrier, in the ceiling of the valency band. The total energy of electrons and holes of the valency band is one of the most important energy properties of crystals, i.e. the Fermi energy or Fermi level. This level is a band in the crystal, below which all energy levels are occupied and above which all energy levels are free, vacant. To oxidize a semiconductor crystal it is necessary to spend an energy exceeding the Fermi energy.

Semiconductive properties of sulphides are closely connected with their actual chemical composition, isomorphic impurities which cause imperfections, with the degree of stoichiometry of the chemical composition. Any isomorphic impurity, even the closest in its nature to the substituted component in the sulphide structure, is characterized by its own electronic structure. In every case it causes considerable changes in the electronic (band) structure of the host mineral. For example, cobalt replacing iron in pyrite FeS_2 has, in comparison with the latter, 7 rather than 6 d-electrons. In the structure of pyrite this impurity is a donor of electrons. As a result of such a replacement in the forbidden gap of pyrite a donor energy level is formed which would make the forbidden gap narrower and increase the concentration of free electrons in the conductivity band. Nickel behaves similarly, supplying even a larger number of electrons into the conductivity band. Pyrite with isomorphic impurity of cobalt and nickel has an electron type of conductivity and is an n-type semiconductor. The donor electron level in the structure of pyrite is formed due to some excess of iron contained in mineral, i.e. the non-stoichiometry of the composition; pyrite of the $Fe_{>1}S_2$ composition belongs to semiconductors with electron conductivity.

Contrarily, the substitution of arsenic for sulphur ($S^{6+} \rightarrow As^{5+}$) in the same pyrite causes the formation in the energy-band structure

of pyrite of the acceptor electron level which is usually near the ceiling of the valency band. As a result, a hole (an electron shortage) is formed in the valency band. Sulphide with an acceptor isomorphic impurity (less charged) has a hole-type (p-type) conductivity. An acceptor level of electrons in pyrite may also be connected with the non-stoichiometry of its structure — a certain excess of sulphur in the mineral composition, which can be determined when deriving a crystalchemical formula of a sulphide according to the chemical analysis data and the results of an X-ray measuring of the arris of the lattice cell. Isomorphic replacement of iron atoms in pyrite by scandium, titanium, vanadium and chromium also causes the formation of a hole-type conductivity of the mineral. All these elements have a considerable deficiency of d-electrons in comparison with the replaced iron. As stated above, nickel serves as a donor impurity when it replaces iron in sulphides. At the same time Fe^{2+} as an isomorphic impurity in nickel sulphides is an acceptor of electrons because of a different number of d-electrons in the atoms of both elements.

In hole-type semiconductors the forbidden gap is wider, the Fermi level is lower, the work of electron release from the structure is greater as compared with electron-type semiconductors. p-type (hole) semiconductors are more difficult to oxidize. In comparison with electron-type semiconductors, they, as a rule, also have higher values of the electrode potential, i.e. actually the Gibbs thermodynamic potential which describes the energy state of the system. These statements, very important for the theory and practice of the bacterial oxidation of sulphides, were confirmed by our experiments on pyrite and arsenopyrite oxidation with the participation of *T. ferrooxidans* [10, 16].

Experimental investigation of bacterial oxidation of sulphides showed that hole-conductivity minerals as compared with their electron-conductivity varieties (p-type pyrite and n-type pyrite), though they have a higher energy barrier, are actively oxidized by *T. ferrooxidans* for a long period of time. Electron-conductivity analogues easily 'emitting' electrons from the conductivity band rapidly reach the stoichiometry of the composition, sharply increase their electrode potential and become difficult for oxidation, in case of pyrite — even for bacterial oxidation.

Experiments have convincingly shown that bacterial oxidation of sulphides proceeds in accordance with electrochemical and corrosion laws.

It has been stated above that minerals' contact with each other is very important for oxidation, including bacterial oxidation. In view of everything mentioned about semiconductive properties of sulphides it should be noted that in different types of ores, even belonging to the same deposit, pyrite of different types of conductivity can be found with rather different electrode potentials. The pyrite with electron conductivity contacting with other minerals can serve as an anode and can be easily oxidized. The hole-conductivity pyrite

being a cathode in galvanic couples is, vice versa, safely protected against oxidation. In this case it plays the role of the oxidizer and acts as a competitor versus living organisms — bacterial cells. Our experiments showed that a polymorphic analogue of pyrite — marcasite FeS_2 (wide-banded, of a hole conductivity usually) — in couples with a great number of sulphides (chalcopyrite, arsenopyrite, bismuthine, pyrrhotite, etc.) always serves as a 'non-decomposable' cathode, safely protected against oxidation and stimulating destructuring of the components which contact with it.

Let us examine two new examples testifying to the connection between the process of bacterial corrosion of sulphides and properties of their actual composition. They are related to the oxidation of monoclinic pyrrhotite and pentlandite the structures of which were described above.

In pyrrhotite $Fe_{<1}S$ due to the mutual convergence of iron atoms in its structure the role of electronic (metallic) bonds increases, as well as the number of free electrons in the conductivity band, the width of the forbidden gap diminishes. Despite the hole conductivity of this sulphide it facilitates its oxidation, controlled by a very low EP which approximated 0.3 V at pH = 2.5. Experiments showed that the initial stage of the mineral oxidation is intensive and is characterized by solubilization of excess sulphur in incompletely oxidized forms (H_2S and HS^-). This results, besides a hydrogen sulphide contamination of the solution, in a sharp pH increase which causes the inhibition of the life activity of *T. ferrooxidans*. Hence pyrrhotite can be efficiently oxidized only by oxygen. It is also obvious that presence of an easily oxidized pyrrhotite in ores is unfavourable for the life activity of microorganisms.

The second example concerns the oxidation of pentlandite. The experimental results are shown in Fig. 2 in the form of plots of the change in the oxidation potential (OP) of the culture solution and the EP of pentlandite in time (12 days). The initial concentration of *T. ferrooxidans* was 10^5 cells per ml. A control experiment was conducted simultaneously.

The main conclusions on the bacterial oxidation of pentlandite were the following:

1) During oxidation nickel was extracted into the solution with a retarded iron leaching. If in the initial sample the ratio Fe:Ni:S was 1:1:2, it was 1:2:2 in the extracted product.

2) Iron was solubilized in the form of $[FeHSO_4]^+$ complex cation, i.e. it was bivalent, sulphur — in the form of sulphate anions.

3) During the experiment pH value remained at the initial level (2.7) which indicated to the parallel hydrogen complexing and its accumulation in the solution in the form of H^+ ions. The latter follows from the preliminary study of the EP-pH dependence of pentlandite, when in an acid state of the solution (pH < 3) the broken line of the plot of the EP change of sulphide was very steep (—0.050), described

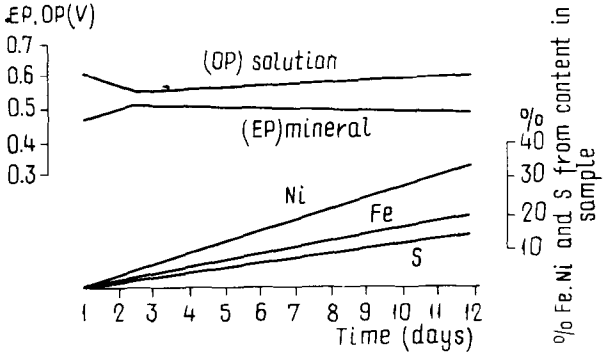

Fig. 2. Curves of change in solution oxidizing potential and pentlandite electrode potential; plots of Ni, Fe and S leaching from sulphide in the process of bacterial oxidation within 12 days

by the following schematic equation of the mineral oxidation (the equation is schematic because it is difficult to take into account the quantitative ratios of different ion forms of Fe, Ni and S in the solution): $Fe_5Ni_4S_8 + 15O_2 + 2H_2O \rightarrow 5Fe^{2+} + 4Ni^{2+} + 8SO_4^{2-} + 4H^+ + 6\bar{e}$.

4) Bacteria grown on pentlandite suspension developed satisfactorily — their ultimate concentration was 10^8 cells/ml.

The obtained data are fully correlated and explained by the peculiarities of the already described crystalchemical constitution of pentlandite. The bacterial stimulation of the oxidation of this sulphide (the structural formula is $(Ni, Fe)_8^{IV}\ Fe^{VI}S_8$) causes a preferential liberation of electrons due to the electronic (metallic) bonds' breakdown in tetrahedron 'stars' of nickel and part of iron, i.e. nickel is solubilized in the first place. The great difference between OP and EP during the first 24 hours of the experiment points to the consumption of liberated electrons from the mineral conductivity band by the bacteria and to the easiness of the mineral oxidation. During this period the first 'tetrahedron' iron was solubilized in the bivalent state. A further breakdown of bonds, liberation of electrons from the valence band of the sulphide structure, oxidation of sulphur and iron — this is a sequence of phenomena which accompany the destructuring of pentlandite. On the eighth day of the experiment the portion of ferric iron in the total balance of iron began to noticeably increase.

Thus, the process and the nature of bacterial oxidation of sulphides which proceeds according to the laws of the electrochemical (corrosion) model depend on the crystalchemical properties of individual minerals and on those paragenetic associations which they form in ores. To prepare ores for bacterial leaching at the sites of mineral deposits a special service should be organized with a task to determine specific technological types of ores for bacterial leaching. The properties of individual minerals (impurities, imperfections, conductivity, types of bonds, composition stoichiometry, etc.) facilitating or retarding the oxidation should be taken into account, as well as mineral associations favourable or unfavourable for the leaching of a compo-

nent. It is also considered necessary to further the experimental study of this problem with the aid of new minerals and their association.

In conclusion, it should be pointed out that the presented data convincingly demonstrate that the interaction between thionic bacteria and sulphides conforms to the laws of redox (electrochemical) processes developing through electron transfer from the inorganic mineral substrate to the bacterial cell. This is accompanied by a breakdown of high covalent chemical bonds in sulphide structures and a synthesis of high covalent organic compounds inside the cell. For living organisms, bacterial oxidation is a vital process developing, for this reason, at a high rate.

A similar conception is derived from the analysis of experimental data concerning destruction of aluminosilicates and quartz using silicate bacteria [17], which also revealed that the nature of bacterial degradation of silicate minerals is controlled by various imperfections in their crystalline constitution (defects, substitution of aluminium for silicon in tetrahedron, isomorphic replacements in the cation portion, structural order, etc.), underlying the degree of chemical bond deterioration in the structure and restructuring of bonds accompanied by electron liberation.

Silicate bacteria, which are heterotrophic microorganisms, develop rather intensively in carbohydrate (organic) media, decomposing them to CO_2 and H_2O. According to the modern conception of the living cell energy mechanism, the latter process represents a biochemical (electrochemical) oxidation [18, 19] whereby electrons released in the restructuring of organic matter chemical bonds, enter the oxidizer system and (in the case of the living cell acting as an oxidizer) are used for synthesizing its biomass in a highly complex reduction process.

There is a probability that in the natural silicate systems (crusts of weathering, soils) silicate minerals (quartz, caolinite, feldspars, etc.) are more directly involved in the vital processes of bacteria present in such systems than is presently thought. At this stage, it is rather difficult to describe the vital activity of silicate bacteria relying on silicate degradation but it might be suggested that in this case the development of the cell is also ultimately based on the process of electron transfer from the inorganic substrate to the microorganism's organic structure. The living cell acts as a most potent electron acceptor in the process.

REFERENCES

1. Yakhontova, L.K., and Grudev, A.P., Hypergenesis Zone of Ore Deposits, Moscow State University (1978) (in Russian).
2. Yakhontova, L.K., and Isaev, Z.Ya., *Proceedings of the Fersman Mineralogical Museum, 27*, 189 (1978).
3. Yakhontova, L.K., Grudev, A.P., and Nesterovich, L.G., *Vestnik*, Moscow State University, No. 3, 66 (1978).

4. Yakhontova, L.K., Grudev, A.P., and Nesterovich, L.G., *Geologiya rudnykh mestorozhdenii*, No. 2, 63 (1979).
5. Yakhontova, L.K., Zeman, I., and Nesterovich, L.G., *Reports of the USSR Academy of Sciences, 253*, 2, 461 (1980).
6. Yakhontova, L.K., Nesterovich, L.G., Grudev, A.P., and Postnikova, V.P., *Reports of the USSR Academy of Sciences, 250*, 3, 718 (1980).
7. Garrels, R.M., Mineral Equilibria at Low Temperatures and Pressures, Inostrannaya literatura, Moscow (1962) (in Russian).
8. Pourbaix, M.N., Atlas d'Equilibres Eléctrochimiques à 25 °C, Paris (1963).
9. Yakhontova, L.K., Nesterovich, L.G., Grudev, A.P., and Sukhantseva, V.S., *Izv. Vysch. Ucheb. Zaved.*, No. 1, 52 (1980).
10. Nesterovich, L.G., The role of microorganisms in the process of the formation of hypergenesis zone of ore deposits, M.Sc. Thesis, Moscow State University (1979) (in Russian).
11. Yakhontova, L.K., Nesterovich, L.G., and Grudev, A.P., *Proceedings of the Fersman Mineralogical Museum, 29*, 133 (1981).
12. Yakhontova, L.K., Sergeev, V.M., Karavaiko, G.I., and Sukhantseva, V.S., In: Ecology and Geochemical Activity of Microorganisms, Pushchino, 99 (1976) (in Russian).
13. Belov, N.V., Essays on Structural Mineralogy, Nedra, Moscow (1976) (in Russian).
14. Kireev, P.S., Physics of Semiconductors, Vysshaya shkola, Moscow (1975) (in Russian).
15. Marfunin, A.S., Introduction into the Physics of Minerals, Nedra, Moscow (1974) (in Russian).
16. Yakhontova, L.K., Nesterovich, L.G., and Grudev, A.P., *Vestnik*, Moscow State University, No. 1, 53 (1980).
17. Yakhontova, L.K., Nesterovich, L.G., Liubarskaya, G.A., Andreev, P.I., Pyzhov, V.Kh., and Blinova, G.K., *Mineral. zhurnal*, No. 2 (1983).
18. Electrosynthesis and Bioelectrochemistry, A.N. Frunkin (Ed.), Nauka, Moscow (1975) (in Russian).
19. Wol'kenshtein, M.V., Biophysics, Nauka, Moscow (1981) (in Russian).

MECHANISM OF OXIDATION OF SULFIDE MINERALS BY THIONIC BACTERIA

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INTRODUCTION

A chemolithotrophic bacterium, *Thiobacillus ferrooxidans*, has been proved to have considerable economic significance in the leaching of many kinds of metals from their sulfide ores since it was discovered in 1947 [1].

On the oxidation and leaching of sulfide minerals by the bacterium, two different mechanisms have been proposed.

One is the indirect mechanism [2, 3] in which the bacterium makes chemical oxidation of sulfide minerals with ferric iron cyclical, by oxidizing ferrous iron formed in the chemical oxidation.

The other is so-called direct mechanism [4] in which the sulfide moiety of sulfide minerals are oxidized directly by the bacterium without the participation of iron.

The author studied on the mechanism of oxidation of sulfide minerals by *Thiobacillus ferrooxidans*, especially on the participation of the two mechanisms under several conditions, and the results are described in this paper.

MATERIALS AND METHODS

1) **Microorganism and growth conditions.** An acidophilic iron-oxidizing chemolithotrophic bacterium, *Thiobacillus ferrooxidans* was used throughout the study. The strain was isolated from acid mine water at Yanahara Mine (Japan) and cultured in 100 ml of 9K medium [5] in a 500 ml shaking flask at 30 °C under shaking.

2) **Metal sulfides.** Metal sulfides tested in this study were MnS, NiS, CoS, PbS, ZnS, and CdS. They were analytically pure grade compounds (99.9 %) and were supplied by Ishizu Pharmaceutical Co., LTD (Japan).

Each metal sulfide was ground to pass through a 200 mesh sieve, sterilized separately in an oven at 180 °C for 30 minutes, and added to autoclaved 9K medium at the time of inoculation of the bacterium.

3) **Preparation of cell suspension.** Cells were grown in 10 l of 9K medium in carboy at 30 °C for 4—5 days under aeration, and the culture was filtered under suction in order to remove the bulk of ferric precipitates. The filtrate was centrifuged (10,000 rpm, continuous centrifuge) and the precipitated cells were washed twice with distilled water, then a small amount of insoluble iron was removed with low speed centrifugation (500xg). The harvested cells were resuspended

in 0.01 M potassium phosphate buffer (pH 7.0) and used for the tests of oxidation of sulfide minerals.

4) Assay methods. Concentration of solubilized metal ions was determined with a Shimadzu Model AA-625-01 Atomic Absorption Spectrophotometer after centrifugation of culture media.

Concentration of ferrous iron was determined colorimetrically by the o-phenanthrolin method [5] using Hitachi Model 101 Spectrophotometer.

For the measurement of cell growth, number of free floating cells in the filtered culture media was counted using a Toma's Haematometer.

Iron- or metal sulfide-oxidizing activity of cells was determined by measuring oxygen-uptake in a Warburg Manometer. Composition of the reaction mixture was as follows: ferrous sulfate or each metal sulfide, 100 μmoles; glycine-H_2SO_4 buffer (pH 2.5 or 3.5), 400 μmoles; cells (grown in 9K medium), 3.2—22.5 mg protein; total volume, 2.8 ml; 20 % KOH, 0.2 ml in center well; gas phase, air; temperature, 30 °C.

RESULTS AND DISCUSSION

1) **Leaching of manganese sulfide.** In the first place, as an example of easily acid-soluble metal sulfides, manganese sulfide was chosen and the leaching of manganese was tested under shaking at 30 °C in 100 ml culture medium.

In the case, growth of cells, solubilization of manganese, oxidation of ferrous iron, and change of pH of culture media were determined in three different culture media, namely, (a) 9K medium, inoculated, (b) 9K medium added with 1 % (w/v) MnS, inoculated, and (c) 9K medium added with 1 % MnS, but not inoculated. The medium (c) was used as control of chemical reaction.

As shown in Fig. 1, the growth of cells and the oxidation of ferrous iron were normal in medium (a). However, the growth of cells was not observed for 7 days after inoculation in medium (b). And the leaching of Mn^{2+} occurred rapidly in medium (b), and the rate was almost the same with that of medium (c) (chemical control). In the media of (b) and (c) pHs rose rapidly to almost 5.0 by the addition of MnS, and during the period the smell of hydrogen sulfide was perceived. Then the pHs were decreased gradually. From these results, it may be thought that manganese sulfide reacts rapidly with sulfuric acid and manganese ions are solubilized as follows;

$$MnS + H_2SO_4 \rightarrow MnSO_4 + H_2S \quad (1)$$

and the pH of the culture medium rises to unsuitable level for the growth of *T. ferrooxidans*.

And autooxidation of ferrous iron will be accelerated also by the high pH.

As manganese ions do not inhibit cell growth or iron oxidase of the bacterium even in high concentration [6], the inhibition of the cell growth in this case will be attributed to the high pH of the medium.

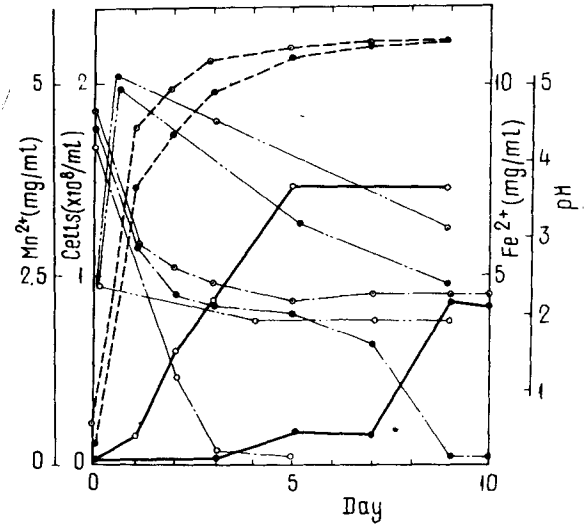

Fig. 1. Leaching of MnS with *T. ferrooxidans:*

_____ Cells, __ _ __ Fe^{2+},
_____ Mn^{2+} (leached), ___ ___ pH
a) 0 — 9K medium, inoculated;
b) ● — 9K medium + MnS (1 %), inoculated;
c) θ — 9K medium + MnS (1 %), not inoculated

In such way, in the case of easily soluble metal sulfides, bacteria will not act so effectively in the leaching of the metals, especially in the early stage.

2) **Leaching of nickel sulfide.** Secondly, the oxidation and leaching of nickel sulfide were tested. Examinations were carried out under the same conditions as in the case of manganese sulfide, except manganese sulfide was replaced with 1 % nickel sulfide. The results are shown in Fig. 2.

As can be seen from Fig. 2, cell growth in 9K medium added with 1 % NiS and inoculated was inhibited for 7 days as in the case of MnS. However, in this case pH of the medium did not rise over the initial pH throughout the culture period. And further, high concentration of nickel ions does not inhibit cell growth and iron oxidase of the bacterium [6], so the growth inhibition is thought to be caused by the pulp of nickel sulfide itself.

The rates of autooxidation of ferrous iron (may be accelerated by nickel sulfide also) and leaching of nickel were almost the same for 7 days in both of the inoculated and not inoculated media.

Then, the pulp density of NiS in the media was lowered to 0.5 % in order to decrease the inhibition of the bacterial growth. The results are shown in Fig. 3. As seen in Fig. 3, the lag phase of cell growth was curtailed to 2 days, but the rate of leaching of nickel was almost the same with that of chemical control for 8 days.

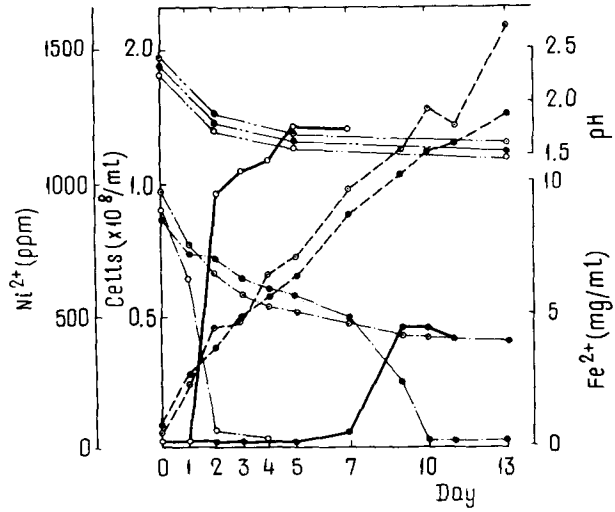

Fig. 2. Leaching of NiS with *T. ferrooxidans* (1):

———— Cells, — — — Fe^{2+},
— — — — Ni^{2+} (leached), — — — — pH
a) 0 — 9K medium, inoculated;
b) ● — 9K medium + NiS (1 %), inoculated;
c) 0 — 9K medium + NiS (1 %), not inoculated

3) **Leaching of cobalt sulfide.** Then, the leaching of cobalt sulfide was tested. Firstly, the leaching of cobalt was examined in standard 9K medium (containing 10 mg Fe^{2+}/ml).

In this case, three different culture media were used as in the case of the leaching of MnS or NiS, namely, a) 9K medium, inoculated, b) 9K medium added with 1 % CoS, inoculated, c) 9K medium added with 1 % CoS, but not inoculated (chemical control).

The results are shown in Fig. 4. From the figure, it may be seen that the bacterium can grow relatively well in 9K medium containing 1 % pulp of CoS (medium (b). However, the rate of leaching of cobalt was not so high compared with the chemical control (medium (c).

The reason was thought to exist on the high rate of autooxidation of ferrous iron in the media. As can be seen from Fig. 4, in the medium (b) ferrous iron is rather strongly oxidized chemically. Then, the ferric iron formed reacts chemically with CoS, and CoS is oxidized and leached out. And, then reduced iron is re-oxidized chemically. In such way, chemical leaching of CoS proceeds cyclically.

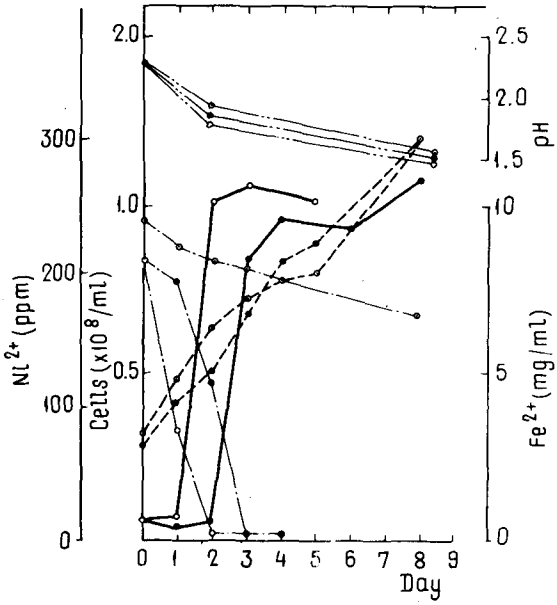

Fig. 3. Leaching of NiS with *T. ferrooxidans* (2):

_____ Cells, ___ _ ___ Fe^{2+},
_ _ _ _ _ Ni^{2+} (leached), ___ _ ___ pH
a) 0 — 9K medium, inoculated;
b) ● — 9K medium + NiS (0.5 %), inoculated;
c) θ — 9K medium + NiS (0.5 %), not inoculated

As ferrous iron is fairly stable in sterilized 9K medium (pH 2.5, without any metal sulfide) under shaking at 30 °C [7], and cobalt ions do not accelerate the oxidation of ferrous iron [6], the chemical oxidation of ferrous iron under acid conditions may be thought to be caused by the pulp of CoS itself.

Then, the concentration of ferrous sulfate in 9K medium was lowered to half of the standard (Fe^{2+}, 5 mg/ml) in order to lower the rate of autooxidation of ferrous iron, and the effect of the bacterium on the leaching of CoS was examined. The results are shown in Fig. 5. Expectedly the rate of chemical oxidation of ferrous iron and chemical leaching of CoS were lowered remarkably. On the other

hand, the rate of biological leaching of CoS was not decreased at all. And so, the biological effect of the bacterium on the leaching of CoS was manifested markedly. From Fig. 5, it may be noticed that the growth rate of the bacterium in medium (b) is almost the same with that in standard 9K medium (medium (a) (about 1.5×10^8 cells/ml).

It means that the bacterium in medium (b) grew on ferrous iron using it as sole energy source. And so, the leaching mechanism of CoS

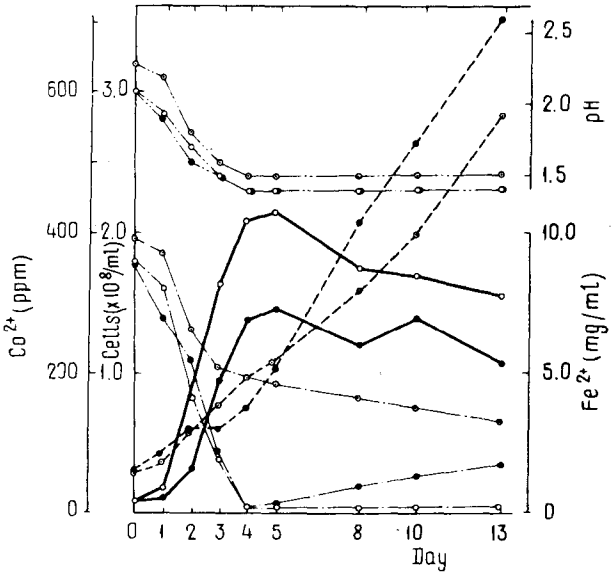

Fig. 4. Leaching of CoS with *T. ferrooxidans:*

_____Cells, __ _ __ Fe^{2+},
_____ Co^{2+} (leached), ___ ___ pH
a) 0 — 9K medium, inoculated;
b) ● — 9K medium + CoS (1 %), inoculated;
c) θ — 9K medium + CoS (1 %), not inoculated

in medium (b) should be indirect one almost exclusively. Because, if the bacterium used greater part of sulfide moiety of CoS as its energy source in addition to ferrous iron, the growth rate of the bacterium should be much higher, as the free energy change per mole in $S^{2-} \rightarrow SO_4^{2-}$ is about 20 times greater than that in $Fe^{2+} \rightarrow Fe^{3+}$.

Then, the leaching of CoS under the conditions of very low iron content of the media was examined.

In this case, 9K medium without ferrous sulfate was used as basal medium. However, as the inoculum which was actively growing culture in standard 9K medium (Fe^{2+}, 10 mg/ml) was used 10 % (v/v) to a fresh medium, iron was brought into the fresh medium from the

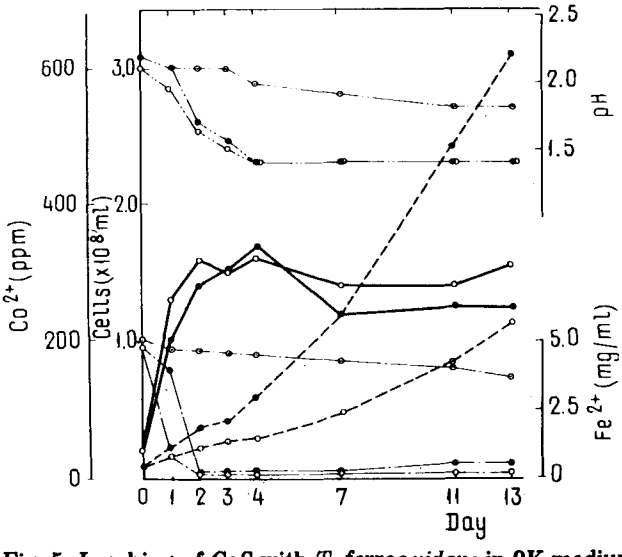

Fig. 5. Leaching of CoS with *T. ferrooxidans* in 9K medium:
_____ Cells, __ _ __ Fe^{2+},
_ _ _ _ _ Co^{2+} (leached), ___ _ __ pH
a) 0 — 9K medium (Fe^{2+}, 5 mg/ml), inoculated;
b) ● — 9K medium (Fe^{2+}, 5 mg/ml) + CoS (1 %), inoculated:
c) ⊖ — 9K medium (Fe^{2+}, 5 mg/ml) + CoS (1 %), not inoculated

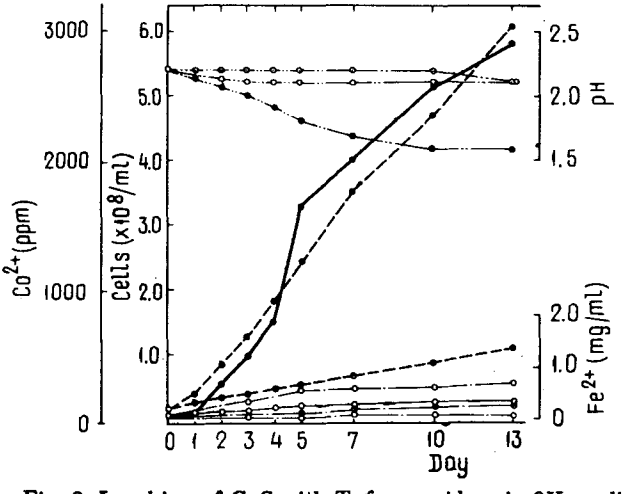

Fig. 6. Leaching of CoS with *T. ferrooxidans* in 9K medium (trace amounts of Fe):
_____ Cells, __ _ __ Fe^{2+},
_ _ _ _ _ Co^{2+} (leached), ___ _ __ pH
a) 0 — 9K medium (trace Fe), inoculated;
b) ● — 9K medium (trace Fe) + CoS (1 %) inoculated;
c) ⊖ — 9K medium (trace Fe) + CoS (1 %), not inoculated

inoculum (final concentration of iron was about 1 mg/ml). Other conditions were the same as those of Fig. 5.

The results are shown in Fig. 6. From the Figure, it may be seen that cobalt was solubilized as much as 3,000 ppm and the number of the bacterial cells reached 6.0×10^8 cells/ml after 13 days in the medium (b).

From the results it may be thought that CoS was oxidized and leached out principally through direct mechanism, as iron content of the medium was too low to support the active growth of the bacterium.

The rate of the participation of indirect mechanism in this case is not clear, but it may participate to some extent, especially at the early stage of the culture. Because, under the conditions of almost no iron in the medium (Fig. 7), the lag phase of the bacterial growth appears as long as 7 days, and the rate of the solubilization of cobalt is very low during the period.

The experiments shown in Fig. 7 were carried out as follows: 9K medium without ferrous iron, but added with 1 % of CoS was inoculated (10 %, v/v) with actively growing culture in standard 9K medium and cultured for 20 days under shaking at 30 °C, and the culture was transferred to a fresh medium described above in the same way. The transfer was repeated several times. During the transfer the iron contamination is decreased exponentially, and the final culture contained almost no iron. In the transfer, about 7—10 days of lag phase was observed and it was not shortened remarkably during several transfers.

However, by the procedure a culture which can grow on CoS as its sole energy source in the absence of iron was obtained. The culture was used as inoculum and the leaching of CoS was tested in the medium (9K medium without ferrous sulfate, but added with 1 % CoS). In Fig. 7, it can be seen that a lag phase as long as 7 days appeared, and also the rate of the leaching of CoS was slowed down compared with the case shown in Fig. 6.

From the results of the leaching of CoS described above, it may be postulated that:

A) High concentration of ferrous iron in the medium inhibits the biological oxidation of sulfide moiety of sulfide minerals by *T. ferrooxidans*, and so in such case the leaching of the sulfide minerals is carried out principally through indirect mechanism, namely by cyclic oxidation of ferrous iron by the bacterium;

B) On the contrary, when the concentration of iron in the medium is too low to sustain active growth of the bacterium, sulfide moiety of the sulfide minerals becomes to be utilized as its energy source, and so the leaching of sulfide mineral is carried out through both direct and indirect mechanisms;

C) Leaching of sulfide minerals in entire absence of iron will not be under natural conditions. It is carried out specially in laboratory or in specific case. Under such conditions the leaching of sulfide minerals

proceeds exclusively through direct mechanism. However, the leaching of cobalt sulfide under such conditions is rather slowed down compared with that achieved in the tests with low level of iron.

4) **Oxidation of metal sulfides** by the cells of *T. ferrooxidans* grown in 9K medium. Oxidizing activity of cells grown in standard 9K medium to several metal sulfides was tested using a Warburg Manometer as described in MATERIALS AND METHODS. The results are shown in Table. Each value shown in Table has been subtracted the value

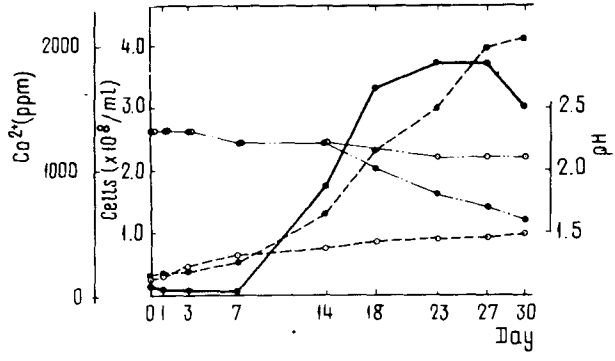

Fig. 7. Leaching of CoS with *T. ferrooxidans* in 9K medium (without Fe):

_____Cells, _ _ _ _ _ pH, _ _ _ _ _ Co^{2+}
a) ● — 9K medium (without Fe) + CoS (1 %), inoculated;
b) 0 — 9K medium (without Fe) + CoS (1 %), not inoculated.
The bacterium passed several times through the medium indicated above was used as inoculum

corresponding to the chemical oxidation (using boiled cells). Endogeneous respiration was negligible. The oxygen-uptake proceeded almost linearly for 120 minutes in every case.

Specific activity of the oxidation to all of the metal sulfides tested was remarkably low compared with that to ferrous iron. However, the order of higher activity was as follows:

$$PbS > CoS > NiS > ZnS > CdS$$

Acknowledgement. The author would like to express grateful thanks to Mr. T. Hatano and Mr. H. Ohtake for their technical assistance.

Table

Oxidizing activity versus metal sulfides of cells of *T. ferrooxidans* grown in 9K medium

Metal sulfide (100 μmoles)	O_2-uptake (μl/mg protein·min)	
	pH 2.5	pH 3.5
CoS	0.047	0.031
PbS	1.482	0.048
CdS	0.016	0.009
ZnS	0.017	0.010
NiS	0.020	0.016
($FeSO_4$)	(64.1)	(35.1)

REFERENCES

1. A.R. Colmer, and M.E. Hinkle, *Science, 106*, 253 (1947).
2. S.R. Zimmerley, D.G. Wilson, and J.D. Prater, US Patent, 2829964 (1958).
3. J.A. Sutton, and J.D. Corric, *Min. Eng., 15*, 37 (1963).
4. M.P. Silverman, *J. Bacteriol., 94*, 1046 (1967).
5. E.B. Sandell, Colorimetric Determination of Trace Metals, 2nd Ed., Interscience Publishers Inc., New York, 362 (1950).
6. K. Imai, In: World Mining and Metals Technology, A. Weiss (Ed.), American Institute of Mining and Metallurgical, and Petroleum Engineers Inc., *1*, 321 (1976).
7. K. Imai, In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma, J.A. Brierley, (Eds.), Academic Press, New York — San Francisco — London, 275 (1978).

PART IV

TECHNOLOGIES OF BACTERIAL LEACHING OF METALS FROM ORES

BACTERIAL LEACHING OF METALS IN TANKS. NON-FERROUS CONCENTRATE TREATMENT: TECHNOLOGY AND FLOW SHEETS

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INTRODUCTION

The process of bacterial-chemical leaching in tanks is intended mainly for utilization in combined concentration flowsheets, which, in addition to routine operations based on the physical separation of free minerals (gravitation, flotation), use chemical and biochemical (oxidation, dissolution, precipitation) and purely microbiological (cultivation of bacteria, their isolation from pregnant solutions and further utilization in the process) methods. Flowsheets which utilize bacterial-chemical leaching in tanks make it possible to tackle the tasks of extracting metals from combined hard-to-dress ores and their derivatives. Bacterial-chemical leaching may be both the main and the accessory means of processing concentrates. When developing technological flowsheets one should take into account a number of questions — from the pretreatment of input raw materials to the output of commercial produce.

Irrespective of the tasks set, one may single out the following stages envisaged in the flowsheets.

1. Preparation of the input materials for leaching.
2. Bacterial leaching.
3. Preparation, processing and regeneration of recycle solutions, their further utilization in the metal leaching process.

Characteristics of these basic stages are examined below.

I. A TECHNOLOGY FOR BACTERIAL LEACHING OF METALS IN TANKS

1. Methods of Metal Extraction

Methods of metal extraction, pyrometallurgic or hydrometallurgic, are inextricably connected with the preparation of input material. They are what determines the method of raw material preparation: concentration and leaching. We shall therefore briefly dwell on the metallurgy of some basic metals: copper, lead, tin, zinc and gold [1].

Metallurgy of copper. Flotation concentrates are the basic materials for the production of copper. According to their composition concentrates are divided into three types: (1) chalcopyrite-pyrite containing 16—20 % copper, 30—35 % sulfur and iron each, zinc and barren rock; (2) chalcopyrite concentrates containing 24—28 % copper, 30—35 % sulfur and iron each, 1—2 % nickel; (3) chalcocite-bornite concentrates containing up to 40 % copper, 10—12 % sulfur and iron each, quartz, aluminosilicates.

Copper concentrates are obtained mainly from combined hard-to-dress ores containing zinc. The zinc content of the concentrates may reach 10—12 % and it is lost during metallurgical conversion. The most appropriate, therefore, is the flowsheet with a preliminary zinc removal. This task may be effectively resolved by a combined technology for ore processing according to the pattern: flotation — selective bacterial leaching of zinc — smelting of copper concentrate to obtain both copper and zinc.

Metallurgy of lead. Virtually all primary lead is obtained from flotation sulfide concentrates. Ores containing lead alone occur extremely seldom, and they contain as a rule, apart from lead minerals, minerals of zinc and copper, barytes and others. The concentrates contain 40—70 % lead, 16—20 % sulfur, 4—15 % iron, 3—13 % zinc, 1—6 % copper.

The crude lead obtained contains 2—8 % admixtures, mainly copper (2—4 %), antimony and arsenic (0.5—1.5 %). The most laborious operation in the refining of crude lead is the removal of copper. Thus, in the production of lead too, it should be probably regarded as the most advisable to use a combined flowsheet of ore processing with the removal of zinc, copper and arsenic from the concentrate before the metallurgical conversion. This task can also be solved by using the process of selective bacterial leaching of zinc, copper and arsenic.

Metallurgy of tin. Bedrock ores, typical of the tin deposits occurring in the USSR, are more labour-intensive as regards extraction and concentration than scattered ones. The tin content of the concentrates (gravitation and flotation), refined and ready for smelting, is 40—70 %, iron 1—14 %, arsenic from 0.1 to 8.0 %, sulfur up to 10 %; other elements are also present. The main method of tin production used today is reduction smelting of tin concentrates. Great difficulties arise when smelting concentrates with a high content of iron, sulfur, arsenic, antimony, bismuth, lead, and copper.

It should be noted that the share of tin-polymetallic ores subjected to processing is continuously growing. Because of their inadequacy, the concentration flowsheets at operating factories fail to recover such valuable metal impurities of tin ores as lead, zinc and cadmium, which are lost with the sulfides during the refining of tin concentrates. Sulfides, including arsenopyrite, account for the main losses of tin. The separation of the sulfide products from different types of ores allows to obtain selective concentrates and complex products into which the total extraction of tin increases to 70—75 % as against 36—69 % when only the tin concentrate is obtained. One flowsheet for processing commercial tin-copper-arsenic products may be the flowsheet using bacterial leaching. This flowsheet allows to selectively extract from the commercial product (2 % Sn, 8 % Cu, 10 % As) a copper concentrate with a 23 % Cu content with the extraction of 86 %, and a 10—15 % tin concentrate with the extraction into it of 85 % of tin [2].

Metallurgy of zinc. Commercial types of zinc-bearing ores are represented by copper-zinc, lead-zinc and copper-lead-zinc ores. Serving as the main raw material for the production of zinc are flotation sulfide concentrates containing 40—56 % zinc, 5—16 % iron, 1—3 % copper and others. The zinc concentrates constitute a rich complex material bearing cadmium, indium, gold, silver, thallium, and selenium.

Copper-zinc-pyrite ores are among the most complex types of nonferrous metal ores, and their concentration technology occasionally precludes the production of quality standard zinc and copper concentrates. Thus, one of the deposits features metacolloid pyritic copper-zinc ores. Among their special features is the development of colloform formations and of finest, even emulsive, intergrowths of basic ore minerals with one another (chalcopyrite, sphalerite), the presence of secondary copper sulfides, a high content of flotation active pyrite.

From the technological viewpoint the pattern of collective copper-zinc flotation is the most suitable for preparation of input material for bacterial leaching: it yields a concentrate containing 9—11 % copper and 11—15 % zinc with at least 90 % of each metal being recovered. In this case the concentrate cannot be processed at copper and zinc plants by routine methods. Bacterial leaching allows to extract into commercial products up to 85 % of copper, 80 % of zinc and cadmium, and is the basic process in the combined flowsheet [3].

Gold metallurgy. Present-day gold metallurgy is based on the cyanide process, widely used in Soviet and foreign industrial practice. There is, however, a certain category of refractory gold-bearing ores whose direct cyanation is inacceptable for technological and economic considerations.

Fig. 1 represents the relation between gold and arsenic contents and the extraction of gold by cyanation for different size classes of a gold-arsenic concentrate. As the Figure shows, there is a close relationship between the content of gold and arsenic. Gold in the concentrate is finely dispersed and occurs predominantly in arsenopyrite in the form

of a mechanical admixture. The close association of finely dispersed gold with arsenopyrite renders the concentrate refractory to the extraction of gold by cyanation. Only 7—10 % of gold is extracted from the concentrate of either the initial size, or reduced to 95 % of the class —0.044 mm. Following cyanation of the sinder in the two-stage sintering

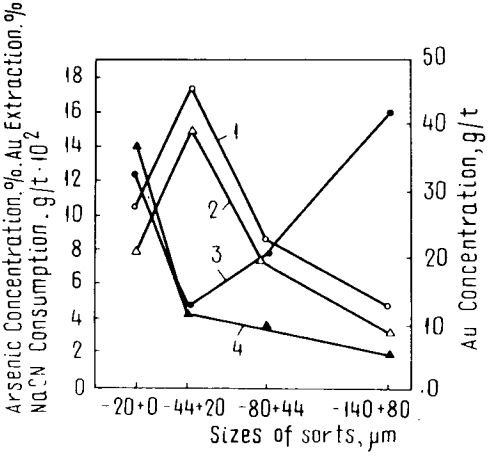

Fig. 1. Gold and arsenic content and the extraction of gold from the input concentrate of different sizes:

1 — gold content, g/t; 2 — arsenic content, %; 3 — extraction of gold, %; 4 — expenditure of cyanide, g/t x 10^2

process the extraction of gold does not exceed 77 %. After the release of gold using bacterial leaching in tanks the extraction of gold from the residue came up to 90—92 %. In this case bacterial leaching is a supplementary process under the combined flowsheet [4].

Thus, modern metallurgical methods of extracting metals in certain cases fail to meet the demands of the day. These demands are determined by the complexity of the ores to be processed. When producing *copper*, zinc defies extraction, when producing *lead*, copper and zinc are inadequately extracted. The metallurgy of *tin* includes the obligatory stage of the refining of concentrates for the removal of noxious admixtures of iron and arsenic; yet during the refining a considerable amount of tin is lost. No effective technology exists for producing copper and zinc from collective copper-zinc concentrates. In the hydrometallurgy of *gold* it becomes necessary to selectively extract gold from sulfides. These tasks may be tackled by the process of bacterial leaching in tanks. Its special characteristic is a high degree of selectivity.

2. Bacterial Leaching

Leaching represents a heterogeneous process whereby dissolved reagents interact with solid substance. The rate of leaching, i.e. the quantity of substance solubilized in a unit of time (or the quantity of reagent consumed in a unit of time), depends on many factors: the concentration of reagents, temperature, speed of agitation, surface of

the solid phase, and other factors, and, as a rule, it changes as the process goes on.

The rate of metal leaching from minerals and other solid substances is determined in most cases not by the rate of chemical reactions taking place at the boundary between the solid and liquid phases, but by the rate of diffusion processes. This is due to the fact that the velocity constant of chemical interaction between the mineral and the reagent is considerably higher than the diffusion rate constant and the process is therefore limited by the delivery of the reagent to the surface dividing the phases, or the withdrawal of the product of reaction.

The rate of leaching, bacterial leaching included, depends on many technological parameters. The experimental study of leaching kinetics is aimed at determining the optimal conditions of controlling the process.

Before the experiment, the limiting stage is unknown, therefore the rate of the process is described formally by a kinetic equation which, for an irreversible chemical reaction, is as follows:

$$-\frac{ds}{dt} = kP^n\Psi(s)$$

where $-\frac{ds}{dt}$ is the rate of decrease in the amount of leach material in the solid phase; k, the reaction rate constant; P, the concentration of the reagent in the solution at the moment of time t; n, the order of reaction by the reagent; $\Psi(s)$, the function which takes into account the surface area and changes in the rate of reaction due to decrease in the surface area and building-up of the solid envelope, etc.

When studying kinetics, the dependence of the degree of leaching on the duration of the process under different conditions is determined experimentally, as well as the basic kinetic parameters (reaction order, rate constant, limiting stage).

The difficulty of describing the bacterial leaching process lies in the need to coordinate the regularities of chemical heterogenic reactions with the kinetic regularities of enzymatic catalysis.

The bacterial leaching process may proceed in the external diffusion mode, in the internal diffusion area, and also in the kinetic region. A study of the kinetics of bacterial leaching allows to determine the limiting stage and consequently to outline ways of controlling the process. The establisment of the limiting stage and determination of the dependence of the leaching rate on basic technological parameters is essential for evaluating optimal leaching conditions, for controlling the process, for calculating the equipment used, and also for establishing the mechanism of bacterial leaching.

The basic factors and methods of intensifying bacterial leaching in tanks. Bacterial leaching may only proceed actively under medium conditions favourable to the life activity of bacteria. The basic factors determining these conditions may be divided into three groups. The first group includes physical-chemical factors: pH of the culture me-

dium, the redox potential temperature, the degree of aeration, the concentration of oxygen and carbon dioxide. The second group includes biological factors: the concentration of bacteria and their activity, the mineral composition of the nutrient medium. It is necessary to use bacterial cultures adapted to leaching conditions, as well as mixed cultures of microorganisms. Technological factors make up the third group. These are the density of the pulp, the method of agitation and aeration, size of material, type of equipment used for the process, etc. We shall only dwell on those factors that are technologically important for the metal leaching process, in tanks in particular.

The process of sulfide mineral oxidation should be conducted in a dense pulp. High pulp density, however, steeply changes the physicochemical conditions of the medium (pH and the redox potential, the concentration of inhibiting substances, etc.), which creates unfavourable conditions for the life activity of bacteria. The creation of optimal conditions for bacterial life activity in dense pulps is possible given their continuous cultivation according to a multistage chemostatic system on the test substrate. Such a system allows to conduct the process at a pulp density of 30 % solid. The multistage system allows also to constantly maintain stable conditions for the growth and life activity of the bacteria. Another advantage of this system is that it envisages multiple utilization of the bacterial biomass adapted to the substrate and to a range of other factors whose parameters change during metal leaching. The bacterial biomass may be isolated from pregnant solutions by separation.

The application of the continuous leaching system considerably improved the efficiency of the bacterial leaching of sulfide minerals. Thus, the extraction rate of zinc and copper from the concentrates was raised to 1,300 mg/l and 750 mg/l an hour respectively [5, 6]. This method of conducting the process allowed also to obtain technologically acceptable rates of arsenopyrite oxidation in concentrates, which is important for utilizing the process in the flowsheet for the removal of arsenic from tin-containing concentrates or for releasing gold in gold-bearing arsenical concentrates.

A considerable intensification of bacterial oxidative processes takes place also under optimized gaseous regime, particularly following introduction of 0.1 to 10 % carbon dioxide into the aerial mixture used for the aeration of the pulp. Carbon dioxide is essential because it is a source of carbon for autotrophic bacteria.

Thus, when leaching zinc sulfides in an atmosphere containing 1 % carbon dioxide, the zinc leaching rate reached 1,150 mg/l, compared to 360 mg/l an hour when normal air was used [5].

Producing strains of microorganisms active under extreme conditions is one of the most important factors in intensifying bacterial oxidation processes when leaching metals from complex ores and their concentrates in tanks.

The last 10—15 years have demonstrated that active strains of *Thiobacillus ferrooxidans* can be obtained by adapting laboratory strains isolated from deposits to extreme factors in conditions similar to those existing in industry. The very process of microorganism adaptation is complex and has not been fully revealed. Adaptation is based on various causes [7] associated both with changes in the bacterial genotype *per se* and with the realization of their genetic potentials (physiological adaptation).

3. Processing and Regeneration of Recycle Solutions, Their Utilization in Metal Leaching

Used in hydrometallurgy for separating metals from solutions is their precipitation in the form of hydroxides or metal sulfides, separation of metals by cementation, and ion-exchange, extraction and other processes.

Of great importance in bacterial leaching in tanks is the study of all possible methods of removing toxic elements from recycle solutions. Preparation and utilization of active recycle solutions is one way to intensify the leaching process because this allows to utilize active bacteria adapted to leaching conditions and solutions without metal ions.

Lime purification of recycle solutions. During bacterial leaching of arsenic-contaminated sulfide concentrates the solution's arsenic content may reach 7 g/l and higher, and iron content — up to 17 g/l. Reutilization of these solutions in a closed leaching cycle requires removal of iron, arsenic and other metals which inhibit bacterial life activity. An analysis of the literature indicates that the lime technique has a number of drawbacks. One of them is that trivalent arsenic is not firmly bound in the precipitate, which results in the formation of precipitates with a low arsenic content. Also important is the matter of burying large quantities of precipitate.

The probable thermodynamic composition of arsenate precipitates can be calculated from the structural diagrammes in the φ-pH coordinates. The results of thermodynamic calculations are given in Fig. 2. This Figure indicates that when lime precipitation of arsenic is used the following compounds may be found in the precipitate: $FeAsO_4$, $CaSO_4$, $Ca_3(AsO_4)_2$, $Fe(OH)_3$. With the exception of iron arsenate these compounds are more easily soluble and therefore are toxic.

Precipitation of metals with sodium sulfide. Taking account of the possible reactions of precipitation of tri- and pentavalent arsenic, bi- and trivalent iron from acid solutions with sodium sulfide, a φ-pH diagramme has been plotted for the sulfur-water, arsenic-water, and iron-water systems. The calculations are given in Fig. 3. The data obtained demonstrate that at a pH value between 0 and 7 the following species may occur in the solid phase: As_2S_3, FeS_2, S^0, and these

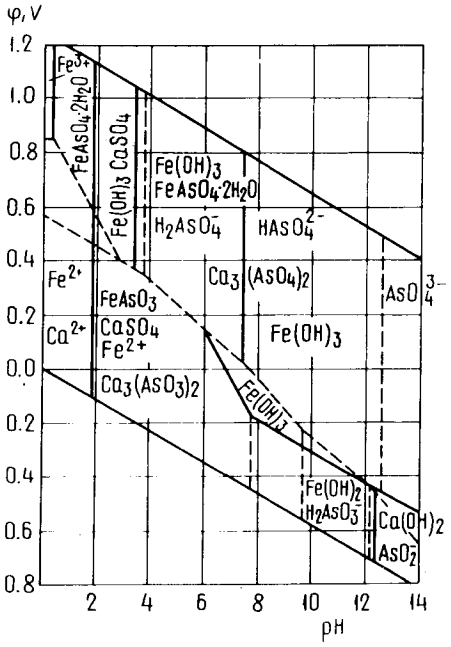

Fig. 2. φpH diagramme for the systems of iron-water, calcium-water and arsenic-water

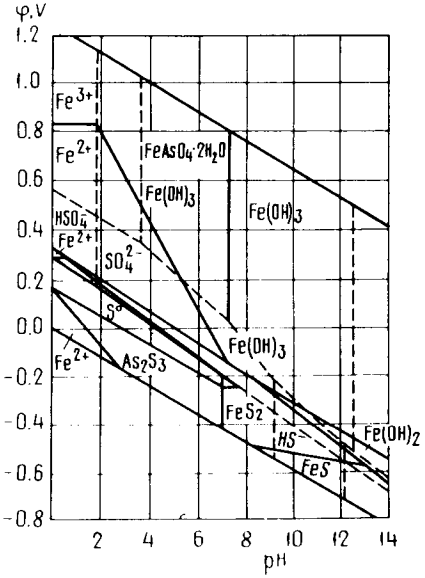

Fig. 3. φpH diagramme for the systems of iron-water, arsenic-water and sulfur-water

species are in equilibrium with the Fe^{2+} ion. An analysis of the possible phase composition indicates that the sulfide precipitate must be less depleted of arsenic than the precipitate obtained during lime purification of arsenic and iron-containing solutions. Arsenic sulfide As_2S_3 contains more than 60 % As, while iron arsenate (the main arsenic-containing compound in lime precipitation) contains about 35 % As. In distinction from the sulfide technique, the precipitate obtained with lime purification contains considerable amounts of calcium sulfate and iron hydroxide. Thus, the separation of arsenic sulfide is better than its separation in the form of iron arsenate, since the arsenic is precipitated in the form of a poorly soluble ($SP_{As_2S_3} = 4 \cdot 10^{-29}$), low toxic compound, with a higher arsenic content of the precipitate.

We have considered the separation from bacteria-containing solutions of two classes of poorly soluble compounds: the precipitation of hydroxides and sulfides. Depending on the material composition of the concentrate being treated for extracting metals from solutions and removing admixtures from them one can use ion exchange and extraction processes, reduction of metals by gases, crystallization from solutions, separation of metals by cementation. The application of one or another method depends on the tasks being tackled in a combined

flowsheet: the production of harmful wastes for disposal, of semi-products or commercial products.

Settling, filtering and washing are essential operations in the bacterial leaching process. These operations are associated with the separation of heterogeneous dispersion systems involving an aqueous solution, implemented by the mechanical transfer of phases. They are not, as a rule, accompanied with chemical reactions; their regularities are determined by hydrodynamics which largely depend on the design of equipment and conditions of their exploitation.

II. CONCENTRATES TREATMENT FLOWSHEET INVOLVING BACTERIAL LEACHING

An important feature of tank leaching is that, when combined with other processing techniques, it requires much higher process rates, incommensurable with the leaching rates of the underground and dump techniques.

As pointed out above, the high selectivity of the process allowing to use it for different purposes in the treatment of concentrates, determines the practical value of bacterial leaching in tanks.

Let us take a few instances of using the tank leaching process for the treatment of concentrates. In all variants a single process resolves fundamentally different production tasks: the recovery of finely dispersed gold from refractory arsenopyrite-pyrite concentrate, removal of arsenic as a harmful admixture from copper-tin products, selective leaching of zinc from hard-to-dress collective copper-zinc concentrates. The flowsheets for the treatment of concentrates are combined so that along with bacterial leaching, dressing and hydrometallurgical processes are used.

1. Treatment of Refractory Gold-Arsenic Concentrates

As pointed out above, bacterial leaching in tanks is one of the most effective methods of releasing finely dispersed gold from arsenopyrite-pyrite concentrates. A gold-arsenic flotation concentrate containing 8.4 % arsenic, 24.1 % iron, and 26.6 % sulfur was subjected to bacterial treatment. The ore portion of the concentrate is represented by sulfide minerals — pyrite and arsenopyrite at a ratio of 2:1. Gold is predominantly associated with arsenopyrite and partially with pyrite.

Experiments were conducted using a continuous-flow scale-up installation; Fig. 4 presents the hardware schematic description of the installation.

The equipment employed is made of acid-resistant steel X18H10T. For bacterial leaching of the concentrate, pulp from contact tank CT-1 (1) is continuously fed by air lift to Pachuka tanks. Between Pachuka tanks, the pulp moves gravitationally, according to a direct-

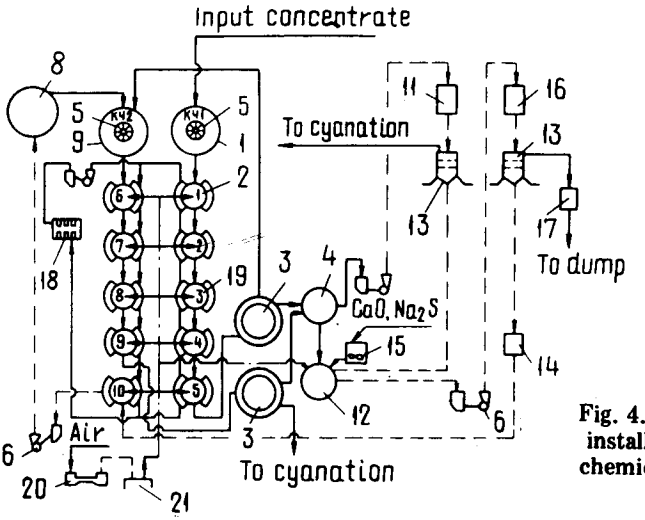

Fig. 4. Diagramme of pilot installation for bacterial-chemical leaching in tanks

flow scheme. The first four Pachuka tanks cover leaching stage I. Leached pulp is fed to dehydrating cone (3) wherefrom solids are air-lifted, if necessary, to the second contact tank (9) equipped with a stirrer (5), and are continuously fed into Pachuka tanks of leaching stage II. Liquid discharge of dehydrating cone (3), via collector (4), is pumped to tank (11), filtered, if necessary, in nutch-filter (13), and is fed to contact tank (CT) (12). Iron arsenates or arsenic sulfide are precipitated there adding corresponding reagents from tank (15). Pulp is transferred by pump (6) to collector (16) equipped with an air stirrer, is filtered in nutch-filter, precipitate being collected in tank (17), to be buried consequently. Filtrate is accumulated in collector (8), and is used for leaching concentrate at stages I and II. Following stage II, pulp is fed to dehydrating cone; solids from the latter are collected in tank (10) and pumped to cyanation. To maintain the desired temperature in Pachuka tanks, each tank is equipped with an external casing (19) with continuous circulation of hot water heated in boiler (18). The pulp in Pachuka tanks is stirred with air from vacuum-pump RMK-2 (20) with receiver (21). Water-cycling vacuum-pump used as a compressor partially humidifies the air, reducing evaporation losses in Pachuka tanks during the leaching. The installation is equipped with an exhaust ventilation device.

The concentrate was leached using *T. ferrooxidans* adapted to the conditions of leaching the gold-arsenic concentrate. The nutrient medium used for growing the bacterial culture contained salts of nitrogen, magnesium, chlorine, phosphorus, and iron. The pH values of the medium were brought to 2.2—2.4 with sulfuric acid.

Bacterial leaching was carried out in a closed cycle by two stages in 9 Pachuka tanks: 4 for the first stage and 5 for the second one.

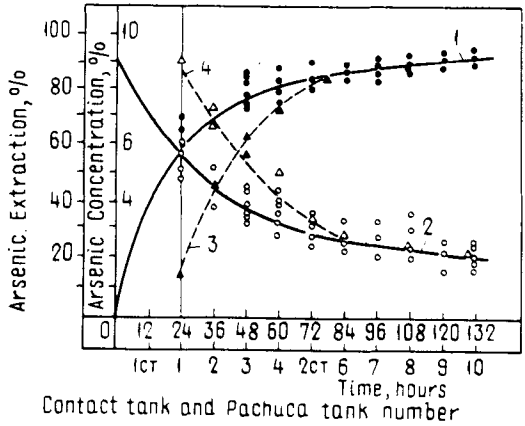

Fig. 5. Specifications of bacterial-chemical leaching of gold-arsenic concentrate on pilot installation:

1 — extraction of As; 2 — sulfide arsenic content; 3 — extraction of As during initial period; 4 — sulfide arsenic content in initial period

Before delivering the concentrate for the second stage the pulp was thickened in a cone thickener. The cone discharge containing arsenic and other elements was channelled to regeneration for further use in the leaching process. The thickened product was delivered to the second leaching stage. The number of bacterial cells in the leaching process was as high as 10^8—10^9 per 1 ml of pulp.

The results of these scale-up tests are given in Fig. 5. This figure demonstrates that the bulk of the arsenopyrite in the concentrate is oxidized in 48 hours during the first leaching stage. The content of arsenic sulfide drops from 8.4 to 3.14 % and its oxidation reaches 77.9 %.

During the 60-hour-long second stage of bacterial leaching the arsenic sulfide content of the concentrate drops from 3.14 % to 2.11 %, while the total oxidation of arsenopyrite is 86.13 %. The main reasons for the reduced arsenopyrite oxidation kinetics during the second stage are:

1) reduced sulfide mineral content of the concentrate delivered for the second stage of leaching;

2) the formation of slimes in the leaching process and precipitation from the solutions of the finely dispersed iron arsenates and hydroxides which cover the surfaces of arsenopyrite;

3) decrease in the pulp's pH from 2.5 to 1.5, and increase in the concentration of arsenic (up to 4.5 g/l), iron (up to 7.0 g/l) and ions of other elements in the solution causing an inhibition of bacterial activity;

4) the removal of iron, arsenic and sulfur from the solutions is also connected with a sharp change in pH values: in the beginning the pH rises from 1.5 to 3.2 and then drops to 1.7 during the second leaching stage. This also inhibits bacterial activity.

Trials have shown that conducting the leaching process in two stages may be justified when the arsenic content of the input con-

centrate exceeds 8.0 %. When conducting the process in a single stage the total leaching time should not exceed 90 hours for obtaining the same results as with the two-stage leaching.

The precipitation of arsenic, iron and sulfur following bacterial leaching was carried out with a 10—20 % lime suspension. At pH 2.9—3.3 at least 90 % of arsenic precipitates.

The precipitate formed during neutralization is separated by decanting and filtration, while the filtrate, acidified to pH 2.0—2.2, is used as a leaching solution.

The residue obtained in the process of bacterial leaching is subjected to cyanation (S:L = 1:2—4; KCN — 0.1 %; CaO — 0.01—0.02 %). With cyanation of the leaching residue, gold extraction amounts to 88—92 %, while in cyanation of the input concentrate, it amounts to 7—10 % only.

Such a higher recovery of gold by cyanation gives grounds for believing that the combined flow sheet involving bacterial leaching for

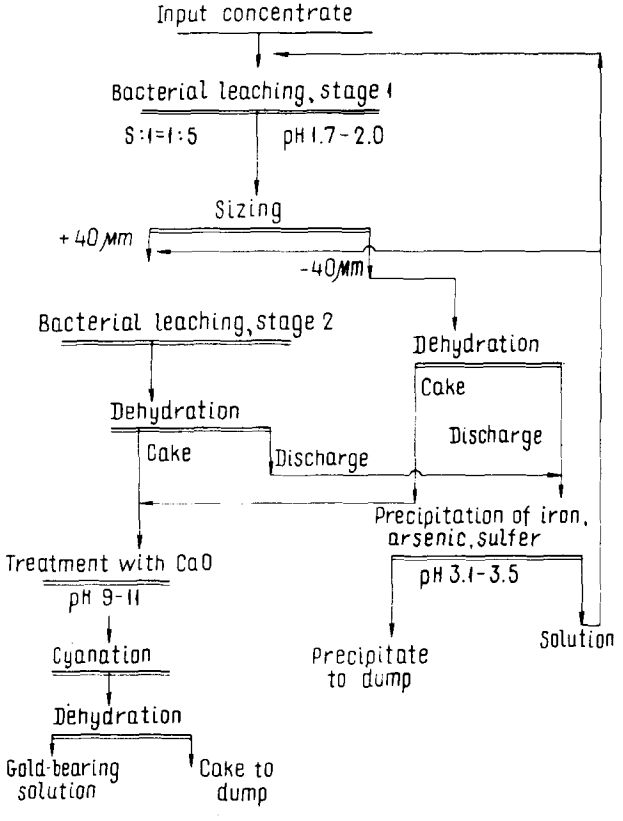

Fig. 6. Diagramme of processing gold-arsenic concentrates by bacterial-chemical leaching

recovery of gold is promising for the treatment of gold-arsenic concentrates (Fig. 6).

The bacterial tank leaching process is particularly important in extracting gold from concentrates with a high arsenic content exceeding 20 % As. At present, an efficient technology for metallurgical treatment of such concentrates is not available.

Bacterial leaching technology for such concentrates requires special regimes to be developed: use of adapted bacterial culture, direct-flow scheme, and concentrated biomass.

Gold-arsenic concentrate of a deposit contained 290 g/t gold, 20.3 % arsenic, 20 % sulfur, 20 % iron and small amounts of quartz, copper, and lead. Particle size of 100 % of the concentrate was — 0.074 mm. Bacterial leaching with a *T. ferrooxidans* culture was carried out in an installation comprising three successive fermentors of 1.5 l each. The pulp was agitated mechanically, and aerated by means of a compressor producing 3 l of air per 1 l of pulp per minute. Leaching was carried out according to a direct-flow scheme. The input concentrate and recycle solutions were fed to the first fermentor (S:L = = 1:4—5), the pulp was discharged from the third fermentor. Solution was separated from leaching residues, bacterial cells were removed, and the solution was channelled to arsenic and iron removal using conventional techniques. The isolated biomass and purified solution were recycled. Leaching residues, treated with sulfuric acid solution to dissolve iron arsenates, were tested for sulfide arsenic content.

Principal leaching parameters:

pulp pH	1.7;
redox potential	600—750 mV;
total iron	8.0—15.0 g/l;
total arsenic	6.0—12.0 g/l;
oxidative activity of solutions versus Fe^{2+}	2.0—5.0 g/l·h;
cell concentration in solution (wet wt)	2.0—5.0 g/l;
temperature	28—30 °C.

It can be seen from the above data that the concentrate leaching regime differed from those of earlier trials. The main difference consisted in the high concentration and activity of bacterial cells. In all the previous cases, the concentration of cells in leach solution did not exceed 1.0 g/l, oxidative activity, 0.5—1.06 g/l·h.

Leaching results revealed that during 50—60 hours, in one stage leaching, the sulfide arsenic content of the residues decreases from 20.3 % to 4.1 % with extraction exceeding 91 %. In the future, subject to optimization of parameters and of scheme, leaching time may be reduced to 20—30 h with the arsenic oxidation exceeding 95 %.

2. Flow Sheet for Treating Copper-Arsenic-Tin Concentrate

The main task in processing copper-arsenic-tin concentrates is the selective removal of arsenic as a harmful admixture and preparation

of copper and tin concentrates from the leaching residues. The flow sheet has been worked out for application at the deposits of the Solnechny concentration plant. The ores of this deposit are distinguished for the diversity of the mineral composition and close inter-impregnation of cassiterite with copper and arsenic sulfides. Arsenic is a harmful admixture and its removal presents considerable difficulties in flotation separation of chalcopyrite and cassiterite.

The flow sheet for processing this concentrate envisaged the removal of arsenic by bacterial leaching combined with the production of standard tin and copper concentrates from the residues by the flotation technique. Copper, arsenic, and iron sulfides occur in the concentrates in approximately equal proportions (chalcopyrite 22.5 %, pyrite 23.5 % and arsenopyrite 24.7 %). The other minerals are represented by quartz, feldspars, tourmaline, etc. The concentrates' chemical composition includes on the average 11 % arsenic, 7.5 % copper, 1.41 % tin, 25.4 % sulfur and 35.2 % iron.

Before starting bacterial leaching the input product is ground so that 98 % is — 0.08 mm in size, and is treated with a sulfuric acid solution for the removal of soluble copper compounds. About 7 % of the total copper in the input concentrate is solubilized. After the separation of the solid and liquid phases the solution containing 3.0 g/l copper is pumped for cementation, while the concentrate is delivered into Pachuka tanks for bacterial leaching. The flow sheet underwent trials in an installation described above for leaching the gold-arsenic concentrate. The installation's output was 50—60 kg/day at an S:L = = 1:5—4. The culture of *T. ferrooxidans*, preliminarily adapted to leaching conditions, was used in the trials.

Regenerated recycle solutions were used for scale-up bacterial leaching of concentrates. In distinction from the flow sheet for the leaching of gold-arsenic concentrates, the regeneration of bacterial solutions in the scale-up version included, in addition to arsenic precipitation, also cementation of copper on iron shavings and the bacterial oxidation of ferrous oxide (regeneration of ferric oxide sulfate) before delivering the solutions to the first Pachuka tank. The neutralization of solutions with pH 1.5—1.6 was conducted with 10 % lime suspension. The trials for arsenic precipitation from solutions demonstrated that at pH 2.8—3.1 at least 95 % of arsenic is precipitated. The precipitate of iron arsenates and hydroxides was separated by filtering, the filtrate containing 1—2.9 g/l iron, 0.2—0.9 g/l arsenic and 2.0—2.7 g/l copper. Lime expenditure for the precipitation of iron and arsenic was 25—30 kg/t of concentrate, with precipitate output exceeding 30 % of the input concentrate.

Following the precipitation of arsenic the copper was subjected to cementation on iron shavings. The solutions were preliminarily acidified with sulfuric acid to a pH of 1.8—2.0. Cementation time was 1.5—2.0 hours. The recovery of copper from the solution reached 80—90 %. Following the precipitation of arsenic and cemented copper

the bacterial iron-oxidizing activity in the barren solutions dropped from 0.29 to 0.1 g/l per hour. In order to restore this oxidative activity, the solutions containing after cementation up to 2.0 g/l Fe^{2+} were delivered into a separate Pachuka tank for aeration at 30 °C until bivalent iron was completely oxidized. A small quantity of the input concentrate was added to the same Pachuka tank. The solutions regenerated in this way were recycled.

The diagramme for testing the flow sheet of bacterial leaching of tin-copper-arsenic concentrate is represented in Fig. 7 and the metal balance in Table 1.

Following removal of arsenic by bacterial leaching, the tin-copper material was subjected to selective flotation separation to obtain individual copper and tin concentrates.

The bacterial leaching-flotation flow sheet provides for selectively producing from an input collective tin-copper-arsenic material (containing 11.0 % arsenic, 1.41 % tin and 8.03 % copper) 20 % tin and 16.0 % copper concentrates with the recovery of 84.6 and 92.3 % of tin and copper from the input material respectively. The arsenic content of the selective concentrates does not exceed 2.0 % and they may be used for the recovery of metals by existing metallurgical techniques.

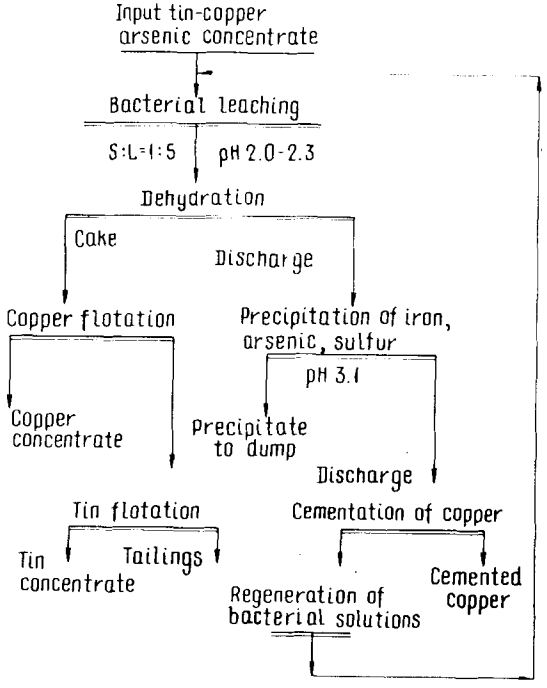

Fig. 7. Diagramme of processing copper-arsenic-tin concentrates

Table 1

Metal balance of flow sheet for bacterial processing of tin-copper-arsenic concentrate

Material	Yield, %	Content, %				Extraction, %			
		sulfide arsenic	total arsenic	tin	copper	sulfide arsenic	total arsenic	tin	copper
Residue following bacterial leaching	82.6	1.7	9.2	1.7	8.28	7.4	69.4	100.0	85.2
Arsenate precipitate	25.4	—	12.9	—	0.79	—	29.9	—	2.5
Cemented copper	1.2	—	0.5	—	82.0	—	0.1	—	11.8
Solution	—	—	0.2 g/l	—	0.2 g/l	—	0.6	—	0.5
Input concentrate	100.0	11.0	—	1.41	8.03	—	100.0	100.0	100.0

3. Flow Sheet for Treating Copper-Zinc Concentrates

The flow sheet for bacterial leaching of zinc and obtaining commercial products has been elaborated for the collective concentrates of metacolloidal copper-zinc ores. The average metal content of the input product was 13.4 % zinc, 6.24 % copper, 0.049 % cadmium, 28.4 % iron, 38.31 % sulfur, 0.4 g/t gold, 47.9 g/t silver and 0.68 % lead.

The size of the input concentrate was 95.7 % of the class of minus 80 μm (82.5 % of the class — 40 μm), with 87—89 % of copper and zinc represented in the particle class of —40 μm.

A typical feature of the concentrate is that sphalerite is represented by finer granules than chalcopyrite and pyrite. A culture of *T. ferrooxidans* was used in the tests in the treatment of copper-zinc concentrates, the bacteria having been preliminarily adapted to copper-zinc concentrate in a continuously operating pilot installation.

It has been demonstrated that in the process of leaching of a collective copper-zinc concentrate, iron, sulfur, cadmium and other elements are solubilized, apart from copper and zinc. Table 2 presents the average analytical results of the liquid pulp phase obtained in the process of leaching of concentrate at S:L = 1:5, and the output in solid terms was 60 kg per day.

Table 2

Analytical results of liquid pulp phase (S:L = 1:5, t = 27—35 °C, output by solid = 60 kg per day)

Parameters	Contact tank (Ct)	Pachuka tank numbers					
		1	2	3	4	5	6
pH	2.41	2.39	2.3	1.98	1.76	1.77	1.78
Fe^{3+}, g/l	0.59	0.68	0.5	1.69	2.25	2.55	3.24
Fe^{2+}, g/l	1.45	0.47	0.55	0.24	0.33	0.45	0.17
ΣFe, g/l	2.04	1.15	1.15	1.93	2.53	3.0	3.41
Concentration of copper in solution, g/l	1.08	1.26	1.41	1.53	1.71	1.83	1.94
Concentration of zinc in solution, g/l	11.36	14.43	16.91	19.19	21.59	22.57	22.44
Bacterial activity in solution in terms of Fe^{2+} oxidation, g/l·h	0.26	0.71	0.85	1.06	1.02	0.9	0.88

Following the removal of iron and partially of sulfur from the solutions, copper and cadmium were precipitated by cementation. For the precipitation of these elements a zinc dust was used, 100 % of size class of minus 0.074 mm.

The cementation of copper and cadmium was carried out at a pH value of about 2.5—3.5.

Precipitation of zinc from solutions. When conducting scale-up trials the method of zinc precipitation from solutions with soda was used. The solution, after extracting copper and zinc from it, was fed to a container added to which was a 20 % soda solution (90 % activity) to obtain pH 6.3—6.6. Maximum precipitation time was 30 minutes with aeration agitation.

Investigations into the precipitation of zinc during scale-up trials demonstrated the reliability of obtaining commercial zinc products with a zinc content of up to 48.6 %. The process is practicable and easily controlled. Admixture content may be regulated by modes of precipitation of iron, copper, and cadmium during preceding operations.

In bacterial leaching of collective copper-zinc concentrates, due to selective leaching of sphalerite, the copper content of the residues increases by an average 12—20 % relative, depending on the quantity of metals solubilized.

With the copper content of the input concentrate being low (6.24 %), even in cases of complete zinc extraction into the solution, it is impossible to produce a standard copper concentrate by means of bacterial leaching. Copper concentrate must be obtained from the leaching residues by flotation.

A quality-quantity diagram was calculated for collective concentrates from the trial results of bacterial leaching flow sheets for copper, zinc, and cadmium (Fig. 8).

Table 3 gives the technological data of scale-up trials, while Table 4 presents the metal balance.

As the data indicate, the proposed flow sheet allows to obtain three commercial products — copper concentrate, copper-cadmium cake and a zinc product. The processing of the first two presents no difficulties. The zinc product may be treated together with rotary-kiln processed ZnO and slag sublimates. Also advisable is the variant in which the hydrate-carbonate material is used as the neutralizer in the preliminary solution neutralization cycle in the hydrometallurgical treatment of zinc cakes at the metallurgical plant.

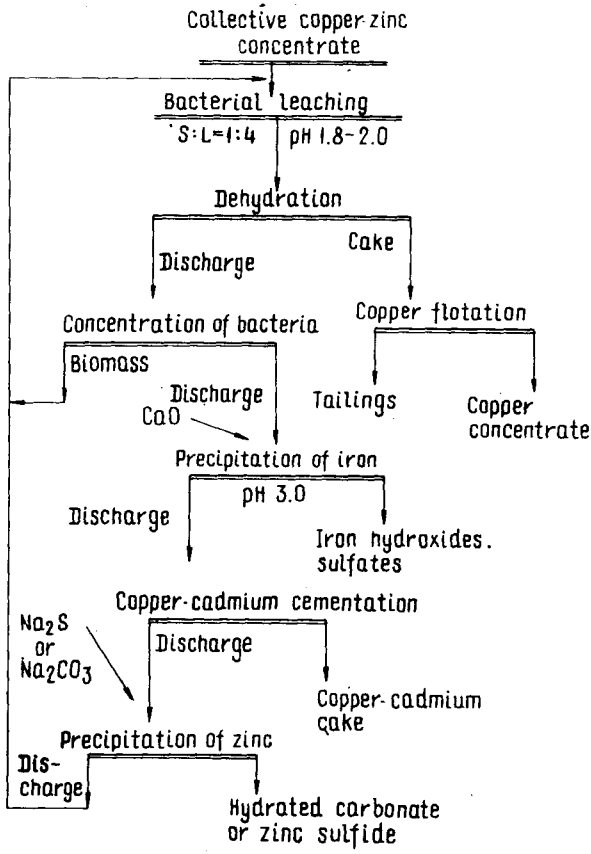

Fig. 8. Flowsheet for bacterial-chemical treatment of collective copper-zinc concentrates

Thus, the investigations carried out demonstrate that the technique of bacterial leaching in tanks is very effective for the treatment of refractory concentrates. Further pilot production tests will allow to evaluate the economics of this method as a basic criterion of its practical utilization for the treatment of ores and concentrates by the method of bacterial-chemical leaching in tanks [8].

Table 3

Bacterial leaching specifications

Material	Yield, %	Content, %		Extraction, %		
		Cu	Zn	Cu	Zn	Cd
Residue following bacterial leaching	75.1	6.99	3.95	84.1	22.1	22.4
Solution after leaching, g/l	24.9	1.98	20.97	15.9	77.9	77.6
Input concentrate	100.0	6.24	13.4	100.0	100.0	100.0

Table 4

Metal balance and flow sheet technological data

Material	Yield, %	Content, %		Extraction, %		
		Cu	Zn	Cu	Zn	Cd
Copper concentrate of basic flotation	25.75	16.33	7.79	67.37	14.97	
Copper-cadmium cake	1.7	55.5	10.8	15.08	1.37*	75.0
Total copper in commercial products	27.45	18.74	7.98	82.45		75.0
Hydrate-carbonate of zinc	24.0	0.18	45.2	0.32	81.09* 77.31	
Copper flotation tailings	49.35	2.12	1.94	16.73	7.13	
Iron hydroxides and sulfates	15.0	0.2	0.5	0.5	0.59	
Input concentrate	100.0	6.24	13.4	100.0	105.15* 100.0	100.0
Zinc dust	1.0	—	69.0	—	5.15*	—

* — Including zinc dust (zinc) added for copper cementation.

REFERENCES

1. Kistyakovsky, B.B., and Gudima, N.V., The Manufacture of Non-ferrous Metals, Metallurgiya, Moscow, 166 (1978) (in Russian).
2. Pol'kin, S.I., and Laptev, S.F., Concentration of Stanniferous Ores and Gravels, Nedra, Moscow, 409 (1974) (in Russian).
3. Pol'kin, S.I., Adamov, E.V., Karavaiko, G.I., Panin, V.V., Andreev, P.I., and Kasinceva, S.I., *Rudy i metale nieżelazne*, *24*, No. specjalny, 15 (1979).
4. Pol'kin, S.I., Yudina, I.N., and Panin, V.V., In: Hydrometallurgy of Gold, Nauka, Moscow, 67 (1980) (in Russian).
5. Torma, A.E., Walden, C.C., and Branion, R.M.R., *Biotechnol. Bioeng.*, *12*, 4, 501 (1970).
6. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.I., The Role of Microorganisms in Leaching Metals from Ores, Nauka, Moscow (1972) (in Russian).
7. Karasevich, Yu.N., Experimental Adaptation of Microorganisms, M.N. Meissel' (Ed.), Nauka, Moscow (1975) (in Russian).
8. Pol'kin, S.I., Adamov, E.V., and Panin, V.V., Technology of Bacterial Leaching of Ores and Concentrates, Nedra, Moscow (1982) (in Russian).

INTENSIFICATION OF BACTERIAL OXIDATION OF IRON AND SULFIDE MINERALS BY A *THIOBACILLUS FERROOXIDANS* CULTURE AT A HIGH CELL CONCENTRATION

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INTRODUCTION

The microbiological method employing *Thiobacillus ferrooxidans* cultures is used at present in dump and underground leaching of copper and uranium. Another promising technique is tank bacterial leaching which is utilized to separate difficult-to-dress concentrates and industrial products and to remove harmful impurities, such as arsenic, from them. A disadvantage of these methods is an insufficiently high rate of the process, which ultimately determines the latter's efficiency. The tank leaching process takes from 7 to 30 days in the case of batch cultivation, while in continuous cultivation the time required is between 100 and 180 hours [1, 2].

The long duration of bacterial leaching is due in part to the low rate of multiplication of *T. ferrooxidans* (the generation time in the pulp is about 6 hours), so that the biomass needed to accomplish oxidation is accumulated slowly. Under conditions of batch or chemostat cultivation the concentration of *T. ferrooxidans* is usually below 10^6-10^9 cells/ml ($0.025^{-0.05}$ g of dry weight per liter). Data on the relationship between the rate of leaching and concentration of bacteria have been reported in the literature [3, 4]. For example, experiments involving biological leaching of copper-zinc concentrates have shown that an increase in the bacterial biomass concentration to 2.4 g/l (dry weight) markedly intensifies zinc leaching, particularly the first stages of the process, and allows to reduce the leaching time by 80 %. A factor that inhibits multiplication of *T. ferrooxidans* is a high concentration of trivalent iron accumulated in the medium as a result of the bacterial metabolism [5]. A cultivator employing electrochemical reduction of trivalent iron makes it possible to obtain the biomass concentration up to 5 g of dry weight per liter [6].

The aim of this work is to investigate the effect of the initial biomass quantity on the rate of oxidative processes in underground leaching solutions and in the course of tank leaching of arsenopyrite-containing concentrates.

MATERIALS AND METHODS

The biomass of *T. ferrooxidans* was grown in a cultivator containing an electrochemical cell which reduces trivalent to bivalent iron at the cathode, bivalent iron being the energy substrate for the bacterial growth. The biomass concentration that can be obtained in the cultivator using a modified synthetic medium 9K, is up to 5.0 g of dry weight per liter [6, 7].

The following *T. ferrooxidans* strains were used:

K-1 — a non-adapted strain grown in the cultivator using electrochemical reduction of iron on the modified 9K medium;

K-4 — a strain adapted to high arsenic concentrations through cultivation on arsenopyrite-containing concentrates;

K-5 — a strain obtained by growing the arsenic-adapted strain K-4 in the cultivator with electrochemical reduction of iron for 72—96 hours.

The biomass concentrations of these strains used in the test work were 2.5; 0.025; and 2.5 g/l, respectively.

Experiments on microbiological oxidation of iron in underground leaching solutions and on leaching of arsenopyrite-containing concentrates were carried out by the batch method in a one-liter reactor equipped with a mechanical stirrer of the turbine type (1000 rpm) and automatic pH control. The temperature was 30 °C and the air was supplied at the rate of 4 liters per minute.

The kinetics of bacterial leaching of iron was studied using industrial and simulated solutions of underground metal leaching having the following composition: $FeSO_4 \cdot 9H_2O$ — 4 g/l; $Al_2(SO_4)_3$ — — 3.8 g/l; $MgSO_4$ — 6.0 g/l; $CaCl_2$ — 1.37 g/l; $NaNO_3$ — 0.6 g/l; Na_2SO_4 — 10 g/l; pH 1.5.

The arsenopyrite-containing concentrate used was a mixture (1:3) of flotational and gravitational concentrates ground to —74 μm and containing 8.5 % arsenic, 18 % iron, 17 % total sulfur, and 20 % carbon. The concentrate was composed of 55 % arsenopyrite and 44 % pyrite. Distilled water with pH 2.0 (adjusted with H_2SO_4) was used as the leaching solution.

Arsenic in solution was assayed iodometrically and in the solid phase, by X-ray spectroscopy, the iron content of the solution was determined by the complexometric technique with EDTA. The amount of biomass was found from the volume of the pellet after centrifugation (g of total biomass per liter), and the dry weight was calculated using an experimentally found coefficient of 0.25 (dry matter content of the total bacterial biomass). The activity of bacteria was determined on the basis of the rate of Fe^{2+} oxidation in 50 ml funnels equipped with a bubbler and containing the 9K medium and 9 g/l iron (15 ml of medium plus 5 ml of the solution).

RESULTS

The rate of oxidation of Fe^{2+} by *T. ferrooxidans* grown on 9K medium with the initial biomass concentration of 0.025 g/l is 5 times lower in underground leaching solutions than in the 9K medium where it equals 0.6 g/l per hour. This can be accounted for by the presence in the solutions of bacterial inhibitors such as NO_3^- and Cl^- ions as well as by the effect of high magnesium and aluminium concentrations. However, when the K-1 strain with the biomass concentration of 1.0 g/l (dry wt) was used, the rate of iron oxidation for both the 9K medium and underground leaching solutions increased to 15 g/l per hour regardless of the content of inhibitors (Fig. 1). The use of a concentrated culture therefore allows not only to markedly increase the rate of bacterial oxidation of iron but also to make the oxidative activity of bacteria resistant to inhibition by ions in the medium.

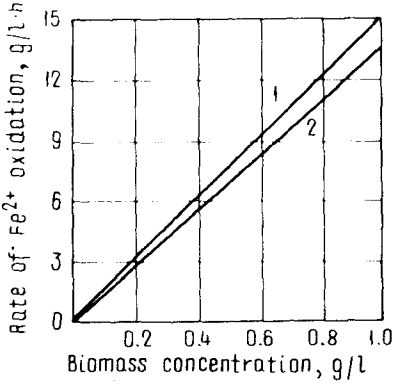

Fig. 1. Effect of *T. ferrooxidans* biomass concentration on the rate of Fe^{2+} oxidation in the solution:

1 — 9K/2 solution; 2 — underground leaching solution

Experiments with bacteriochemical leaching of arsenopyrite-containing concentrates by the tank method were performed in order to find out the relationship between the rate of arsenic leaching and the initial biomass concentration of *T. ferrooxidans* (in the range between 0 and 5.0 g of dry weight per liter), solid:liquid ratio (1:10 to 1:2.5) and ferrous sulfate content (up to 20 g of iron per liter). Also studied was the effect of electrochemical cultivation on the adaptive properties of the bacteria.

It has been shown that the rate of arsenic extraction is a function of all the three parameters studied (Figs. 2, 3, 4). When the iron concentration in the solution is optimal (10 g/l) and S:L equals 1:5, the growth of the initial biomass concentration of strain K-1 from 0 to 5.0 g/l increases arsenic extraction in 27 hours from 0.7 to 8.0 g/l. However, biomass concentrations in excess of 2.5 g/l are inexpedient since the rate of arsenic extraction is increased only slightly.

Experiments aimed at finding an optimal S:L ratio have shown that maximum arsenic extraction for the initial biomass concentration

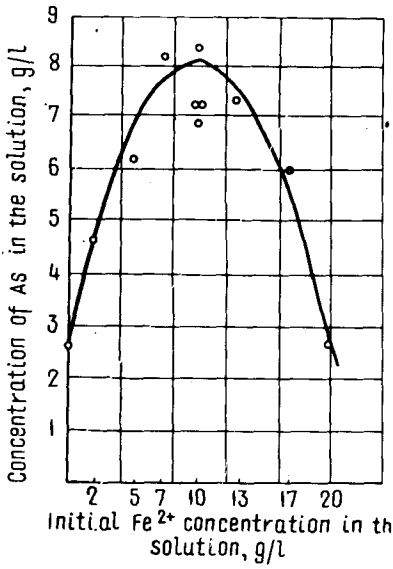

Fig. 2. Effect of initial Fe^{2+} concentration in the solution on the rate of arsenic leaching:

Time — 27 hours; S:L = 1:5; biomass — 2.5 g/l dry weight (strain K-1)

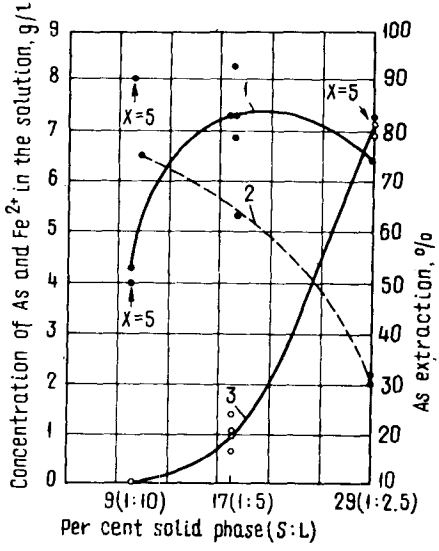

Fig. 3. Effect of pulp density (per cent solid) on the rate of arsenic leaching (1) and variation in Fe^{2+} concentration in the solution (2):

1 — as concentration after 27 hours of leaching; 2 — extraction of As after 27 hours of leaching; 3 — residual Fe^{2+} concentration in the solution. Biomass — 2.5 g/l dry weight (X) (strain K-1)

of 2.5 g/l occurs at S:L = 1:10 and minimum extraction, at S:L = = 1:2.5 (Fig. 3). The rate of Fe^{2+} oxidation also drops at higher pulp density (Fig. 3, curve 3). Since at S:L = 1:10 65 % of arsenic is extracted during 27 hours as compared to only 20 % for the high pulp density (S:L = 1:2.5), one may suppose that it is the whole complex of pulp components rather than arsenic ions alone that inhibit the bacterial oxidation at high pulp densities. An increase in the initial biomass concentration to 5 g/l (2-fold) at S:L = 1:2.5 does not significantly enhance arsenic extraction, suggesting that the inhibition at this pulp density is not due to a deficiency of bacteria. However, in spite of the high rate of oxidation at low S:L ratios, this process is not profitable economically. Therefore, our experiments with leaching of arsenopyrite-containing concentrates suggest that the pulp with S:L = = 1:5 is most suitable for the purpose. At this pulp density arsenic extraction in 27 hours is 55 % (for the initial biomass concentration of 2.5 g/l and 10 g/l iron).

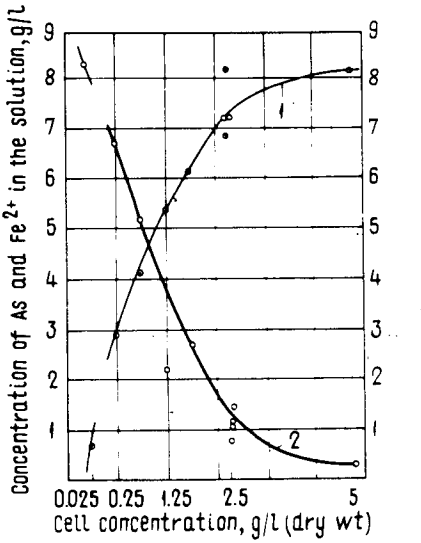

Fig. 4. Effect of bacteria concentration (strain K-1) on the rate of arsenic leaching (1) and variation of Fe^{2+} concentration in the solution (2);

Time — 27 hours; S:L = 1:5; $Fe^{2+}_{init.}$ = 10 g/l

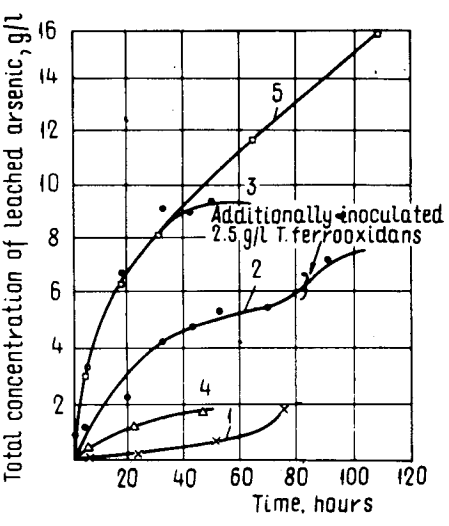

Fig. 5. Kinetics of arsenic leaching from a gold-arsenic concentrate (S:L = 1:5):

X — bacterial biomass; 1 — K-4 strain, X=0.025 g/l; 2 — K-1 strain, X=2.5 g/l, $Fe^{2+}_{init.}$ = 0; 3 — K-1 strain, X=2.5 g/l, $Fe^{2+}_{init.}$ = 10 g/l; 4 — no bacteria, $Fe^{2+}_{init.}$ = 10 g/l; 5 — K-5 strain, X=2.5 g/l, $Fe^{2+}_{init.}$ = 10 g/l

Fig. 5 shows time course of oxidation for the non-adapted strain K-1 (2.5 g/l) (Fig. 5, curve 3) and adapted strain K-4 (Fig. 5, curve 1) which has been grown on the concentrate (at 0.025 g bacteria per liter, 10 g/l iron and S:L = 1:5). In the case of strain K-1 leaching occurs at a high rate immediately after inoculation of the pulp with a large amount of biomass, and the highest rate of arsenic extraction is observed during the first 4—6 hours. However, when a small amount of biomass of the adapted strain K-4 was used, the oxidation rate increased slowly, which was due to multiplication of the bacteria and accumulation of the bacterial biomass in the pulp. Therefore, a high-rate tank leaching of arsenopyrite concentrates cannot be effectively accomplished with cultures having the concentration of 0.025 g/l (or 10^6—10^7 cells/ml) because the rate of oxidation processes, irrespective of the adaptation of bacteria to substrate, sharply rises only after accumulation of the biomass, which markedly lengthens the overall time of leaching.

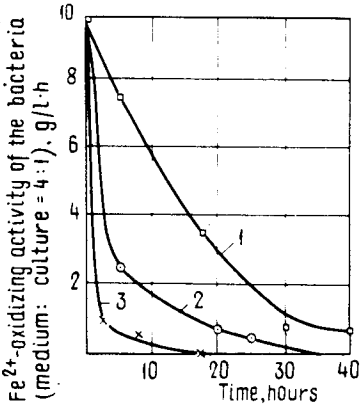

Fig. 6. Changes in bacterial activity in the course of leaching of a gold-arsenic concentrate:

1 — K-5 strain, $Fe^{2+}_{init.}$ = 10 g/l; 2 — K-1 strain, $Fe^{2+}_{init.}$ = 10 g/l; 3 — K-1 strain, $Fe^{3+}_{init.}$ = 20 g/l

To accumulate a large amount of biomass of concentrate-adapted bacteria, the strain K-4 was grown in the electrochemical cultivator on a modified 9K medium for 96 hours. At the initial stage of the experiment, the strain K-5 thus obtained (cell concentration was 2.5 g/l) oxidized the arsenopyrite concentrate at the same rate in the presence of 10 g/l Fe^{3+} as did the non-adapted strain K-1 (Fig. 5, curves 3 and 5). However, after 30 hours when 7 g/l arsenic was accumulated in the medium the oxidative activity of strain K-1 began to fade away rapidly while strain K-5 was still active and by 65 hours there was 9.5 g/l arsenic in the solution, the extraction efficiency being 80—90 %. Besides, the concentrate-adapted strain K-5 retained its activity at a sufficiently high level (about 1 g/l·h) even at 11.5 g/l arsenic in the solution while the non-adapted strain K-1 was completely inactive at 7.3 g/l arsenic (Fig. 6, curves 1 and 2).

The rate of iron oxidation did not increase after a fresh biomass (2.5 g/l) was added 70 hours after the beginning of leaching (Fig. 5, curve 2).

Therefore, to ensure a high efficiency of leaching of arsenic-containing sulfide concentrates it is necessary to use a high concentration of bacteria adapted to all extreme factors in the dense pulp (arsenic, pH, S:L, etc.).

As mentioned above, the bacterial leaching can be accelerated by supplying the pulp simultaneously with the concentrated biomass and bivalent iron at the concentration between 0 and 20 g/l. In the course of 2 to 3 hours all of the bivalent iron was oxidized by bacteria to trivalent iron. Maximum rate of arsenic extraction was obtained after feeding the pulp with 9—11 g/l bivalent iron together with 2.5 g/l biomass of strain K-1 (Fig. 5, curve 3). Arsenic solubilization during 30 hours was two times higher than in the absence of iron (Fig. 5, curve 2). These results suggest that the presence of trivalent iron which is an oxidizer of the surface of sulfide minerals, creates better conditions for leaching.

A further increase in iron concentration up to 20 g/l results in slowing down of the oxidative process (Fig. 2, curve 1). At 20 g/l iron only 2 g/l arsenic is solubilized during 6 hours, and the process then is abruptly decelerated due to inhibition of bacterial activity with high concentration of trivalent iron. That high Fe^{3+} concentrations are indeed inhibitory to the oxidative activity of the bacteria also follows from the fact that at 20 g/l iron the bacterial activity drops from 10 to 1 g/l·h after one hour (Fig. 6, curve 3). At 10 g/l iron the activity dropped only to 5.3 g/l·h after one hour and after 12 hours it remained at the 1.0 g/l·h level (Fig. 6, curve 2).

To reveal the role of the chemical factor in biological leaching, 10 g/l trivalent iron was added together with thymol as antiseptic. In this case only 1.7 g/l arsenic is solubilized in 30 hours as compared to 8.0 g/l for biological leaching of the same duration (Fig. 5, curves 3 and 4). The efficiency of chemical leaching is thus markedly lower compared to the biological process, confirming the importance of *T. ferrooxidans* as the biochemical catalyst of leaching.

CONCLUSION

For intensification of iron oxidation in dump and underground leaching solutions as well as for tank leaching of refractory arsenopyrite-containing concentrates, high density of bacterial biomass (2.5 g/l) adapted to all factors occurring in the leaching process, may be efficiently used.

The intensity of leaching of arsenopyrite-containing concentrates is markedly enhanced after adding to the pulp 10 g/l Fe^{2+} together with the concentrated biomass. If the initial concentration of Fe^{2+} is increased to 20 g/l, the leaching process is inhibited because of the rapid generation of Fe^{3+} which is a competitive inhibitor of bacterial oxidation processes.

The chemical oxidation of arsenopyrite is relatively slow, and cannot ensure a high efficiency of leaching.

REFERENCES

1. Pol'kin, S.I., Yudina, I.N., Panin, V.V., Kostyukova, O.N., and Korostyshevsky, N.B., In: Hydrometallurgy of Gold, Nauka, Moscow (1980) (in Russian).
2. Yudina, I.N., Kostyukova, O.N., Gorenkov, N.L., Panin, V.V., and Kim, D.Kh., *Tsvetnye metally*, *8*, 109 (1979).
3. Kulebyakin, V.G., Bacterial Leaching of Sulfide Minerals, Nauka, Novosibirsk (1978) (in Russian).
4. Linnik, O.B., Pol'kin, S.I., Skakun, T.O., Denisov, G.V., and Zimenko, G.K., *Izvestiya vuzov, Tsvetnaya metallurgiya*, *4*, 10 (1982).
5. Kelly, D.B., and Jones, C.A., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, Academic Press, New York, 19 (1978).
6. Kovrov, B.G., Denisov, G.V., Sekacheva, L.G., and Belyi, A.V., In: Chemosynthesis in a Continuous Culture, Nauka, Novosibirsk, 34 (1978) (in Russian).

SCIENTIFIC FUNDAMENTALS OF TECHNOLOGY OF TANK LEACHING OF URANIUM

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CONVENTIONAL TECHNIQUES OF URANIUM EXTRACTION

The extraction of uranium from its ores is currently carried out at commercial scales in agitated stirred leach tanks in open systems for sulfuric acid and in closed systems (autoclaves) for carbonate and bicarbonate leachings [1]. The choice between acid and alkaline processes is essentially dictated by the type of mineralization of the ore and the associated economics. Sulfuric acid is the most frequently used leachant [2] and the alkali solutions are preferred for ores associated with large quantities of acid consuming components such as carbonates and oxides [3]. The hexavalent uranium is readily soluble in the above leach solutions while the tetravalent uranium must first be oxidized by a suitable oxidant to the hexavalent state before dissolution occurs. The oxidation process is recognized to be an electrochemical reaction involving cathodic reduction of the oxidant and the anodic oxidation of tetravalent uranium [4—6]:

Anodic oxidation:

$$2UO_2 \rightarrow 2UO_2^{2+} + 4e^- \tag{1}$$

Cathodic reduction:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \tag{2}$$

Sum reaction:

$$2UO_2 + O_2 + 2H_2O \rightarrow 2UO_2^{2+} + 4OH^- \tag{3}$$

The most frequently used oxidants are manganese dioxide and sodium chlorate [7—9], which are oxidizing ferrous ion to the ferric state that is interacting with tetravalent uranium, as shown in equations 4 to 6:

$$2Fe^{2+} + MnO_2 + 4H^+ \rightarrow Mn^{2+} + 2Fe^{3+} + 2H_2O \tag{4}$$

$$6Fe^{2+} + ClO_3^- + 6H^+ \rightarrow Cl^- + 6Fe^{3+} + 3H_2O \tag{5}$$

$$U^{4+} + 2Fe^{3+} \rightarrow U^{6+} + 2Fe^{2+} \tag{6}$$

The minute amount of iron required in the process is always available from the ore, most frequently in form of pyrite and metallic iron

abraded from crushing and grinding operations. In more recent years, the use of hydrogen peroxide as a possible alternative of the above oxidizing agents has been proposed [10, 11].

In acid leaching, the ore is pulped to 60—70 % solids with a solution containing 3—50 $g \cdot l^{-1}$ free acid and 0.5—3 $g \cdot l^{-1}$ of oxidant. The leach stirred tanks are often rubber lined to prevent corrosion. The leaching is carried out at 50—80 °C. The hexavalent uranium is dissolved in $UO_2(SO_4)_3^{4-}$ complex species in presence of excess of sulfate ion:

$$UO_2 + 1/2\ O_2 + H_2SO_4 \rightleftharpoons H_4\ [UO_2(SO_4)_3] + H_2O \qquad (7)$$

The carbonate leaching is generally carried out in closed circuit, in which, the leach solution is continuously recycled. The ore is pulped to 30—60 % with a leach solution containing 40—60 $g \cdot l^{-1}$ Na_2CO_3 and 10—20 $g \cdot l^{-1}$ $NaHCO_3$ in stirred autoclaves at 75—95 °C under pressure of air at about 120—160 psi . Uranium is dissolved in $UO_2(CO_3)_3^{4-}$ complex species.

$$UO_2 + 1/2\ O_2 + 3Na_2CO_3 + 2NaHCO_3 \rightleftharpoons Na_4[UO_2(CO_3)_3] +$$

$$+ 2Na_2CO_3 + H_2O \qquad (8)$$

The bicarbonate prevents the formation of sodium hydroxide which can reprecipitate uranium in form of diuranate.

A generalized and simplified flowsheet of these uranium leach processes is given in Fig. 1.

The recovery of uranium from the acid solutions is generally accomplished by solvent extraction or by solid ion exchange, as shown in the next equilibrium equation:

$$U + E \rightleftharpoons EU \qquad (9)$$

In the first stage of this process, uranium, U, is transferred from the aqueous phase to the ion exchanger, E, as some complex, UE. This requires that the equilibrium in equation 7 be shifted to the right (loading). After separation of phases, uranium is recovered from the exchanger (stripping or elution) by an aqueous solution, which requires that the equilibrium in equation 9 be shifted to the left. Uranium is then precipitated by ammonium, magnesium or sodium hydroxide, for example:

$$2UO_2SO_4 + 6NaOH \rightarrow Na_2U_2O_7 + 2Na_2SO_4 + 3H_2O \qquad (10)$$

The sodium diuranate is then dried and calcined to yield U_3O_8.

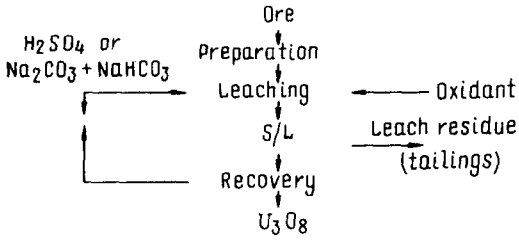

Fig. 1. Generalized flowsheet for the extraction of uranium ore by acidic or alkaline processes

The recovery of uranium from the carbonate leach solution can be accomplished directly from the clarified leach solution by addition of sodium hydroxide:

$$2Na_4UO_2(CO_3)_3 + 6NaOH \rightarrow Na_2U_2O_7 + 6Na_2CO_3 + 3H_2O \quad (11)$$

Drying and calcining of sodium diuranate is similar to those indicated before.

BACTERIAL LEACHING TECHNIQUE

Bacterial mediated uranium extraction technique differs mainly from the conventional chemical approach by the fact that the iron oxidation (ferrous to ferric) is accomplished by the microorganisms. The ferric ion is the oxidation agent [12] for uranium as shown in equation 6. Therefore, we can write:

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4 \quad (12)$$

$$2FeSO_4 + H_2SO_4 + 1/2\ O_2 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O \quad (13)$$

Therefore, the bacterial action in the solubilization of uranium is an indirect involvement. It provides and constantly regenerates the oxidizing agent. In this respect, the role of bacteria is similar to the catalysts in chemical processes.

The best known microorganism involved in the solubilization of uranium from rocks and minerals is *Thiobacillus ferrooxidans*, which was first described in 1947 [13]. This microorganism plays also an important role in a variety of metal sulfide-bearing leaching processes [14—23]. The bacteria oxidize the pyrite associated with the uranium ore and resulting sulfuric acid and ferric sulfate [24—26]:

$$2FeS_2 + 7\ 1/2\ O_2 + H_2O \rightarrow Fe_2(SO_4)_3 + H_2SO_4 \quad (14)$$

Ferric sulfate is known to oxidize pyrite [27]:

$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2S \quad (15)$$

The elemental sulfur produced in equation 15 will further be oxidized by the bacteria to sulfuric acid [28, 29]:

$$S + 1\ 1/2\ O_2 + H_2O \xrightarrow{\text{bacteria}} H_2SO_4 \qquad (16)$$

Laboratory scale studies have well demonstrated this capability of *T. ferrooxidans* [30—32]. The conditions, with regard to kinetic factors, for the bacterial oxidation of pyrite and metal sulfides are very important [33—37]. The laboratory techniques employed included shake flask [38, 39, 43], leach columns [33, 34, 40, 41], batch [42, 44], and continuous reactor [45] leachings.

Industrial bacterial leaching of uranium-bearing ores and waste materials were carried out in heap, dump, and in situ underground operations [46—52]. The economic advantage of biological over non-biological processes was specified [53]. The rate of uranium dissolution was related to the efficiency of bacterial pyrite oxidation.

TANK LEACHING TECHNIQUE

This method is particularly useful for evaluating the leachability of uranium ores and provides for easy control of all the important parameters influencing the bacterial activity. The interpretation of the results of tank leaching experiments also may contribute to a future acceptance of the biohydrometallurgical leaching technique for recovery of metals from minerals, which are presently recovered by the conventional extractive hydro- and/or pyrometallurgy.

INFLUENCE OF OXYGEN

Bacterial leaching of a uranium ore is an oxidation process which requires transfer of large amounts of oxygen and carbon dioxide from air into the liquid medium. Oxygen is required for oxidation of ferrous iron and metal sulfides, and carbon dioxide for the growth of microorganisms [54, 55]. For example, according to the next reaction:

$$S^{2-} + 2O_2 \xrightarrow{\text{bacteria}} SO_4^{2-} \qquad (17)$$

32 g sulfide to be oxidized to sulfate, require 64 g oxygen to be transferred to the leach medium. One can anticipate that the most efficient bacterial leaching will be achieved when the bioreactor operates with the fastest rate of oxygen and carbon dioxide mass transfers, provided that the other growth conditions are maintained at optimum levels. Oxygen is a rather insoluble gas, the acid nutrient medium [56] saturated with air at 35 °C contains approximately 6 ppm of oxygen [57].

Therefore, to take maximum advantage of bacterial activity, the oxygen mass transfer into the leach solution should be optimized. A measure of the oxygen mass transfer performance of a given leaching tank is expressed by the volumetric oxygen mass transfer coefficient, k_La, which can be evaluated experimentally [58, 59] using sodium sulfite solutions and the following form:

$$\frac{dC}{dt} = k_La(C^* - C) \tag{18}$$

where dC/dt is the rate of oxygen mass transfer, C* is the concentration of dissolved oxygen at saturation, C is the actual dissolved oxygen concentration of the leach medium and a is the interfacial area between liquid and gas per unit volume of liquid. After the k_La value has been determined for different air flow rates, V, and agitation rate (rpm), n, a plot of k_La versus A_i (aeration index) can be established as shown in Figure 2. The aeration index can be expressed as follows [61]:

$$A_i = \frac{\frac{\pi}{4} V D^2 H}{n D_i^3} \tag{19}$$

where D is the inside diameter of the leach tank, H is the liquid depth and D_i is the impeller diameter. As equation 19 indicates the aeration index is directly proportionate to the air flow rate and inversely proportional to the rate of agitation. The maximum in the k_La corresponds to a definite figure of aeration index, which expresses the best values for air flow velocity and rpm. Once these operational data (k_La, V and n) are determined for a given leach tank, these should be kept

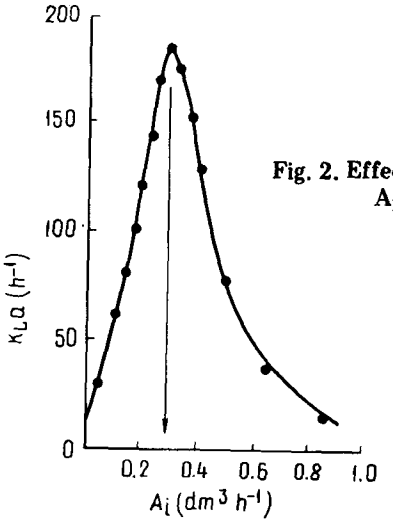

Fig. 2. Effect of index of aeration, A_i, on k_La [60]

constant throughout the bacterial extraction of uranium in order to avoid limitation in the availability of oxygen. Similar determination can be carried out for carbon dioxide mass transfer using potassium hydroxide solutions. If higher oxygen and CO_2 concentrations are required, the partial pressure of O_2 and CO_2 should be increased in air used for aeration.

INFLUENCE OF TEMPERATURE

The temperature range for the optimum activity of *T. ferrooxidans* is limited to 28—35 °C [39], depending upon the origin of the strain of microorganisms and the composition of the ore (substrate). Once the optimum temperature is known, this should be maintained automatically constant during the tank leaching of uranium ore. Therefore, it is preferable to use leach tanks equipped with heating and cooling devices. Deviations from the optimum temperature will slow down the rate of uranium extraction, as there are two competing factors to be considered in this process:

a) Below the optimum value: the usual rise in the rate of uranium extraction with increase in the temperature as described by the temperature coefficient, Q_{10}:

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{T_2 - T_1}} \tag{20}$$

where k_1 and k_2 are reaction rate constants at temperatures T_1 and T_2 respectively. The Q_{10} — value is two if the reaction rate constant of uranium extraction doubles for each 10 °C rise in the temperature.

b) Above the optimum temperature: as the temperature increases the rate of thermal death of bacteria is increased. Above the optimum temperature, the thermal death (denaturation) becomes faster than the uranium extraction, as a result, slowing down the rate of extraction.

INFLUENCE OF pH

The bacterium, *T. ferrooxidans*, is acidophilic and grows well in the pH range of 1.5 to 3.5 [14]. When grown on uranium-bearing minerals, the optimum pH is situated around 2.3 [39]. However, uranium ores containing large amounts of alkaline gangue should first be neutralized with sulfuric acid [42] to permit the bacterial growth. The microbiological leaching of uranium ores containing iron sulfides generates enough acid and ferric sulfate to self-maintain the process if properly initiated.

The tank leaching setup for uranium extraction should preferably be equipped with automatic control of pH. This is especially important in the initial phase of the leaching. Important information can be gained for the microbial leaching of uranium ores, by carefully studying the Eh — pH diagrams uranium — iron — sulfur systems [62, 63].

INFLUENCE OF NUTRIENT CONCENTRATIONS

Several nutrient media [56, 64, 65] are described in the literature, of which, the most frequently used one is from Silverman and Lundgren [56]. In a sulfide ore leaching situation only ammonium sulfate and phosphorous source are required, because all other nutrients (KCl, $MgSO_4$, $Ca(NO_3)_2$) are available from the ore as impurities [66, 67]. In the case of uranium ore leaching, it is possible that only ammonium sulfate will be required in the leach solution as suggested previously [52].

CONCLUSION

Bacterial leaching of uranium ore is a commercially proven process, which involves the bacterially mediated oxidation of pyrite inclusions producing sulfuric acid and ferric sulfate (leaching agents): this process is practiced in heap, dump and in situ leachings for the recovery of uranium in South Africa, Portugal, Canada and is being introduced to practice in many other countries. The tank leaching technique was not yet attempted on an industrial scale for the bio-leaching of uranium ores. However, the present standing of this new branch of hydrometallurgy, called biohydrometallurgy, is ready to be used by the industry. The tank leaching of uranium could be carried out industrially by using an adapted version of the conventional tank leaching equipment. At the present time the commonly known thio-bacteria *T. ferrooxidans* and *T. thiooxidans* are expected to be used in the leaching of uranium ores. If, however, the bioengineering of these microorganisms succeeds and more resistant (to hydrogen and heavy metal ions) and faster metabolizing bacteria are produced, a further progress to industrialization of this tank leaching technique will be achieved.

REFERENCES

1. Merritt, R.C., Extractive Metallurgy of Uranium, Colorado School of Mines Research Institute, Golden, 1 (1971).
2. Mattus, A.J., Jr., and Torma, A.E., *Metall, 34*, 33 (1980).
3. Mattus, A.J., Jr., and Torma, A.E., *Hydromet., 5*, 179 (1980).
4. Nicol, M.J., Needes, C.R.S., and Finkelstein, N.P., In: Leaching and Reduction in Hydrometallurgy, A.R. Burkin (Ed.), Institute of Mining and Metallurgy, London, 1 (1975).

5. Ibid., 12 (1975).
6. Hiskey, J.B., *Trans. Inst. Min. Met. Section C, 88*, C145 (1979).
7. Shabbir, M., and Robins, R.G., *Nucleus, 4*, 86 (1967).
8. Pinkney, E.T., *South African Ind. Chem.*, Nov., 264 (1956).
9. Ashbrook, A.W., In: Proceedings of the Short Course on Extractive Metallurgy of Uranium, R.T. McAndrew and W.C. Cooper (Eds.), University of Toronto, Toronto, 29 (1978).
10. Eligwe, C.A., Torma, A.E., DeVries, F.W., In: Proceedings of the Fourth Annual Uranium Seminar, Corpus Christi, Texas, Published by the American Institute of Mining, Metallurgical and Petroleum Engineers, Inc., New York, 65 (1980).
11. Lawes, B.C., *In Situ, 2*, 75 (1978).
12. Dutrizac, J.E., and MacDonald, R.J.C., *Miner. Sci. Eng., 6*, 59 (1974).
13. Colmer, A.R., and Hinkle, M.E., *Science, 106*, 253 (1947).
14. Silverman, M.P., and Ehrlich, H.L., *Adv. Appl. Microbiol., 6*, 153 (1964).
15. Kuznetsov, S.I., Ivanov, M.V., and Lyalikova, N.N., Introduction to Geological Microbiology, McGraw Hill Book Co., Inc., New York (1963).
16. Zajic, J.E., Microbial Biogeochemistry, Academic Press, New York (1969).
17. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.I., Bacterial Leaching of Metals from Ores, Technicopy Ltd., Stonehouse (1977).
18. Murr, L.E., Torma, A.E., and Brierley, J.A., (Eds.), Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, Academic Press, Inc., New York (1978).
19. Schwartz, W., (Ed.), Conference Bacterial Leaching, Verlag Chemie, Weinheim (1977).
20. Trudinger, P.A., and Swaine, D.J., (Eds.), Biogeochemical Cycling of Mineral-Forming Elements, Elsevier Scientific Publishing Co., Amsterdam (1979).
21. Ito, I., Bacterial Leaching, Kodansha Scientific Co., Tokyo (1976).
22. Trudinger, P.A., Walter, M.R., and Ralph, B.J., (Eds.), Biogeochemistry of Ancient and Modern Environments, Australian Academy of Science, Canberra (1980).
23. Ehrlich, H.L., Geomicrobiology, Marcel Dekker, Inc. (1981).
24. Miller, R.P., Napier, E., Audsley, A., and Daborn, G.R., *Inst. Min. Met. Bull., 674*, 217 (1963).
25. Mouret, M., *Rev. Ind. Miner., 50*, 415 (1968).
26. Barbic, F., Proc. Symp. on Recovery of Uranium, IAEA, Vienna, 279 (1971).
27. Nelson, H.W., Snow, R.D., and Keyes, D.B., *Ind. Eng. Chem., 25*, 1355 (1933).
28. Torma, A.E., *Adv. Biochem. Eng., 6*, 1 (1977).
29. Silver, M., *Can. J. Microbiol., 16*, 845 (1970).
30. Silverman, M.P., *J. Bacteriol., 94*, 1046 (1967).
31. Lowson, R.T., Bacterial leaching of uranium ores, A review. Australian Atomic Energy Commission, Lucas Heights, AAEC/E – 356 (1975).
32. Atkins, A.S., In: Ref. 18, 403 (1978).
33. Ebner, H.G., and Schwartz, W., *Z. Allg. Mikrobiol., 14*, 93 (1974).
34. Ebner, H.G., Schwartz, W., *Erzmetall, 26*, 484 (1973).
35. Rossi, G., *Resoconti Assoc. Miner. Sarda, 76*, 1 (1971).
36. Zajic, J.E., and Ng, K.S., *Dev. Ind. Microbiol., 11*, 413 (1970).
37. Tuovinen, O.H., *Atomic Energy Rev., 10*, 251 (1972).
38. Tomizuka, R., and Takahara, Y., Proc. IV. Symp. Internat. Ferment. Technol., 513 (1972).
39. Guay, R., and Silver, M., *Process Biochem.*, Dec–Jan., 8–11 (1980/81).

40. Harrison, V.F., Gow, W.A., and Hughson, M.R., *J. Met.*, *18*(11), 1189 (1966).
41. Bruynesteyn, A., and Vizsolyi, A., Paper presented at the SMEAIME Fall Meeting in Denver—November 18—20, 1 (1981).
42. Bosecker, K., and Wirth, G., In: Ref. 22, 577 (1980).
43. Guay, R., Silver, M., and Torma, A.E., *European J. Appl. Microbiol.*, *3*, 157 (1976).
44. Guay, R., Silver, M., and Torma, A.E., *Biotechnol. Bioeng.*, 727 (1977).
45. McCreedy, H.H., Harrison, V.F., and Gow, W.A., *Can. Min. Met. Bull.*, *66* (682), 135 (1969).
46. MacGregor, R.A., *Can. Min. Met. Bull.*, *59* (649), 1 (1966).
47. Matic, M., and Mrost, M., *South African Ind. Chem.*, *18*, 127 (1964).
48. Mashbir, D.S., *Min. Congr. J.*, Dec., 50 (1964).
49. Anonymous, *Eng. Min. J.*, Oct., 75 (1967).
50. Popoff, D.R., *Can. Nucl. Technol.*, July—Aug., 31 (1966).
51. Mrost, M., and Lloyd, P.J., In: Proc. of Symposium on Recovery of Uranium, IAEA, Vienna, 223 (1971).
52. Duncan, D.W., Bruynesteyn, A., *Can. Min. Met. Bull.*, *64* (704), 32 (1971).
53. MacGregor, R.A., *Min. Eng.*, *21*, 54 (1969).
54. Torma, A.E., and Bosecker, K., *Progr. Ind. Microbiol.*, *16*, 77 (1982).
55. Lundgren, D.G., and Dean, W., In: Ref. 20, 211 (1979).
56. Silverman, M.P., and Lundgren, D.G., *J. Bacteriol.*, *77*, 642 (1959).
57. Olson, T.M., Ashman, P.R., Torma, A.E., and Murr, L.E., In: Ref. 22, 693 (1980).
58. Finn, R.K., *Bacteriol. Rev.*, *18*, 254 (1954).
59. Tsao, G.T., *Biotechnol. Bioeng.*, *10*, 765 (1968).
60. Guay, R., Torma A.E., and Silver, M., *Ann. Microbiol.* (Inst. Pasteur), *126B*, 209 (1975).
61. Aiba, S., Humphrey, A.E., and Millis, N.F., Biochemical Engineering, Academic Press, New York, 133 (1965).
62. Garrells, R.M., and Christ, C.L., Solutions, Minerals and Equilibria, Harper and Row, New York (1965).
63. Pourbaix, M., Atlas of Electrochemical Equilibria in Aqueous Solutions, Pergamon Press, New York (1966).
64. Postgate, J.R., *Lab. Practice*, *15*, 1239 (1966).
65. Leathen, W.W., Kinsel, N.A., and Braley, S.A., *J. Bacteriol.*, *72*, 700 (1956).
66. Torma, A.E., Walden, C.C., and Branion, R.M.R., *Biotechnol. Bioeng.*, *12*, 501 (1970).
67. Tuovinen, O.H., Niemelä, S.I., and Gyllenberg, H.G., *Biotechnol. Bioeng.*, *13*, 517 (1971).

TECHNICAL ASPECTS AND PROBLEMS OF THE CHEMICAL AND BACTERIAL LEACHING OF LOW-GRADE AND REFRACTORY COPPER ORES

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INTRODUCTION

The theory and practice of dump and underground copper leaching are intensively studied nowadays. Research efforts in many countries are aimed at the development of ways and methods of modelling, optimization, intensification and prediction of the process indices in full-scale conditions. Possibilities for the improvement of mining-technological leaching conditions, wider application of dump and underground leaching and comprehensive utilization of the mineral resources are sought for.

GENERAL DESCRIPTION OF THE LOW-GRADE AND REFRACTORY COPPER ORES FOR LEACHING

By the technological features ores can be divided into the following basic types:

First type: ores, in which copper is represented basically by oxidized copper minerals or chalcocite. At this stage, practically all full-scale installations for dump and underground leaching extract copper from this type of ore. Depending on the content of pyrite and host rocks, soluble in sulphuric acid, the consumption of the latter changes but does not exceed 5 t/t of extracted copper.

Second type: mixed oxidized and sulphide ores. The copper here is represented by a wide range of copper-bearing minerals: oxides, carbonates, silicates, copper and iron hydroxides, secondary and primary copper minerals. The pyrite content does not usually exceed 2 %. The host rocks contain components, soluble in sulphuric acid.

Copper can be effectively extracted from sulphide and oxidized ores with a complex mineral composition by dump or underground leaching. Great difficulties are encountered in the extraction of copper from hydroxides which can be divided into three types (by their solubility): containing copper soluble in sulphuric acid; containing copper soluble in cyanides; and containing copper insoluble in these solvents (~50 % of the copper content of iron hydroxides). The composition of mixed oxidized and sulphide ores determines the specific technological features and problems of the application of chemical and bacterial leaching. For this type of ore the individual application of chemical or bacterial leaching is impossible, in view of the high content of minerals

soluble in sulphuric acid, and the low content of copper oxides and pyrite. Most suitable is the integrated chemical and bacterial leaching with the addition of sulphuric acid, which is conducive to the intensified bacterial oxidizing processes, the sulphur acid consumption for the extraction of 60—70 % of copper is 20—40 kg/t of ore.

Third type: sulphide copper-pyrite ores. Basic copper minerals are chalcopyrite and secondary sulphides. The characteristic feature of these ores is a comparatively high pyrite content (~10 %), which makes only biocatalytic oxidizing processes suitable for copper extraction. As a result of the bacterial oxidation of pyrite the required amounts of sulphuric acid and ferric sulphate are obtained, thus pH of the leaching solutions is maintained constantly below 2, while the concentration of the ferric iron may reach 10 g/l and more. The leaching proceeds in two stages: in the first stage the secondary sulphides are mainly removed and the copper extraction may reach up to 5 % per month (in pilot-plant conditions from ore with a size of —100 mm); in the second phase the chalcopyrite is removed and the copper extraction rate is about 1 % per month. The efficiency of leaching of this ore type depends mainly on the copper content, on the relative proportion of secondary sulphides and the ore amount.

Fourth type: low-grade ore with primary sulphides and low pyrite content. The copper here is represented basically by chalcopyrite. A typical feature of this ore is that due to the low chalcopyrite and pyrite content the possibility of copper extraction through bacterial leaching depends mainly on the sulphuric acid consumption for maintaining low pH values and on the rate of copper extraction. The leaching of chalcopyrite from these ores, even in laboratory conditions from ore with a size of —10 mm, proceeds at a low rate — below 1 % per month, which, combined with the requisite use of 20—30 kg sulphuric acid/t of ore makes the process economically unattractive at present.

TECHNOLOGICAL PROBLEMS OF THE CHEMICAL AND BACTERIAL LEACHING OF LOW-GRADE COPPER ORES

The copper extraction through chemical and bacterial leaching depends on many technological factors. If copper is represented by various copper minerals and the host rocks feature high solubility in sulphuric acid, the leaching proceeds in three stages. In the first stage it is necessary to supply a certain quantity of sulphuric acid to neutralize alkaline ore components and attain pH < 3.0. In this period minerals soluble in sulphuric acid are mostly removed. When pH of the leaching solution drops below 2.5, the intensive bacterial oxidation starts. As a result, copper is extracted both from the oxidized copper minerals and from the secondary sulphides. In the third stage mainly chalcopyrite and poor-soluble oxidized copper minerals and compounds are leached.

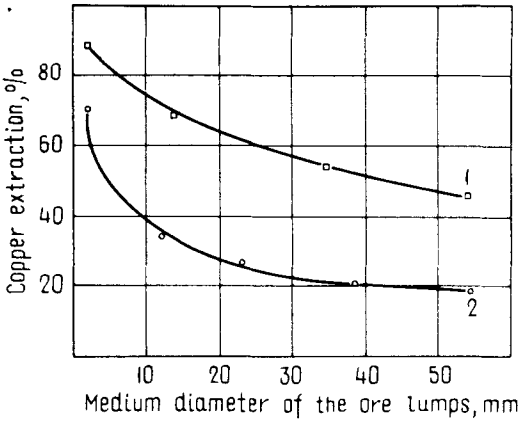

Fig. 1. Relationship between the ore lumps size and copper extraction kinetics. (Leaching time 150 days):

1 — oxide and secondary sulphide copper minerals in ore with increased porosity; 2 — ore with disseminated mineralization in dense host rocks

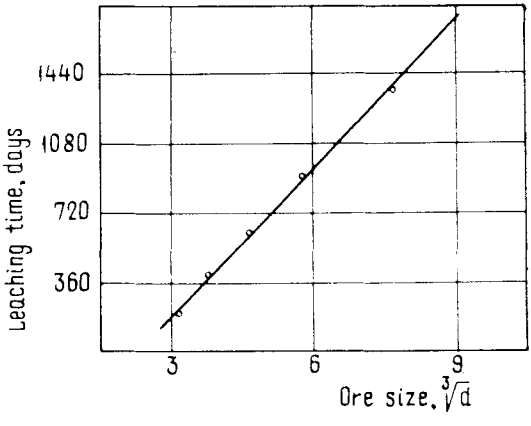

Fig. 2. Relationship between the ore size and the leaching time for 70 % copper extraction

(d — maximum ore lump size, mm)

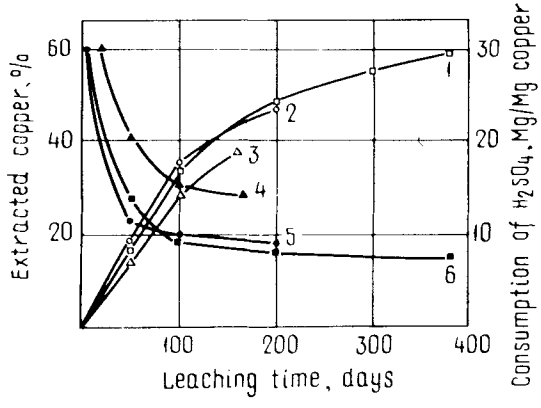

Fig. 3. Relationship between copper extraction and relative sulphuric acid consumption and leaching time of sulphide ore with different sizes:

1 and 6 — 400 mm; 2 and 5 — 200 mm; 3 and 4 — 100 mm

Copper from the secondary sulphides may be extracted at the highest rate under optimum conditions for bacterial oxidation. For example, in leaching ore sized 30—0 mm in a column 1 m high with a solution, containing Fe^{2+} = 4—8 g/l, *T. ferrooxidans* 10^6 cells/cm^3 and pH = 1.5—2.1 for 420 days 90 % of copper is extracted from chalcocite and covellite, 15 % from chalcopyrite and 80 % from the oxidized copper minerals. For the same leaching period about 55 % of pyrite is removed.

The rate of chemical and bacterial leaching is dependent to a great extent on the ore granulometry which determines the degree of solution seepage and the oxygen diffusion. As may be seen from the experimental data on Fig. 1, the copper extraction kinetics is proportional to the mineral surface exposed to the action of the leaching agents. The copper extraction rate from unit area depends also on the copper minerals' type. If the oxidized and secondary copper minerals predominate and the host rocks have a higher porosity, the rate of copper extraction decreases nearly by one order (Fig. 1). When the minerals are dispersed and compact host rocks are dense, leaching proceeds in two stages. The first stage featuring a high leaching rate, in which the copper extraction exponentially decreases until a certain ore size is attained, is followed by a stage with a lower rate of leaching, in which the extraction is reduced by one order. In other words, after a definite ore size is attained the prevailing factors, affecting the kinetics and degree of copper extraction, are micro- and macrostructure of the ore particles, host rock porosity, etc.

The experimental data from Table 1 (Figs. 2, 3) show, that the ore size increase entails not only the prolonged leaching time, but also the greater sulphuric acid use. This relationship is not always valid because of the essential influence of the ore mineralogy. For example, while leaching a sulphide ore tending to decay, a higher copper extraction is obtained at lower sulphuric acid use, provided the ore size is minus 400 mm rather than minus 100 mm (Fig. 3).

Table 1

Ore size effect on copper extraction and sulphuric acid consumption (deposit "Tzar Asen")

Classes, mm	Average ore lump diameter, mm	Time for 50 %-extraction of copper, days	Sulphuric acid consumption, kg/t of ore
−5	1	18	32
−20	9	145	22
−50	20	180	26
−100	45	250	35

Experimental conditions:

1. Height of ore material in a column — 2 m;
2. Leaching solution composition: treated after cementation + + H_2SO_4 5 g/l + *T. ferrooxidans* (10^6 cells/cm^3);
3. Sprinkling solution composition — 20 l/t per day.

The ore column height also affects to some extent the kinetics of copper extraction. This is particularly indicative of ores with the increased content of rock-forming minerals and iron hydroxides soluble in sulphuric acid, because the movement of the leaching solution causes considerable changes of pH and ion composition. For example, at the height of an ore column, taken in the "Medet" deposit (Bulgaria), equalling 11 m the hydrolysis of $Fe_2(SO_4)_3$ and accumulation of Fe^{3+} in the upper layers contribute to the better copper extraction if leaching is performed with a solution recycled after copper cementation, rather than with a regenerated ferric sulphate solution (Fig. 4). When the height of an ore column is 7 m, the copper extraction is constant, regardless of the iron form (Fe^{2+} or Fe^{3+}) in the supplied sulphuric acid solution of *T. ferrooxidans*. Up to this height, *T. ferrooxidans* oxidizes Fe^{2+} to Fe^{3+} and the copper extraction from sulphide minerals increases as a result of the slight change of pH and ion composition of the solution. Consequently, a specific integrated scheme for sprinkling "Medet" mine dumps was developed, which ensures 7 % extraction of copper at the sulphuric acid consumption being 30 kg/t of ore.

The pilot-plant experimental data show that the copper extraction from various groups of minerals is related to the ore column height (Table 2). As the height of an ore column increases, the extraction of copper from oxidized copper minerals diminishes as a result of a great difference between the initial and final sulphuric acid concentration. The lower extraction of oxidized copper in the first meters is attributed to the precipitation of Fe^{3+} on the mineral surface in the form of sulphate hydroxides. High solubility of iron hydroxides in the sulphuric

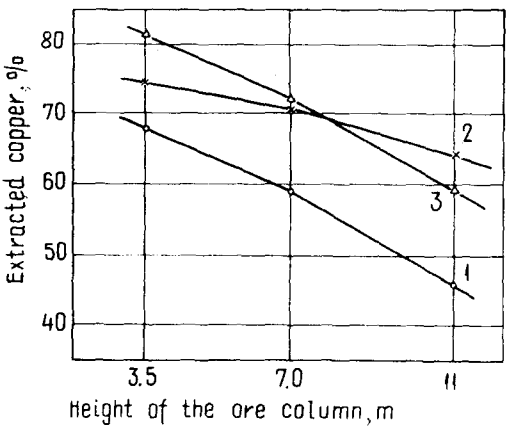

Fig. 4. Influence of the ore column height on the extraction of copper (sulphuric acid consumption 35 kg/t ore):

1 — H_2SO_4 5 g/l + *T. ferrooxidans*; 2 — H_2SO_4 5 g/l + + Fe^{2+} = 2—3 g/l + *T. ferrooxidans*; 3 — H_2SO_4 5 g/l + + Fe^{3+} = 2—3 g/l + *T. ferrooxidans*

acid and the emergence of zones with the increased pH lead to the higher rates of copper extraction if the leaching solution contains 5 g/l sulphuric acid, Fe^{3+} = 3—5 g/l and *T. ferrooxidans* 10^6 cells/cm^3 The uniform extraction of copper from the sulphide minerals may be obviously associated with the low height of ore in columns, and the favourable conditions for reduction-oxidation processes.

Table 2

Effect of the ore column height on the copper extraction

Height, m	Copper, extracted from minerals, %				Total copper released, %
	oxidized copper minerals	copper in iron hydroxides and oxidized bonded copper	secondary sulphides	chalcopyrite	
3	75	58	90	6.5	72.0
5	86	49	80	6.5	65.5
8	76	47	84	7.5	64.5
10	65	37	80	7.5	56.5

Experimental conditions:
1. Ore size: 75—0 mm;
2. Leaching time: 250 days;
3. Composition of the leaching solution:
H_2SO_4 5 g/l + Fe^{2+} 4—6 g/l + *T. ferrooxidans* 10^6 cells/cm^3.

POSSIBILITIES FOR INTENSIFICATION AND OPTIMIZATION OF THE CHEMICAL AND BACTERIAL LEACHING

The kinetics of the copper extraction depends on the type of copper minerals, the character of host rocks and the leaching solution composition. Therefore, the biocatalytic and chemical oxidizing processes, as well as the dissolution of copper minerals may be intensified in two ways:

a) by affecting the solid phase by thermal, electrical, oxidizing and other processes which could lead to a change of the structure and energy of the crystal lattice of copper minerals, as a result their solubility increases;

b) by increasing the redox reactive capacity of a leaching solution.

Since the leaching technology is mostly applied nowadays to low-grade ores with a low copper content, the intensification of copper extraction by affecting the liquid phase is believed most promising from technological and economic points of view.

The increased redox reactivity of a solution may be attained by establishing optimum proportions of H_2SO_4, O_2, Fe^{3+}, *T. ferrooxidans* concentrations or through the introduction of additional oxidizers.

For example, when leaching ores from tne "Medet" mine with the sulphuric acid solution of ferric sulphate, containing 2—3 g/l of Fe^{3+}, 10 g/l of H_2SO_4 and 10^5—10^6 cells/cm^3 of *T. ferrooxidans*, the leaching rate increases by a factor of 1.5 in comparison with a solution containing 5 g/l of sulphuric acid, but the sulphuric acid consumption to ensure 50 %-extraction of copper increases by a factor of 1.4 (Fig. 5). Reactions and structural changes in the host rocks, as a result of which the chemical and bacterial oxidizing processes are hampered, are to be blamed for this. For ores requiring small quantities of sulphuric acid and featuring high seepage properties, the high rate of copper extraction is observed even at the sulphuric acid concentration of 1 g/l.

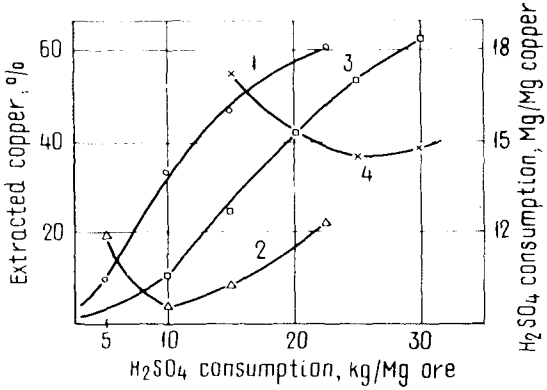

Fig. 5. Influence of the sulphuric acid concentration on the rate of bacterial and chemical extraction of copper from sulphide-oxide ores:

1 and 2 — H_2SO_4 5 g/l;
3 and 4 — H_2SO_4 10 g/l

The contribution of sulphuric acid into the intensification of chemical and bacterial leaching is determined by the comprehensive effect it produces. In leaching chalcocite and bornite with a solution, containing Fe^{3+} = 4 g/l and *T. ferrooxidans* — 10^6 cells/cm^3, it was established that at pH = 1.5 the rate of copper extraction is 90 mg/h, and at pH about 2.5 = 3 — 4.5 mg/h. The positive effect of low pH values may be attributed to the fact that no basic iron sulphates can be formed, which contributes to the quick diffusion and adsorption of O_2, Fe^{3+} and *T. ferrooxidans* on the sulphide mineral surface. It is known that the oxidizing processes in leaching depend strongly on the concentration of dissolved oxygen and its adsorption on the mineral surfaces while the oxygen concentration is controlled not only by pH values, but by the Fe^{3+} concentration, too. For example, in leaching chalcocite in agitated flasks it was established that if Fe^{3+} concentration is 2 g/l, the solution contains approximately 4 mg/l of oxygen, and if the Fe^{3+} concentration is 6 g/l the oxygen content is 2 mg/l. The catalytic role of Fe^{3+} in this case may be explained by its ability to increase the chemisorption of oxygen on the mineral surface.

The rate of copper extraction from copper sulphides enhances considerably when the *T. ferrooxidans* strains, adapted to solutions with low pH and showing the increased capacity for sulphur oxidizing,

are used. Utilizing such strains in the conditions of low pH and optimum O_2, Fe^{3+} concentrations, the copper extraction from both chalcocite and chalcopyrite increases.

To intensify the chemical leaching of secondary copper sulphides, Cl^- and NO_3^- ions can be used for leaching ore, in which copper is represented by chalcocite; the addition of Cl^- and NO_3^- ions in a concentration up to 0.5 g/l increases the rate of copper extraction by a factor of 1.2—1.4.

All the above data show that, regarding the ore minerological composition, the optimum proportions of H_2SO_4, O_2, Fe^{3+} and *T. ferrooxidans* concentrations should be found for leaching intensification.

TECHNICAL AND ECONOMIC ASPECTS, TECHNOLOGICAL PARAMETERS AND ORGANIZATION OF THE FULL-SCALE APPLICATION OF CHEMICAL AND BACTERIAL LEACHING

At present, geological prospecting includes not only high-grade (> 0.3 % of Cu) ores, but also low-grade and refractory ores. Therefore the actual metal reserves in deposits to be exploited have grown significantly (by 5 to 20 %). There are deposits, from which the copper may be extracted only by dump or underground leaching.

In most cases the construction of installations is considered economically expedient when more than 1,000 t of copper are to be produced annually. To attain this level 5 to 10 million tons of ore should be leached simultaneously.

The chemical and bacterial leaching efficiency is determined by many factors and the most important of them are:

— ore and host rocks mineralogy and chemistry; copper content of ores;

— mining-technical conditions; geological and hydrogeological characteristics of ores and host rocks;

— physical, physico-chemical and kinetic conditions of the process application;

— utilization of selective solvent, interacting weakly with host rocks.

Most suitable for leaching are ores, in which copper is represented as oxides and secondary sulphides and the host rocks have low alkalinity (sulphuric acid consumption does not exceed 25—30 kg/t of ore), high permeability and low degree of clogging.

While constructing new installations it is very important to know the time period required for the extraction of 60—80 % of copper, when the forecasting method of indices and technico-economic appraisal of the leaching efficiency is used. It is impossible to establish an integrated functional relationship between copper extraction and time, satisfying all possible cases of leaching. The experimental data analysis shows that with regard to the type of copper minerals, the character

of mineralization, the ore size and the ore column height the kinetics of extraction is different. When leaching ore veins where copper oxides and secondary sulphides predominate over the chalcopyrite and copper-containing iron compounds, the copper extraction from a unit area will be constant. In this case the extraction may be expressed by the relationship:

$$E = 1 - e^{-kt}, \text{ where}$$

E is copper extraction;
t is a leaching time;
k is a rate of copper extraction, determined experimentally for the given ore size.

If the copper minerals are evenly distributed and the host rocks are massive, then the initially high rate of leaching is gradually subsiding. In this case, the copper extraction in the individual periods can be expressed as follows:

$$E = at - bt^2, \text{ where}$$

t is a leaching time;
a and b are coefficients having different values for every leaching stage.

These relationships obtained during pilot-plant studies, may be helpful for predicting copper leaching parameters for full-scale plants only if the specific conditions of every mine, and particularly of ore size and leaching solution composition, are considered.

The low-grade ores mined from open pits and underground mines are actually leached in dumps. The spoil is stored on an impermeable terrain with a high mechanical stability or on specially allotted sites. For oxidized copper ores the thickness of a spoil layer should not exceed 6 m, and for sulphide ores — 15 m at a total dump height being 50—60 m. The increase of the dump height does not lead to a higher copper content of the pregnant solution. The leaching solutions containing sulphuric acid, Fe^{3+} and *T. ferrooxidans* are distributed over the dump surface by sprinklers, injection boreholes or irrigation trenches and ponds. The water recycling principle is used here and the solution consumption varies from 3 to 10 l/m^3 per hour. More often the pH of solution is controlled, and in some cases only the flow rate after cementation. When ferrous and ferric iron as well as aluminium and magnesium salts, are present, it is necessary to supply sulphuric acid to arrest sedimentation. It is experimentally established that Fe^{3+} concentrations up to 10 g/l do not signally diminish the leaching rate. The operation of the industrial installation at the "Vlaikov Vrah" mine (Bulgaria) shows that part of the iron settles down in the first few meters (2—4 m), as a result the Fe^{3+} content of the pregnant solutions during the last 5—6 years is constant (3—5 g/l). The iron sedimentation has a negative effect on the oxygen penetration in the dump; thus, *T. ferrooxidans* are spread only within a 5—8 m horizon from the surface.

To intensify leaching and ensure even distribution of the leaching solution, boreholes, drilled at a depth of 2/3 of the dump height, may be used. Compressed air is introduced in the borehole together with the leaching solution, which makes the leaching process more intensive. The rate of copper extraction increases also by introducing "rest" periods between consequent sprinklings.

Depending on the ore mineral and chemical composition copper extraction at dump leaching ranges from 5 to 20 % a year. The unit costs of the obtained copper are 2—3 times lower in comparison with copper produced by flotation, and depend basically on the copper content of ore, sulphuric acid consumption, copper extraction and installation capacity.

Underground leaching is employed for low-grade ore deposits, areas of pillars or tails left after underground mining. Underground leaching is used in the following cases:

1. Copper leaching in blocks without ore breaking. This method is applicable only for ores which have naturally high water permeability (permeability factor of the ore-bearing rocks no less than 0.5 m/day) and porosity no less than 5 %. No special preparation of the ore material is needed.

2. Leaching of unbroken ore blocks, with artificial enhancement of water permeability in the ore. This method is applied if the permeability factor is below 0.5 m per day and porosity is below 5 %.

3. Underground leaching of crushed and shrinkaged ores. It is used in deposits with low-efficient porosity (< 5 %) and low ore filtration properties ($K_f < 0.5$ m/day).

4. Combined methods of underground leaching.

An alternative of the underground leaching is the "*in situ*" leaching of deposits that have not been exploited by other means. In this case, the whole ore body is blasted and leaching is realized "*in situ*", or the leaching is made by boreholes without disturbing the ore massif.

Solutions obtained after dump or underground leaching contain from 0.3 to 2.0 g/l and more of copper, 2—30 g/l of Fe (including 3—5 g/l of Fe^{3+}), 5—6 g/l of zinc, 0.5—1.0 g/l of solids; pH is 1.5—3.5. The copper from the solutions is recovered by cementation, extraction or sorption.

Copper cementation is accomplished in different facilities regarding the type of the settler, solution composition, the required productivity. Most popular are cementing pans, cone cementators, rotating drum cementators, vats and tanks with mechanical agitation. The concentrate produced by cementation contains approximately 70—80 % copper and the copper recovery from the effective solution is 90—98 %.

The application of the sorption process for copper recovery from solutions with pH = 2—3 is restricted at present. This is due to the fact that ion-exchange resins adsorb also Fe^{3+}, which makes the technology for obtaining a high-grade final product more complicated. The method

can be applied successfully (after solution neutralization to pH = 4—5) in cases, when tail solutions are not utilized, but are treated and disposed into water bodies.

The great selectivity of extragents in comparison with the sorbents makes it possible to obtain high-grade copper at low costs. Cathode copper may be obtained from solutions containing more than 1 g/l of copper at pH = 1.5—3, if extragents, such as β-oxybenzophenoximes (ABF, Lix—64N) are used. The method of solvent extraction of copper favours the maximum sulphuric acid utilization in leaching, high and selective copper extraction, the limited accumulation of additional quantities of ferrous iron in the circulating solutions, the obtaining of final products with a high copper content (99.9 %).

CONCLUSION

High efficiency of copper ore leaching makes this method most promising for expanding the mineral raw-material base.

This urges to seek new more efficient technologies based on the wide use of mechanization, automation, management and control of the process of chemical and bacterial copper leaching.

REFERENCES

1. Merazchiev, G., Transactions of the NIPRORUDA Institute, Sofia, 156 (1975) (in Bulgarian).
2. Shchirkov, P., Merazchiev, G., Semkov, N., and Nestorova, P., *Rudodobiv*, *30*, 1, 15 (1975).
3. Shchirkov, P., Merazchiev, G., Semkov, N., Nestorova, P., and Vulkova, M., Transactions of the NIPRORUDA Institute, Sofia, 176 (1975) (in Bulgarian).
4. Khalezov, B.D., and Nezhivykh, V.N., Transactions of the UNIPROMED' Institute, Sverdlovsk, *20*, 129 (1977) (in Russian).
5. Khalezov, B.D., Transactions of the UNIPROMED' Institute, Sverdlovsk, *22*, 104 (1979) (in Russian).
6. Khalezov, B.D., and Chudakov, V.G., *Tsvetnaya metallurgiya*, No. 5, 32 (1973).
7. Lunev, L.I., and Rudakov, I.E., Underground Systems of Metal Leaching, Tsvetmetinformatsiya, Moscow (1974) (in Russian).
8. Harris, J.H., Proc. Austral. Inst. Min. Met., *230*, 81 (1969).

TECHNOLOGY OF BACTERIAL DUMP LEACHING OF METALS FROM ORES

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DESCRIPTION OF DUMP LEACHING TECHNOLOGIES

The earliest publications on the production of copper from copper pyrite ores by dump leaching at Rio-Tinto mine in Spain date back to 1752 [1—4]. The ore contained 48 % sulphur and 1.5 % copper.

At the beginning of the present century, the leaching practice at Rio-Tinto reached considerable proportions, when nearly 20 million tons of ore in separate dumps of 100,000 tons each, was irrigated at a time.

The dumps were spread over a natural water-confining slope in the form of terraces. Spaced at 5 to 10 m from one another, culverts (canals) of 300 x 300 mm cross-section were made out of large pieces of ore, and were arranged lengthwise and transverse to the dumps' foundations. At points of culvert intersection, exhaust pipes were installed spaced 12 to 15 m from one another. These devices provided natural ventilation of the dumps, encouraging oxidation processes whereby the ore temperature rose to 50—60 °C. Before being dumped, the ore was crushed to the size of 50—100 mm. The height of the dumps varied from 6 to 12 m. The dumps were then covered with a layer of fine ore for a uniform percolation of solutions. Water was supplied to the dumps by way of lengthwise-transverse ditches. The dumps were kept saturated with solutions as long as the leaching of copper was sufficiently intensive, then irrigation would be shifted to other dumps, while the surface of the first one was levelled.

The solutions contained, g/l: copper — 1—4, ferrous iron — 20, ferric iron — 1, free sulphuric acid — 10.

Upon the extraction of copper from them, the solutions were reused for leaching. The water consumption rate amounted to 570 l per 1 ton of ore a year. During the first two years, 60 % of copper was extracted; in the course of 7—9 years, this figure increased to 80—85 %. Some of the ore dumps, containing most "refractory" (i.e. difficult-to-leach) copper minerals, were leached for up to 30 years. The residual copper content of the ores reached 0.3 %. The leaching tailings, containing up to 0.3 % copper, were used as pyrite basic material.

In 1900 and later in 1916, dump leaching operations were undertaken in Bisbee for ores containing 1.33 % Cu [1].

The test dump contained 9,487 tons of run-of-the-mine ore spread over an inclined site, the maximum and minimum height of the layer being 8 m and 1.5 m, respectively. 68 tons of copper, i.e. around 26.9 %, was extracted during the first seven months. The net cost of 1 ton of copper was 75 dollars.

The total amount of copper extracted roughly had the following breakdown by years, in %; 1st year — 40; 2nd year — 20; 3rd year — 15. Even though the installation was experimental, copper production was profitable.

In 1924, Felps Dodge Corporation based at Copper Queen (Bisbee) applied a process of dump leaching of copper from the ore deposit of Sacramento Hill. Two dumps were formed, of 2 and 1.4 million tons of ore, the copper content being 0.89 and 0.68 %, respectively. The dumps' measurements were: 548 x 213.4 m and 426.7 x 182.9 m, maximum height 10.4 m and 22.9 m, the gradient 1—5 and 6—10 %.

The dumps were irrigated with the aid of ponds by mine drainage solutions at the rate of 2,080 m^3 per day. Sulphuric acid was added at the rate of 0.09 kg per 1 m^3. The composition of pregnant solutions was as follows, g/l: copper —4.79; ferrous iron — 1.44; ferric iron — 5.39; acid — 0.06—3.6. Water evaporation losses measured 15—18 %.

In the course of 5 years of operation from the two dumps that originally contained 24,690 tons of copper, 12,430 tons of Cu (50.35 %) was extracted, i.e. an average of 10 % a year.

In 1929, copper leaching operations were started on a dump containing 9.1 million tons of stripped rock, its copper content being 0.26 %.

Copper was extracted from solutions in tanks and launders by cementation. The composition of cementation copper was as follows, %: Cu — 70; SiO_2 — 1.2; Al_2O_3 — 3.0; Fe — 6.8; CaO — 0.3; S — 1.2; H_2O — 23.0. The successful application of dump leaching of poor ores and of stripped rock in Bisbee served as the basis for further development of this copper production technique.

At Douglas (Arizona, USA) the afore-mentioned Felps Dodge Corporation undertook experimental leaching of copper from a dump containing 25 tons of sand tailings that were obtained following their enrichment in a Dorr classifier. There was 0.52 % copper in these products, including 0.19 % oxide copper. The height of the dump was 2—2.5 m. Results of the leaching were promising enough, and therefore the dump leaching of oxide ores was started at the old Burro Mountain Mine in Tyrone. The dump contained 20,722 tons of ore and 2.7 % copper. The ore was spread over a slope in the form of terraces, on a specially prepared site made up of clay rocks impregnated with fuel oil. The ore was irrigated by acidified solutions with the aid of ponds, the copper being extracted from the solutions in launders by cementation, whereupon the solutions were again fed to the dump. During the first year, 271.15 tons of copper was extracted, i.e. 24.1 %.

In Russia, dump leaching was first realized in 1874 at Kedabeck Mine (Caucasus) [4]. Two groups of dumps were formed. One of them consisted of pre-roasted sulphide chalcopyrite ore, the other — of the same ore, but not roasted. For roasting, the ore was put through a sorting grizzly and dumped onto firewood. Roasting continued for

2—3 months. Then the ore was spread over sites inclined at 5—8°, and culverts of Rio-Tinto type were arranged.

The dumps were irrigated with water from the mines and with tailing solutions following cementation. Irrigation of the ore was conducted by means of ponds for 10 to 30 days, whereupon the next site was irrigated. Operations were conducted in the warm season, from mid-March to mid-November. Every year, at the close of the season, the ore was shovelled up. During 60 years of operation (1874—1934) 14,000 tons of copper was produced by leaching at Kedabeck, i.e. its extraction from the ore amounted to 60 %. Dump leaching at other mines took place in later periods. For example, in 1939—1941, copper dump leaching experiments were undertaken in the Urals, involving dumped ores at Pyshma, Belorechensk and Novo-Levinsk.

Two dumps were subjected to leaching at the Belorechensk Mine in 1941. The copper content of the ore was up to 1 %. The ore was irrigated with the mine drainage water, sprayed over the dump. 16 % of copper was extracted during the seven months of tests.

A dump with about 1,000 tons of ore was subjected to leaching at the Pyshma Mine in 1940. The ore was spread over a base composed of a 100 mm layer of crushed stone, a 100 mm layer of concrete, and of a boarding. The dump dimensions were 16 x 8 m (top) and 22 x 15 m (bottom), the height, 3 m. Vertical and horizontal ventilation ducts of 300 x 300 mm cross-section were provided.

The ore was leached with solutions containing 5—10 g/l sulphuric acid. Irrigation continued for four months, which allowed to extract 13.5 % of copper. In 1941, however, dump leaching operations in the Soviet Union were suspended because of the Second World War, and were resumed as late as 1972 (Blyavin Mine), 1973 (Nikolaev Mine), 1975 (Volkov and Kounrad Mines), 1979 (Kal'makyr Mine).

By now, the process has been introduced at over 20 mines of the world, including four in the Soviet Union (Table 1) [5—8]. In the mid-1970s, world copper production using a dump leaching technique was 280,000 tons, the United States accounting for 230,000 tons. So vast a scale of production has been made possible due to an enormous quantity of ore being processed at a time. Thus, quantities of rock being leached at a time at the US mines are as follows, million tons: Bingham Canyon — 200; Silver Bell — 27; Butte — 30; Bagdad — 40; Bisbee — 43. The amount of copper produced is 70; 3.5; 6; 7; 6.4 thousand tons a year, respectively. The ores being leached differ in the nature of mineralization and in copper content. For example, Bingham Canyon ores are represented by typical sulphide disseminations, mainly chalcopyrite, the copper content of the dumped ore being below 0.4 %; at Bagdad, the ores are low-grade oxide; at Butte — low-grade mixed ores.

There exist various technological schemes of dump leaching, but, in principle, all of these include the following basic operations: the

Table 1

Data on dump leaching of copper at different mines

Mine, deposit	Principal copper minerals of the ores	Ore reserves, million tons	Final product, production capacity, thousand tons
Silver Bell, Arizona, USA	Chalcocite, chrizocolla	27.0	Cement copper, 3.5
Butte, Montana, USA	Chalcocite	30.0	Cement copper, 6.0
Bagdad, Arizona, USA	Chrizocolla, malachite, azurite	40.0	Copper powder, 7.0
Esperanza, Arizona, USA	Chalcocite, chalcopyrite	17.0	Electrolyte copper, 5.4
Yerington, Weed Heights, Nevada, USA	Chrizocolla	27.0	Cement copper, 22.0
Inspiration, Arizona, USA	Chrizocolla, azurite, malachite	27.0	Electrolyte copper and cement copper, 64.0
Bingham Canyon, Utah, USA	Chalcopyrite	3,629	Cement copper, 70.0
Bisbee, Arizona, USA	Chalcocite, azurite, malachite	43.0	5.4
Castle Dome, Arizona, USA	Chalcopyrite, chalcocite	44.0	—
Copper Cities, Arizona, USA	Chalcopyrite, chalcocite	—	—
Bluebird, Arizona, USA	Oxide ores	—	Electrolyte copper, 5.4
S.-Xavier	Oxide ores	—	Cement copper
Lavender, Arizona, USA	—.	—	8.8
Ram Jungle, Australia	Chalcopyrite, chalcocite, bornite	—	Cement copper, 1.2
Sierro-Verde	Oxide ore		33
Rio-Tinto, Spain	Chalcocite, chalcopyrite	Not available	Cement copper, 8.0
Elephant Mines, Rhodesia	Oxide ore	Not available	Electrolyte copper, 0.08
Ray, Arizona, USA	Chalcocite	169.0	Cement copper, 24.0
		Total:	279.78

leaching of copper from a dump, extraction of copper from solution, recirculation of tailing solutions.

Figs. 1 and 2 show the technological scheme of copper dump leaching at the Bingham Canyon Mines [5, 9, 10—12]. The peculiarity of the scheme was that part of the solutions, once copper was extracted from them, was subjected to removal of iron and aluminium salts. This operation yielded leach solutions that were cleaner and enriched with up to 3 g/l ferric iron, which, ultimately, helped to boost the leaching process. However, this treatment is costly, and according to the available information, it has been discarded. The characteristic feature of the installation is that the launders normally used for copper cementation, are replaced by cone-type precipitators.

Fig. 3 shows the dump leaching process scheme used by the mines at Bluebird. This was the first enterprise ever to discard the copper

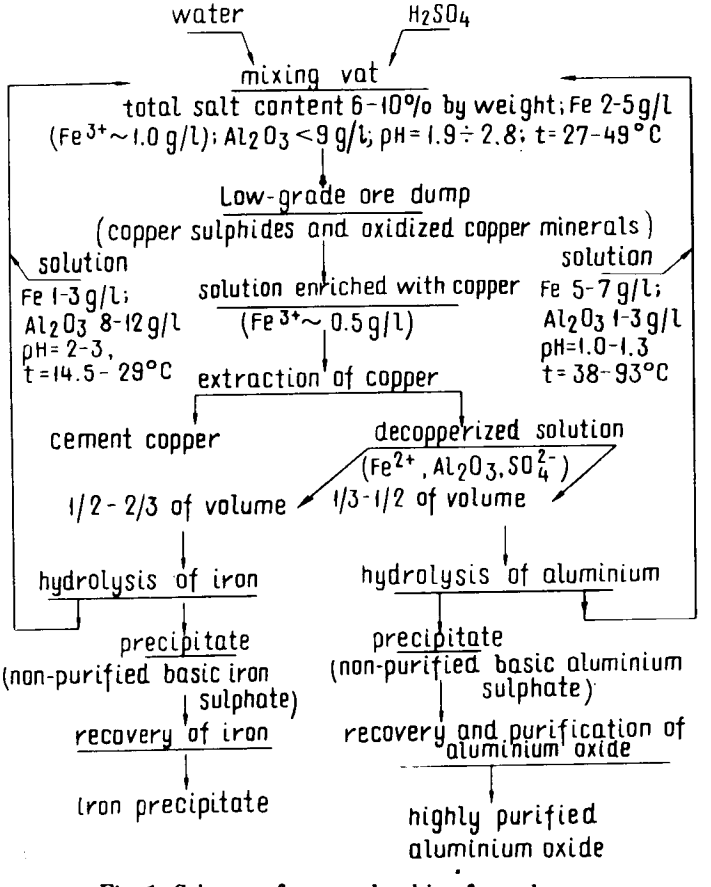


Fig. 1. Scheme of copper leaching from dumps at Bingham Canyon Mine

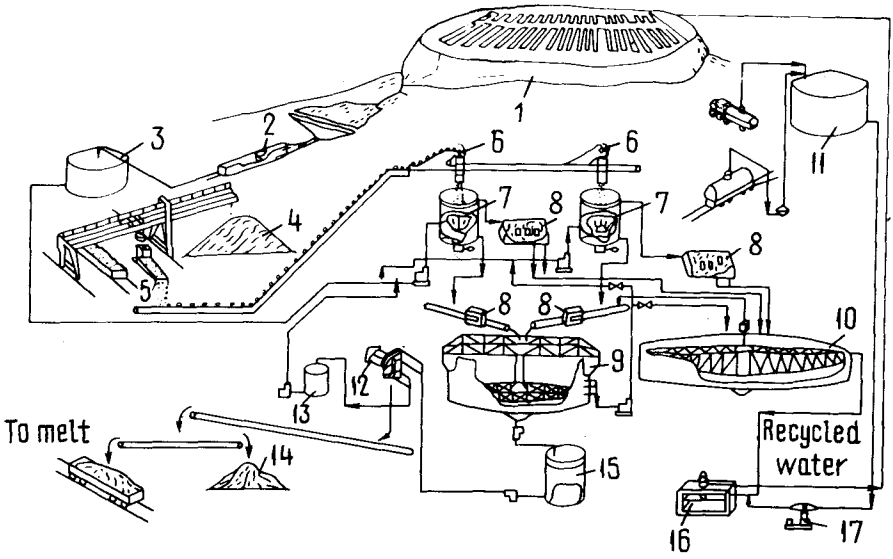
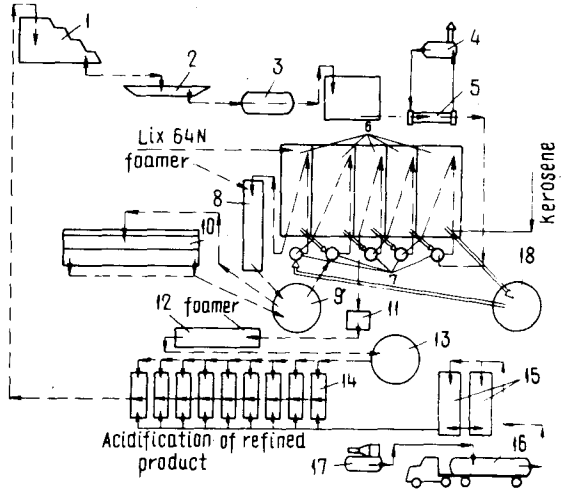


Fig. 2. Copper precipitation shop at "Utah" plant of Bingham Canyon Works, Kennecot Copper Corp.:

1 — ore pile base; 2 — launder; 3 — head tank; 4 — iron scrap; 5 — feeder; 6 — tipper; 7 — cone-type cementing machine; 8 — screen; 9 — thickener; 10 — settling basin; 11 — sulphuric acid tank; 12 — pressure filter; 13 — sump; 14 — store-room; 15 — intermediate capacity; 16 — main pumping station; 17 — acid pump

Fig. 3. Technological scheme of "Bluebird Works":

1 — dump; 2 — pond for concentrated solution; 3 — filter; 4 — boiler; 5 — heat exchanger; 6 — settling basins; 7 — mixers; 8 — flotation machine; 9 — tank for electrolyte; 10 — electrolysis baths; 11 — sump for refined product; 12 — flotation unit; 13 — tank for refined product; 14 — mixers; 15 — tanks for storing acid solutions; 16 — trucks for carrying acid solutions; 17 — compressor; 18 — tank for saturated organic extracting agent



cementation technique in 1968, and to introduce copper extraction with subsequent production of cathode copper from re-extracts [13—19].

Leaching is applied to poor ore containing 0.5 % copper. Copper in this ore is represented mainly by chrizocolla. The dumps are positioned on a natural ground provided with ceramic perforated pipes for draining the solutions. 13 dumps have been formed, containing 120,000 tons of ore each, their maximum height was 60 m. Solutions are supplied through PE pipes, the ore being irrigated with the aid of needle-type sprinklers. Daily solution input for each dump is up to 1,100 m^3. Irrigation lasts for 2—2.5 months, whereupon the upper ore layer is ripped and left to dry up for 1 month. The copper-containing pregnant solution is filtered and passed through a heat-exchanger to maintain the temperature of 27 °C, while the copper is extracted. Daily capacity of the installation is up to 11,500 m^3 of solution. To recover copper, extractors of the mixer-settler type are used. The copper extraction process consists of three stages (with a 7 % Lix-64 solution in kerosene); copper is then re-extracted in two stages with used electrolyte, then the organic phase is washed at the third stage. The ratio of the organic and water phases at the extraction stage is 1:1; at the re-extraction stage, 4.5:1. Electrolysis of re-extracts is conducted in 48 baths furnished with lead anodes, the combined power of rectifiers being 1,500 kW. Every 6 days, cathode copper is collected, the metal content being 99.9 %. Current efficiency is 88.9 %, voltage in the bath, 2 V. Maximum electricity consumption is 2,440 kWh/t. With an annual copper production being 6—7 thousand tons, reagent consumption amounts to 60 tons, that of kerosene, 900 tons, whereas the total cost of the extraction and electrolysis is US $ 260 per 1 ton of copper.

Fig. 4 shows the generalized scheme of dump leaching sites of low-grade oxide, mixed and sulphide, and of high-grade difficult-to-dress sulphide copper-zinc ores. In mixed ores (Nikolaev Mines, USSR), 77.5 % of copper is represented by oxide compounds: chalcanthite ($CuSO_4 \cdot 5H_2O$), malachite ($CuCO_3 \cdot Cu(OH)_2$), azurite ($2CuCO_3 \cdot Cu(OH)_2$), and partially by chrizocolla ($CuSiO_3 \cdot n\ H_2O$); 15 %, by secondary sulphides: chalcocite (Cu_2S) and covellite (CuS), and 7 %, chalcopyrite ($CuFeS_2$).

In the dump of low-grade sulphide ore, copper is represented by: oxide compounds — 6.5 %, secondary sulphides — 22.6 %, and primary sulphides — 71 %. The dump of high-grade ores contains 5 % oxide copper compounds, 36.5 % secondary and 58.5 % primary sulphides.

In all cases, zinc is represented by zincite (ZnO) and sphalerite (ZnS), the phase ratio being similar to that of copper. Table 2 shows the chemical composition of ores [23—25].

All oxide minerals of copper and zinc are known to be soluble in the water solutions of sulphuric acid. The process of dissolution is a diffusive one. The products of dissolution, such as CO_2, SiO_2, Cu_{met} provide additional resistance slowing down the rate of disintegration.

Table 2

Chemical composition of ores

Dump	Component, %						
	Fe	S	SiO_2	Al_2O_3	CaO	MgO	As
No. 1	34.00	38.50	11.80	7.27	0.07	—	0.06
No. 2	16.10	16.00	43.90	8.00	0.98	0.98	0.06
No. 3	31.9	38.2	7.37	0.33	—	—	0.14

Sulphide minerals are oxidized in an acid medium only in the presence of oxidants (mainly, Fe^{3+} and bacteria). Intermediate products of reaction, e.g. CuS with chalcocite, $CuFeS_2$ with bornite, or final products like elemental sulphur with covellite, sphalerite or chalcopyrite are the substances that are chemically stable and hard-to-remove from the surface. They sharply inhibit the process of oxidation and dissolution of copper and zinc minerals. Oxidation of sulphide minerals may be accelerated either chemically or physically. In the former case, in the course of leaching, application of solutions containing ferric sulphate and enriched with atmospheric oxygen, as well as iron and sulphur-oxidizing microorganisms may be recommended. To obtain fresh mineral surface, blasting may be used, resulting in ore crushing and redistribution in the dump.

Mixed ore is spread over a natural clayey bedding, while sulphide ore is placed on a compacted layer of fine pyrite ore, 30 to 50 cm thick, with a 2—8° gradient. The height of the dump varies from 15 to 25 m. The copper is leached by recycle solutions, acidified with sulphuric acid whose content does not exceed 1—2 g/l. The solutions are supplied through PE pipes and are sprayed in ponds arranged on the dump surface. The area of each pond is from 400 to 800 m^2. The irrigation density is 30—40 l/t of ore. The interval between two irrigations lasts 4—6 days [26]. During the first two years, mixed ores only were irrigated. This was followed by the leaching of sulphide ore dumps. The dumps were irrigated as follows: after cementation, acidified solutions were fed to a mixed ore dump, then, on to a low-grade sulphide ore dump, and, finally, to a high-grade sulphide ore dump. As a result, copper content of solutions increases by 1.5—3 times. This technique also allows to increase the ferric iron content of effluents from oxide ore dumps, and to reduce 2—3 times the volume of solutions subjected to cementation.

Using this technique in the first year to irrigate the first two dumps, the copper concentration was raised from 0.8—1 to 1.69—1.9 g/l.

Annual extraction of copper averaged 10—12 % (from mixed ores) and 7—8 % (from sulphide ores). Ore soaking, seepage and evaporation losses accounted for 15—20 % of the daily amount of solutions used for cementation. These losses are offset by mine drainage solutions. Table 3 shows operating data on the installation.

.Dump leaching is conducted during warm months of the year from April to October, i.e. 180—200 days a year. As a result of solution recycling, other elements are also accumulated and by the end of the season, their content may be, g/l: iron, 40; Al_2O_3, ~5; CaO, ~0.5; MgO, ~5.

130 to 500 g/l iron is added to the solutions due to cementation during a single season. However, its content does not exceed 40 g/l (for other mines of the USSR, this figure is 5—15 g/l max). The reason is that in the course of mass exchange between the solution and dumped ore, a "self-purification" of the solutions takes place. The excess iron precipitates in the dump in the form of hydroxides, basic ferric sulphates [$Fe_2(SO_4)_3 \cdot Fe_2O_3$] [34] and even in the form of a jarosite group (e.g. $K(Fe_3(SO_4)_2 \cdot (OH)_6$) [36]. The sample testing of various mines showed that in the course of dump leaching, the iron content of the top layers may sometimes increase 2—2.5 times.

In the process of leaching, the ore becomes finer, the percolation rate slowing down 3 to 6 times in 3—4 years compared to 0.5—1 m/h in the first year of operation. To recover original percolation rate, every year at the beginning or at the end of the season, a 0.3—0.5 m thick top layer of the dump is bulldozed off (2—3 m thick layer in the 5th or 6th year of operation is removed by mechanical shovel). For the same purpose, holes spaced at 8 x 8 m or 10 x 10 m and 5—6 m deep are drilled annually and the ore is blasted. As a result, the desired rate of solution percolation and the optimum leaching regime with regard to density and frequency of irrigations are maintained. The copper-containing solutions are drained in the ponds, where the sludges settle, the sludge content of the solutions being up to 0.3—1.2 g/l. It is noteworthy that the sludge removal is maximum during the first years of operation, and decreases to the minimum value in subsequent years.

The ponds for intermediate solutions are normally dug out in clay and have a storage capacity of 2—3 thousand m^3. Two ponds were made of an acid-resistant concrete having special water-proofing characteristics. One of these accumulated the head solutions, the other, the tailing ones. Copper was extracted from solutions in drum-type cementing machines of a Soviet make (Fig. 5) [21, 22]. Efficiency of copper extraction from the solutions was up to 95—98 %. For cementation, it is best to use scrap not thicker than 0.4—0.5 mm, keeping the rate of scrap feeding at a constant level of 0.8—1.0 t/m^3 of the solution in the apparatus (Fig. 6). Usually, packages of detinned body-stock, transformer steel or roofing iron are utilized [27].

A drum-type cementing machine has a number of advantages over other devices used to extract copper from solutions (launders, baths, cones). With a drum-type cementing machine, the rate of copper extraction increases 15 times, because the contact between the solution and the scrap lasts only 4 to 6 minutes. A continuous process, fully mechanized, is, therefore, possible. As a result, scrap consumption diminishes 1.5—2 times, and capital costs involved in the construction of the cementation installation are reduced considerably.

At the Nikolaev Mine, cement copper is precipitated in vertical settling tanks; at other mines, in conventional thickeners. The settling rate of the finest copper particles ($\leqslant$ 0.01 mm) is 1.2 cm/min. Combined losses (with draining) reach 0.5—0.6 %, and are represented by fractions of 0.006 mm. The copper product is sufficiently fine (see Table 3).

Table 3

Granulometric composition of the copper product

Particle size, mm	Percentage	Particle size, mm	Percentage
+0.177	5.60	+0.015	13.40
+0.102	35.50	+0.010	1.30
+0.029	37.10	—0.010	3.5

The chemical composition of cement copper is as follows, %: Cu, 77—86; Fe, 4—6; CaO, 0.1; SiO_2, 1.7; MgO, 0.3; Al_2O_3, 0.75. When discharged from the settling tank, copper comes into contact with atmospheric oxygen, and up to 15—20 % of it is oxidized. From the settling tanks, copper is discharged once or twice a month with a grab, put in containers and hauled to a smelter where it is smelted direct to produce blister copper or matte, in converters or in combination with copper concentrates, respectively [35].

Recycle solutions (Table 4) accumulate up to 3—7 g/l zinc. Industrial tests were carried out to extract zinc from solutions. Part of the solutions (about 20 %) underwent a zinc extraction procedure. The solutions were joined with a precipitating agent in a reactor under conditions of continuous stirring for 8—10 minutes. The zinc residue was separated from the solution in a settling tank until the solid-to-liquid ratio reached 1:4—1:5. The pulp was then supplied to the fluidized bed fern where a 4 % moisture concentrate was obtained, the zinc content being 45—50 %. Concurrent with this, the zinc concentrate was enriched with the extracted cadmium and indium. Following the extraction of zinc, cadmium, and indium, the solutions were mixed with those that underwent cementation, and the mixture was fed to the dump.

Thus, the process used for copper dump leaching at the Nikolaev Mine allows an extraction of several metals from copper-zinc ores.

CRITERIA JUSTIFYING THE APPLICATION OF DUMP LEACHING

Introduction of dump leaching starts with a geological survey of the deposit resource base.

Until recently, the geological prospecting of the deposits was carried out without taking into consideration the hydrometallurgical methods for processing the ores.

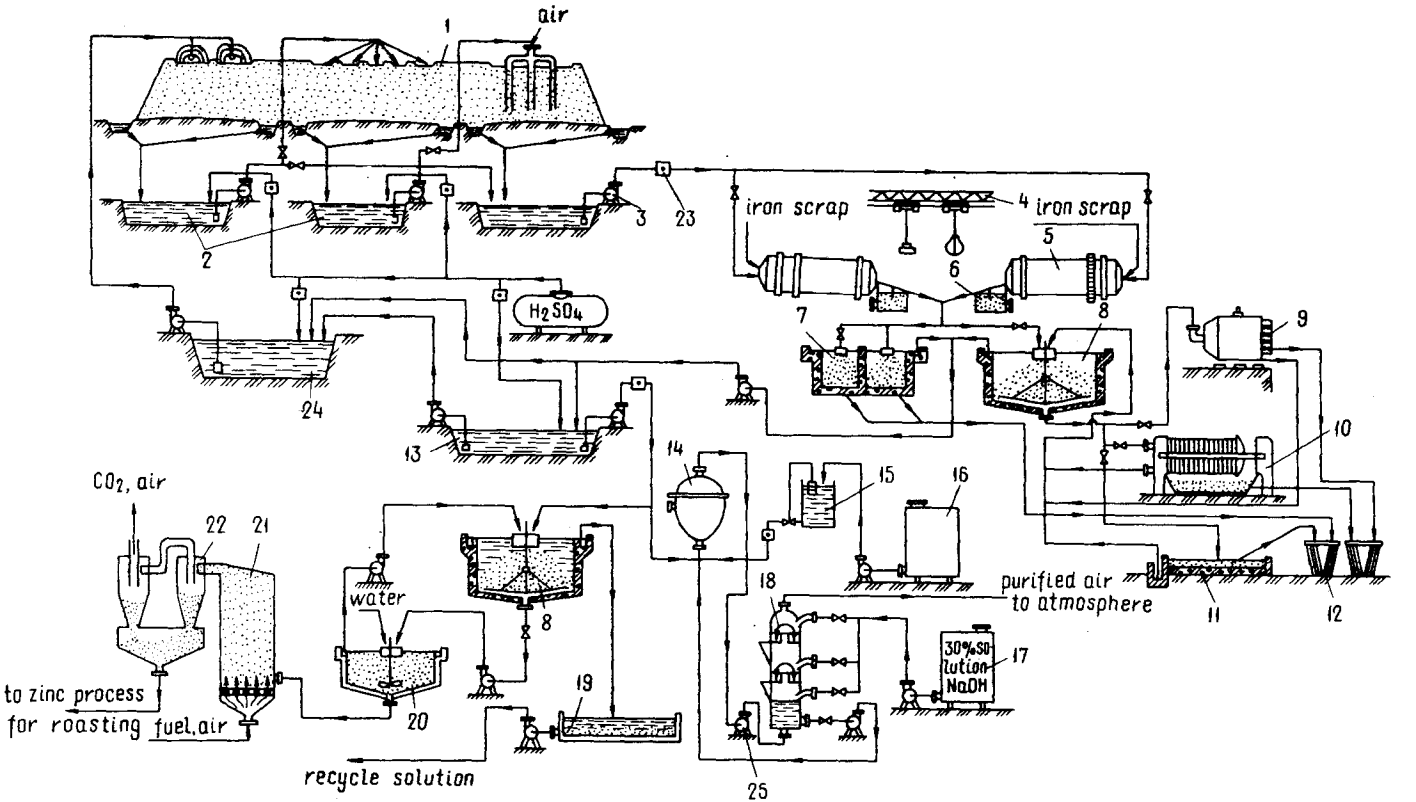

air
1
2
3
23
iron scrap
4
iron scrap
5
6
7
8
9
10
H_2SO_4
24
13
14
15
16
CO_2, air
22
21
water
8
18
purified air
to atmosphere
11
12
30% Solution NaOH
17
to zinc process
for roasting
fuel, air
20
19
recycle solution
25

Fig. 4. Scheme showing hardware linkage of copper ore dump leaching sites (USSR):

1 — ore dumps; 2 — ponds with head solutions; 3 — pumps; 4 — overhead travelling crane; 5 — drum-type cementing units; 6 — traps for small items of scrap; 7 — copper settling basins; 8 — copper thickeners; 9 — centrifuges; 10 — pressure filters; 11 — ponds for precipitate drying; 12 — containers with cement copper; 13 — sumps for zinc solutions; 14 — reactor for zinc precipitation; 15 — discharge tank for reagent; 16 — reagent storage; 17 — tank for alkaline solution; 18 — column for gas neutralization; 19 — pond for recycle solutions; 20 — repulping units for precipitate washing and settling; 21 — fluidized bed furnace; 22 — multiclones; 23 — flow-gauges; 24 — leaching solution preparation; 25 — fan

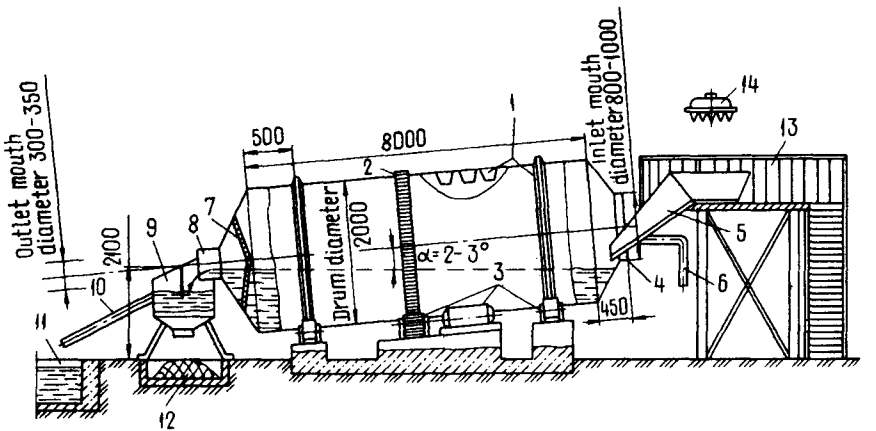


Fig. 5. General view of cementing unit:

1 — drum with rods; 2 — ring gear; 3 — electric drive; 4 — inlet mouth; 5 — charging launder; 6 — feed pipeline; 7 — screen; 8 — outlet mouth; 9 — scrap trap; 10 — draining pipeline; 11 — settling basin; 12 — scrap receiver; 13 — charging platform; 14 — magnetic washer

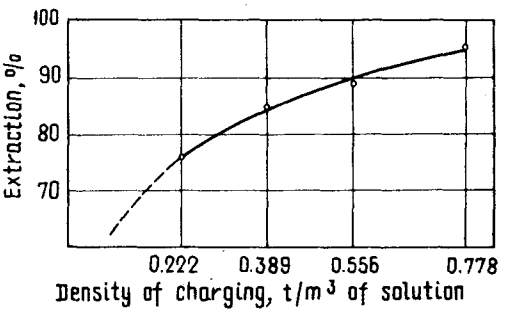


Fig. 6. Relationship between efficiency of copper extraction and density of drum charging with scrap

Table 4

Dump leaching of copper at the East Kazakhstan integrated copper smelter and chemical plant

Description	Year of operation				
	1976	1977	1978	1979	1980
Composition of head solutions (following leaching), g/l:					
copper	1.12*	0.89	0.51	0.81	0.55
zinc	3.32	7.48	6.99	5.29	6.01
ferrous iron	22.06	27.79	19.75	23.38	29.83
pH	2.2	2.83	2.2	2.3	2.2
ferric iron	2.94	2.3	1.71	1.85	1.6
Composition of tailing solutions (following cementation), g/l:					
copper	0.17	0.15	0.1	0.15	0.08
zinc	3.32	7.48	6.99	5.29	6.01
ferrous iron	25.9	28.9	20.7	25.5	31.5
pH	3.1	3.4	3.1	3.3	2.9
ferric iron	0.49	0.6	0.38	0.46	0.36
Extraction of copper from solutions, %	85	83	80	82	85
Specific consumption of scrap iron (t/t copper)	1.4	1.6	1.7	1.8	1.5
Sulphuric acid consumption (t/t copper)	0.31	0.84	2.4	1.2	1.4
Copper content of precipitate, %	86	85	77.2	78	79

* — Copper content in the first year of operation (1973) was 2.33 g/l.

While preparing the deposits for operation, no account was taken of the reserves of low-grade ores and of difficult-to-dress oxide ores. This resulted in the stockpiling of dozens and even hundreds of millions of tons of non-standard raw materials that contained from dozens of thousands to several million tons of copper. For example, there are 1.3 billion tons of low-grade ores containing 0.4 % copper at the Bingham Mine in the United States. The processing of low-grade ores, especially those where copper is largely represented by oxide compounds, using a conventional method "enrichment-smelting" is frequently not feasible technically and not profitable economically.

Geological prospecting now is conducted considering not only high-grade ores ($>$ 0.3—0.4 % Cu), but also all kinds of low-grade, oxide and difficult-to-dress ores. As a result, the real reserves of metal contained in the ore deposits likely to be processed rise considerably (from 5 to 20 %). There exist deposits that can be processed only by dump or

underground leaching. With such an approach, all raw material that does not come to the dressing plant, is stocked separate from the barren rock on special sites and is subjected to leaching.

It is not easy to introduce dump leaching at those mines, where low-grade ore leaching was not envisaged originally. In some cases, low-grade ores are stocked on calcareous rocks, at other mines, they are dumped on water-permeable soils. Hauling such ores to new sites involves considerable costs. Where low-grade ores are kept together with barren rock, such raw material can be considered wasted.

The study of the raw material base begins with an assessment of the quantity and quality of available ores. A prospect of producing over 1,000 tons of copper a year by leaching is generally considered to be a justifiable scale of production, economically. Production of this magnitude calls for the leaching of 5 to 10 million tons of ore at a time, depending on its quality. The most suitable will be ores having the following characteristics:

(a) copper is represented mainly by oxide minerals, or by easily oxidizable secondary sulphides;

(b) low acid consumption of host rocks (acid consumption not exceeding 20 kg of acid per 1 ton of ore);

(c) fine dissemination of minerals;

(d) good permeability for leaching solutions (rate of percolation through the dump 1—3 m/day);

(e) decomposability when affected by leaching solutions;

(f) presence of limonite ($FeO(OH) \cdot nH_2O$) which is dissolved by sulphuric acid and supplies the solution with Fe^{3+};

(g) composition of the ore, encouraging the development of micro-organisms to intensify the process of leaching.

On the basis of summarizing the experience of leaching of various ores it became possible to establish average intensity of copper extraction into the solution, depending on the mineralogical and general chemical composition of the ores.

The rate of copper leaching from run-of-the-mine ores is as follows (annual):

(a) especially "refractory" chalcopyrite and bornite ores, around 5 %;

(b) mixed ores that, along with oxides, contain 50—80 % primary and secondary copper sulphides, around 10 %;

(c) mixed ores, containing only oxide and secondary sulphide minerals of copper (essentially, chalcocite), around 20 %;

(d) oxide ores, containing 80—90 % copper oxide minerals, up to 40—50 % [29].

Once the study of the ore resources on the basis of the geological prospecting data is over, verification of quantitative and qualitative data concerning raw materials, and representative sampling for testing are undertaken. If the deposit is operated simultaneously with the formation of low-grade ore dumps specifically for leaching, the sampl-

ing of the ore can be done using the conventional procedures. When the ore is kept in dumps that previously were not intended for leaching then, depending on the method used for dump formation, either a multiple surface sampling or deep sampling utilizing core drills, percussion/cable tool drills or auger drills is carried out [20, 28]. The weight of the sample taken for investigation in case of a uniform distribution of metal is determined by an existing empiric formula: $q = 0.06\ d^2$, where q is weight of sample, kg; d, maximum size of ore piece.

SOME INFORMATION ON STRATEGY AND METHODS OF INVESTIGATIONS IN THE FIELD OF METAL LEACHING TECHNOLOGY

Investigations are usually conducted in four stages: research, scale-up laboratory, semi-industrial and experimental-industrial.

The objective of the research stage is to get an idea of the nature of the ore, to find proper solvents and oxidants, to reveal the independent variables that influence the process of leaching, to establish approximate ranges of variation of these factors, and to determine the role of bacteria in the leaching of metals from ores.

Scale-up laboratory investigations are conducted with a view to achieving maximum optimization of the leaching regimes using mathematic methods of experiment planning and data processing.

Semi-industrial tests are arranged to finalize the leaching conditions using the samples of ore of industrial size (5 to 15 tons); also, at this stage, a complete technological scheme is field tested: leaching — extracting metals from the solutions — recycling tailing solutions. These tests are conducted in percolation columns or on small dumps of ore.

And, finally, experimental-industrial tests are carried out at the mine so as to further finalize all regimes of the technological scheme, performance of basic equipment, taking into account local peculiarities, etc. The tests are carried out on a dump containing from a few dozens of thousands to 1—2 million tons of ore to obtain commercial product.

As an illustration, the tests that involved the leaching of ore at the Kounrad deposit (USSR) are described below.

The primary objective of the first stage was to establish the amenability of ore for leaching, i.e. to study the chemical and phase composition of the ore, to investigate its mineralogy and petrography, to determine the acid consumption of the ore, rate of dump irrigation and the length of intervals between the irrigations; besides, physical characteristics of the ore were established: porosity, specific gravity and bulk weight, hardness, maximum moisture capacity, etc.).

Three types of disseminated ores were studied (sulphide, mixed and oxide), their chemical composition averaged as follows, %: Fe, 2.1—3.3; S, 1.08—2.85; SiO_2, 65.17—74.76; Al_2O_3, 14.3—16.2; CaO, 0.03—0.86; MgO, 0.04—0.45.

The data on the phase and granulometric composition of the ore are given below (Tables 5, 6).

Before being loaded into percolators, each sample weighing from 5 to 15 kg was made up of material of different sizes, to include 5 to 6 different fractions in proportion to their actual distribution, to ensure the uniformity of the granulometric and matter composition of the ore sample in all percolators. The percolators are made of rigid PVC or of organic glass (Fig. 7); they are 400—700 mm high, and are 100—150 mm in diameter.

Table 5

Phase composition of the ore, %

Type of ore	Oxide compounds	Secondary sulphides	Primary sulphides
Sulphide	10.2	82.0	7.8
Mixed	15.1	83.0	1.9
Oxide	39.9	45.7	13.8

Table 6

Granulometric composition of the ore

Fraction, mm	Content, %
−30+10	52—58.3
−10+5	12.8—15.9
−5	29—33

Later, the following parameters were determined: rate of copper leaching, sulphuric acid consumption depending on its concentration in the solution, frequency of irrigations and the interval between them.

Results of the investigations are shown in Figs. 8, 9, 10 and 11. It appears from these that the independent variables for the optimization of the leaching process are within the following ranges:

interval between irrigations (T) ..... 2—6 days
density of irrigation (specific solution consumption, V) ..... 30—70 l/t
acid concentration in solution (H_2SO_4) ..... 5—20 g/l.

The upper and lower limits of the established parameters are chosen to obtain "optimal" solutions containing $\geqslant$ 0.5 g/l copper and $\geqslant$ 0.5—1.0 g/l sulphuric acid. It is clear from Fig. 11 that utilization of up to 85 % of tailing solutions for copper leaching practically has no effect on the intensity of copper extraction.

At the second stage of investigations, the Box-Wilson method was used to determine optimum parameters for copper leaching from the ore [30, 31]. The practical meaning of applying this full factorial experiment procedure consists in that a minimal number of experi-

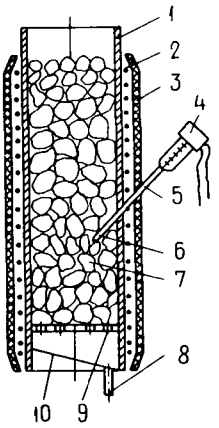


Fig. 7. Percolator:

1 — pipe, dia 160 mm; 2 — Ni-Cr alloy heater; 3 — thermal insulation; 4 — contact thermometer; 5 — branch pipe; 6 — oil; 7 — ore; 8 — drain branch pipe; 9 — false bottom; 10 — inclined bottom

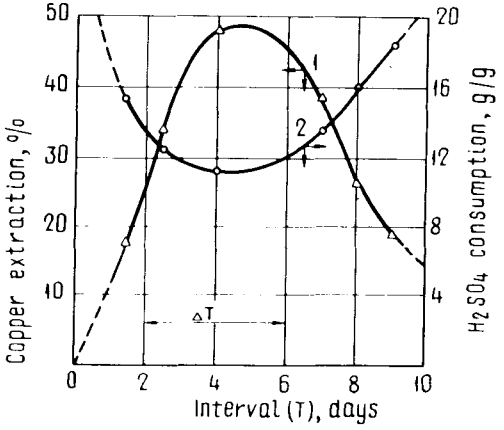


Fig. 8. Relationship between copper extraction (1), sulphuric acid consumption (2) and length of interval (T) between irrigations:

duration of leaching 75 days; acid concentration $C_{H_2SO_4}$ — 25 g/l; density of ore irrigation V = = 140 ml/kg; optimum length of interval ΔT 2—6 days

ments yield maximum of data on the process of leaching. This method allows not only to assess the impact of each factor under study on the leaching process, but also to evaluate the interaction between them.

The optimum conditions of the process were studied at the four stages of copper leaching from the ore: from 0 to 30 %; from 30 to 50 %; from 50 to 60 % and from 60 to 70 %. Results of these investigations are shown in Figs. 12, 13, and 14.

It is noteworthy that in the course of leaching of oxide ore the density of and the intervals between irrigations varied insignificantly, around 50 l/t and 4.5—5.5 days, respectively. Variation of the sulphuric acid consumption was more pronounced: from 5 g/l at the beginning, to 1—2 g/l at the end of the experiment.

In the case of sulphide ore leaching, these parameters register a more substantial change: the interval between dump irrigations increases from 5 to 7 days, while the H_2SO_4 consumption declines from 10 to 1 g/l.

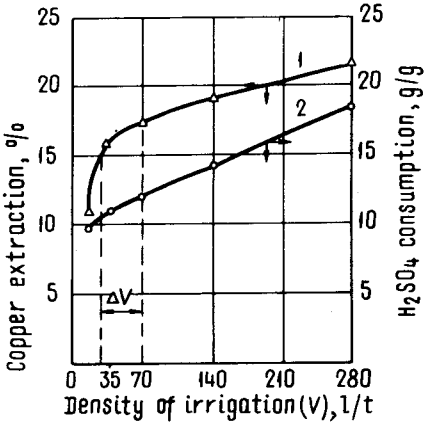


Fig. 9. Relationship between copper extraction (1), sulphuric acid consumption (2) and density of irrigation:

duration of leaching 20 days; $C_{H_2SO_4}$ — 25 g/l, and T — 2 days; ΔV = 30—70 ml/kg — range of optimum irrigation density

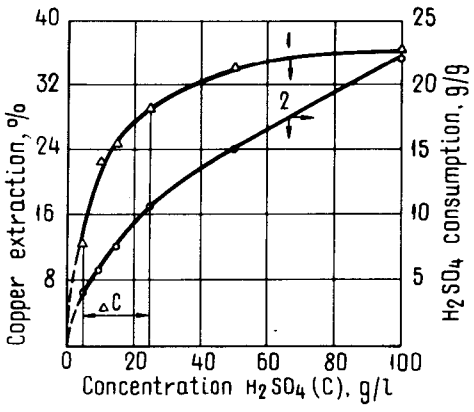


Fig. 10. Relationship between copper extraction (1), sulphuric acid consumption (2) and $C_{H_2SO_4}$:

duration of leaching 34 days; T — 2 days; V = 70 ml /kg; $\Delta C_{H_2SO_4}$ — 5—25 g/l — range of optimum acid concentration

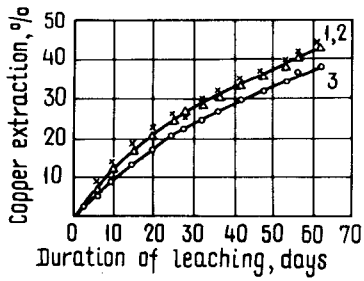


Fig. 11. Relationship between copper extraction and duration of leaching:

T — 4 days; $C_{H_2SO_4}$ — 5 g/l; V = = 70 ml/kg with tailing solution recycled

(curve: 1 — no recycling; 2 — 70 % of tailing solutions recycled; 3 — 85 % of tailing solutions recycled)

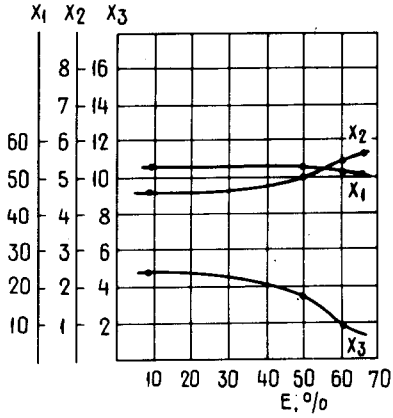


Fig. 12. Relationship between optimum levels of factors (X_i) and efficiency of copper extraction, when leaching oxide ore at deposit II:

X_1 — consumption of solution used for ore irrigation, l/t; X_2 — duration of leaching cycle, days; X_3 — concentration of sulphuric acid in solution, g/l

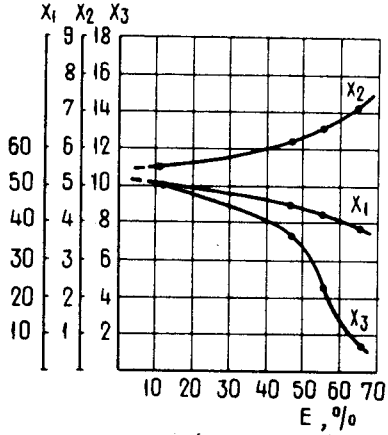


Fig. 13. Relationship between optimum levels of factors (X_j) and efficiency of copper extraction, when leaching sulphide ore at deposit II:

X_1 — consumption of solution used for ore irrigation, l/t; X_2 — duration of leaching cycle, days; X_3 — concentration of sulphuric acid in solution, g/l

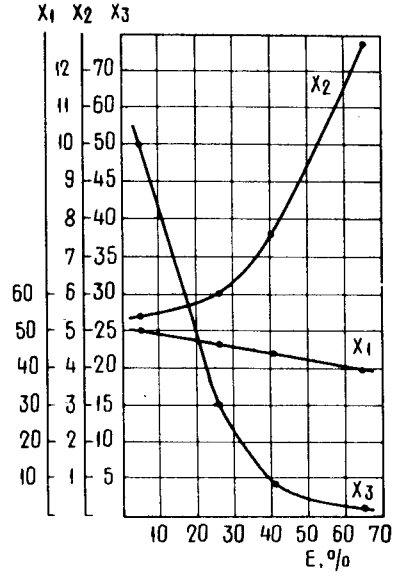


Fig. 14. Relationship between optimum levels of factors (X_j) and efficiency of copper extraction, when leaching mixed ore at deposit I:

X_1 — consumption of solution used for ore irrigation, l/t; X_2 — duration of leaching cycle, days; X_3 — concentration of sulphuric acid in solution, g/l

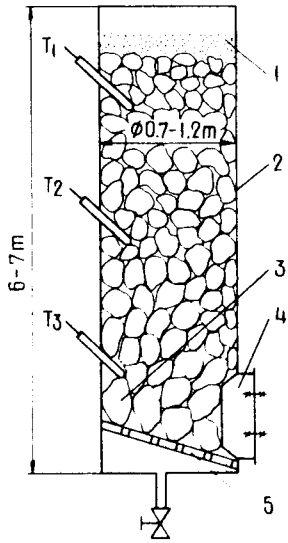


Fig. 15. Column for ore leaching:

T_1, T_2, T_3 — thermo-couples for measuring ore temperature inside column; 1 — layer of fine ore used to control rate of solution percolation; 2 — column body; 3 — coarse ore; 4 — discharge hatch; 5 — false bottom

Particularly noticeable are time-related changes in the leaching of a mixed disseminated ore (Kal'makyr Mine, USSR). For example, the H_2SO_4 consumption varies from 50 to 1 g/l, the interval between irrigations increases from 5 to 14 days, whereas the density of irrigation drops from 50 to 40 l/t of ore.

The parameters of microbiological leaching are examined in a similar fashion. An optimum concentration of microorganisms in the solutions, their temperature and chemical composition and other characteristics are determined.

The third stage of investigations involved samples with a maximum size of ore particles minus 400 mm in columns 0.7—1.2 m in diameter and 6 to 7 m high (Fig. 15). The primary objective of these analyses is verification of the optimum leaching conditions resulting from the second stage. Results of the studies are shown in Figs. 16 and 17. It is

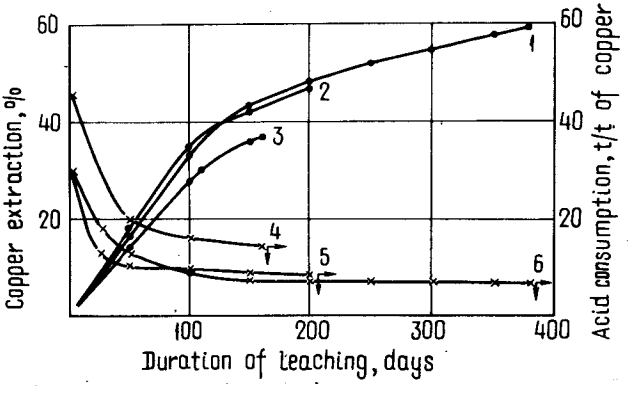


Fig. 16. Relationship between copper extraction, sulphuric acid consumption and duration of leaching sulphide ore pieces of different sizes:

1, 6 — 400; 2, 5 — 200; 3, 4 — 100 mm

noteworthy that, for the ore of the Kounrad deposit, the intensity of copper leaching did not depend on the size of ore pieces in the range from minus 100 to minus 400 mm (Fig. 16), while for the ore of the Kal'makyr deposit this relationship is well-pronounced (Fig. 17). Apparently, where ore is likely to be reduced in size of its own accord, it should not be crushed before leaching.

Experimental-industrial test sites in most cases constitute the first stage of a future dump leaching production site. Various copper leaching and extraction techniques were studied on such sites. Also, experimental industrial tests helped to improve methods of irrigating dumps, using ponds, spraying and a combination of both; to study the technology of copper leaching from the ore dumped on different natural soils, or on specially arranged sites made of clay, pyrite fines, bitumen, etc. [23, 28, 29]; and to investigate the techniques that serve to intensify the leaching process (dump aeration and blasting) [23—25]. Another important task was to determine the significance of microorganisms in dump leaching practices.

Solutions at the leaching sites were found to contain from 10^4 to 10^7 cells/ml of bacteria oxidizing Fe^{2+}, S^0, and sulphide minerals. The dominant species is *Thiobacillus ferrooxidans*. The number of bacterial cells depends on the specific conditions of the technological scheme, ore composition, solutions applied, temperature and season of the year. The minimum number of microorganisms occurs after cementation, and the maximum, in the effluents from the dumps [32, 33].

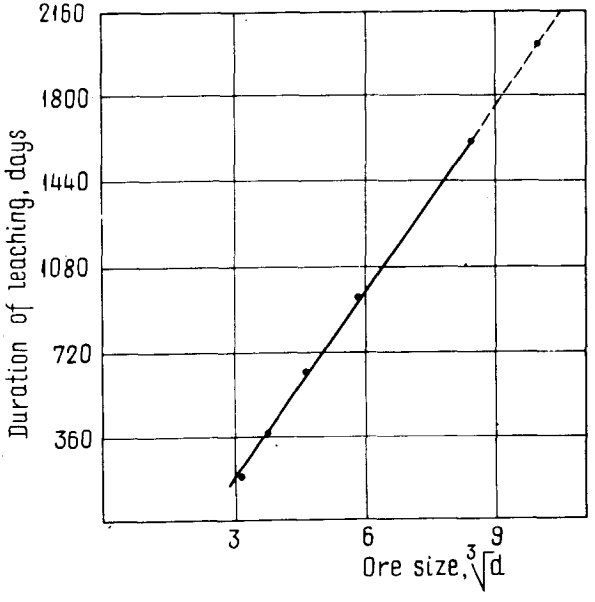


Fig. 17. Relationship between duration of leaching and size of ore pieces at the level of 70 % extraction efficiency (d – maximum size of ore piece, mm)

The sampling of ore across the depth of the dump indicated that maximum in-depth penetration of microorganisms does not exceed 5—8 m, which, apparently, is due to limited penetration of atmospheric oxygen. The number of microorganisms in the solutions obtained after the leaching of low-permeability clay dumps (Kal'makyr Mine) is much lower compared with those obtained from the dumps having a high permeability (Volkov, Kounrad Mines).

Results of microbiological studies conducted on the leaching sites made it possible to formulate recommendations on ways to intensify the process of leaching through intensifying the microbiological processes. This may be achieved either by improved extraction of copper from the solutions used for leaching (Volkov Mine), or by pre-oxidizing iron ($Fe^{2+} \rightarrow Fe^{3+}$) with microorganisms, while aerating the solutions in regenerating ponds, providing excess iron is subsequently removed from the solutions (Nikolaev Mine), and, finally, pumping air inside the dump (Fig. 4).

The following may be regarded as the major trends of dump leaching development:

(a) intensification of the leaching process through the use of microorganisms (both iron- and sulphur-oxidizing); application of various chemical and physical methods of treatment (e.g. the use of ozone, electric fields, etc.);

(b) ensuring a more versatile use of copper ore (in addition to copper and zinc, extraction of lead, cadmium, molybdenum, gold, and silver);

(c) elaboration of technological schemes and application of leaching to lead-zinc, nickel and gold ores;

(d) elaboration and improvement of methods (mainly, sorption, extraction, electrochemical and autoclave) used to extract metals from solutions;

(e) elaboration of efficient and cheap methods and engineering solutions in the construction of impervious foundations, of various hydraulic works for the formation of new and utilization of old dumps.

REFERENCES

1. Dennis, W.H., *Mine Quarry Engng.*, *13*, 5, 140 (1947).
2. Weed, P.S., *Mining Engineering*, *8*, 7, 721 (1956).
3. Grinvud, K.K., *Engineering and Mining Journal-Press*, *121*, 13, 518 (1926).
4. Baraboshkin, S.N., Hydrometallurgy of Copper, Metallurgizdat, Moscow (1941) (in Russian).
5. Siniaver, B.V., and Tseidler, A.A., Hydrometallurgy of Copper, Tsvetmetinformatsia, Moscow (1971) (in Russian).
6. Basic Technico-economic Indicators of Performance of Non-ferrous Metallurgy Plants in the Capitalist and Developing Countries, Tsvetmetinformatsia, Moscow (1973) (in Russian).
7. Proc. of Workshop "Copper-75", sponsored by US Commercial Bureau in cooperation with the USSR Chamber of Commerce (9—11 December, 1975).
8. Sinel'shchikova, N.V., and Makarova, S.N., Hydrometallurgy of Copper and Nickel, Tsvetmetinformatsia, Moscow (1976) (in Russian).
9. Seck, H.R., *Engng. and Mining Journal*, *168*, 8, 75 (1967).
10. Hydrometallurgical Processing of Oxide Ores and Dumps in USA, Review of literature from 1966 to 1969, GINTSVETMET Report (1969) (in Russian).
11. Sheffer, H.W., and Evans, L.G., Bureau of Mines, Information Circular, No. 8341, US Department of the Interior (1968).
12. "Non-ferrous metallurgy" Bulletin, No. 12, 50 (1967) (Abstract of a review: P.C. Merrit (Ed.), *Mining Engineering*, *18*, 6, 70 (1966).
13. Power, K.W., In: Proceedings of the International Solvent Extraction Conference, 19—23 April 1971, *2*, 1409 (1973).
14. Smith, C., Development of a new metal extraction process, Lecture delivered at West Australian University (1970).
15. Krüger, J., *Chemie Ingenieur Technik*, 4, 154 (1973).
16. Bird, J.M., *Can. Min. J.*, *90*, 11, 17 (1969).
17. Dayton, S. (Ed.), *Engng. and Mining Journal*, *176*, 6, 101 (1975).

18. Coler, H.R., *Mining Congress Journal*, 2, 90 (1972).
19. Tholé, B., *Erzmetall, 29*, 10, 453 (1976).
20. Tokmin, B.M., Khalezov, B.D., and Dolzhenko, A.A., *Tsvetnaya metallurgiya*, 7, 43 (1977).
21. Khalezov, B.D., Choudakov, V.G., and Vetrenko, E.A., Author's Cert. 380728 (USSR), Publ. B.I., No. 21, Moscow (1973).
22. Khalezov, B.D., Choudakov, V.G., and Vetrenko, E.A., Author's Cert. 417507 (USSR), Publ. B.I., No. 8, Moscow (1974).
23. Shalamov, V.M., Khalezov, B.D., Novomlintsev, A.M., and Yefimova, V.I., *Tsvetnaya metallurgiya*, 23, 17 (1974).
24. Khalezov, B.D., Nezhivykh, V.A., Shalamov, V.M., Novomlintsev, A.M., and Pavlichenko, G.A., *Tsvetnaya metallurgiya*, 7, 39 (1976).
25. Khalezov, B.D., Nezhivykh, V.A., Novomlintsev, A.M., Shalamov, V.M., and Pavlichenko, G.A., In: Proc. Institute UNIPROMED', *20*, 129, Sverdlovsk (1977).
26. Khalezov, B.D., Shourygin, Yu.A., and Khroustalev, L.Z., Author's Cert. 601397 (USSR), Publ. B.I., No. 13, Moscow (1978).
27. Khalezov, B.D., and Choudakov, V.G., *Tsvetnaya metallurgiya*, 5, 32 (1973).
28. Shourygin, Yu.A., Khalezov, B.D., Khroustalev, L.Z., and Semenov, G.P., *Tsvetnaya metallurgiya*, 11, 50 (1974).
29. Khalezov, B.D., In: Proc. Institute UNIPROMED', Further development of technology for non-ferrous metal ores production and processing, Coll. articles, Sverdlovsk, 104 (1979) (in Russian).
30. Nalimov, V.V., and Chernova, I.A., Statistical Methods of Experiments Planning, Nauka, Moscow (1965) (in Russian).
31. Poustyl'nik, E.I., Statistical Methods of Analysis and Processing of Observations, Nauka, Moscow (1968) (in Russian).
32. Mikhailova, T.L., and Pestovskikh, N.V., *Tsvetnye metally*, 3, 40 (1981).
33. Karavaiko, G.I., Abakumov, V.V., Krasheninnikova, S.A., Mikhailova, T.L., Piskunov, V.P., and Khalezov, B.D., *Prikladnaya biokhimiya i mikrobiologiya, 17*, 1, 73 (1981).
34. Baraboshkin, S.N., *Tsvetnye metally*, 6, 83 (1939).
35. Zav'yalov, M.M., Lotosh, V.E., Kharitidi, G.P., Khalezov, B.D., and Tokmin, B.M., Author's Cert. 541850 (USSR), Publ. B.I., No. 1 (1977).
36. Brent, W., and Rees, D., In: International Symposium on Copper Extraction and Refining, Las Vegas, 922 (1976).

MODELLING PRODUCTION PROCESSES IN BACTERIAL LEACHING

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At the present state of metal extraction technology bacterial leaching becomes commercially feasible mainly when the process involves stationary solid mass (leaching by percolation of the liquid phase), i.e. when the process requires minimum power. These features determine its place in the general classification of production processes. In terms of the nature of the solution flow about the rock, leaching processes with a stationary solid phase may be divided into several groups [1, 2]. However, in each separate case, the requirement of an easy access of leaching solutions to the entire rock body in question as well as the requirements of aeration favour only a minimal preparation of the ore, which may be relatively coarse-grained. Thus, the cost of rock preparation dramatically drops minimizing the overall expenditures. In practice, solid phase contains grains of different size, the leaching solution flowing through pieces of rock. Evidently, the hydrodynamics of solution flow through the rock body is very complicated which accounts for failures of the common engineering approach and makes unreliable theoretical output estimates by scaling up the equations that are based on the similarity theory and may even allow for specific diffusion processes and for specific surface chemical reactions. Leaching systems like some other production processes require for their assessment specific models incorporating characteristic features of those systems. Development of such models with precisely determined parameter values may lead to an accurate forecast of the technological output of the system or help to evaluate design parameters for new systems of bacterial leaching.

When bacterial leaching is applied it is necessary, first of all, to assess the quality of the liquid phase, i.e. the content of active components therein. Concentration of the said components in the solution is closely related to the amount of bacteria and their activity in the system under consideration. Of equal importance is the use of bacterial mutants oxidizing sulfide minerals and Fe^{2+} at high rates.

Monitoring the development and spread of laboratory-bred bacterial strains acquires particular importance when they are used under industrial conditions, e.g. in dumps where wild strains do occur. The introduction of active bacterial strains may intensify oxidation of primary sulfides at the given mining site.

Let us consider the most simple case when the given population, comprising n_0 bacteria, starts developing into a homogeneous population (without mutation). Let us also assume that at time intervals t_j ($j = 1, 2, ..., N$) spontaneous increase of cells due to the multiplication of the initial population n_0 is complemented by introduction of

external cells of the same bacteria. We can further assume that one bacterium gives progeny independently of other members from the same generation, and the progeny produces a new generation under the same conditions. In this case the growth of the population can be described [3] by a branched Markovian process whose generating function we denote by F(s,t). As the population development from any initial bacteria is assumed to be independent and with the same distribution, the generating function of the sum of the processes is equal to the product of the functions. Consequently, the generating function for the size of the population at time t produced by the initial n_0 cells shall be equal to $[F(s,t)]^{n_0}$. The bacteria coming from outside are of the same type as the initial population and appear at discrete time points t_j, therefore at a time point t each j-th migrant produces the progeny with the generating function $F(s, t-t_j)$. It follows that after time interval t from the beginning of the process the entire population has generating function $[F(s,t)]^{n_0} \cdot \prod_{j=1}^{N} F(s,t-t_j)$. The problem can be made more complicated if the external bacteria arrive at random time intervals which are Poisson distributed. Then our task is to express the generating function of the total population size at time interval t after the beginning of the process in terms of the generating function of a single bacterium progeny and the parameter of the Poisson distribution. Thus the problem of determining the population size at any time interval t is reduced to a purely mathematical problem of estimating the generating functions for the processes in question. The latter supplemented by specific relations between the size and quality of the biomass on the one hand, and solution parameters on the other, characterizes the liquid phase of the system.

A simple assessment of changes in the solid phase occurring in leaching of some of its components can be based on the following model [4].

Let us assume that the prepared rock body to be processed by the solution comprises particles whose size is defined by the effective diameter D. Naturally, the grains are of different sizes so that the solid phase is characterized by a certain size distribution of grains. Actually, any granular rock hypothetically contains a sufficient amount of particles of any size from the maximum grain size to a minimum one (close to zero), that is, value D is assumed to be continuously distributed between D^{max} and 0. Let us further assume that the mineral containing the component to be leached is finely and uniformly distributed throughout the rock, as in the case of porphyric copper-pyrite ores. Alternatively, the rock body can be divided into rock types, our model being applicable to each of them. For each type there is a specific relationship between the recovery of the component and the time of leaching, $\epsilon = f(t)$.

Another assumption is that the depth of solution penetration into the monolithic rock is proportional to the rate of recovery; the depth

of solution penetration at the given time after the onset of leaching may be determined as a size of the largest granulometric class completely leached by the solution. The latter can be expressed as follows:

$$\frac{D}{2} = \alpha \int_0^t \frac{\partial \epsilon}{\partial \tau} d\tau$$

where D — effective diameter of particles leached during time interval t. Proportionality factor α can be found from the size of the largest piece of rock utterly leached by the time t:

$$\frac{D_n}{2} = \alpha \int_0^{t_n} \frac{\partial \epsilon}{\partial \tau} d\tau$$

and

$$\alpha = \frac{D_n}{2\epsilon\,(t_n)}$$

Then the maximum grain size leached during the time interval t will be represented by:

$$D = \frac{D_n}{\epsilon(t_n)} \int_0^t \frac{\partial \epsilon}{\partial \tau}\, d\tau$$

Now, the portion of the rock body processed over time t comprising particles with grain-size below D(t) can be found from the grain-size distribution function for the ore by considering the fraction of particles in the range zero to D(t). In rock pieces of greater size the thickness of the leached layer is $\frac{D(t)}{2}$. The amount of the reacted solid phase for a particle of the i-th class of grain size is expressed by the difference between the masses of two spheres: the first with diameter D_i (the entire particle) and the second with diameter $(D_i - \frac{D(t)}{2})$ (unleached part of the particle). Summing up the masses through all classes from D_i to D^{max} we obtain the second portion of the processed matter represented by the reacted mass of partially leached grains. Thus, under certain physical and chemical conditions it is possible to estimate the amount of the processed solid phase.

The algorithm described above can provide a basis for computation techniques. The computed values, however, differ greatly from the measured parameters. The reason does not lie solely in idealized description of granular rock through spherical pieces, but in the assumption that the entire surface of all rock pieces is accessible to the solution, i.e.

— touching surfaces of different pieces are negligibly small;
— the entire volume of rock cavities is filled with the solution, and
— there is no silting by inert argillaceous mass.

These conditions may actually obtain in column or smaller scale experiments for relatively short periods of leaching, with typical rock pieces chosen and used and unexpected silting by random flows excluded.

We have already mentioned that the general model of transformations in the solid phase depends on the rate of metal extraction. Our assumption was that the recovery is proportional to the depth of solution penetration into the solid phase. In certain test experiments where the mass transport and mass transfer is known, the outcome of leached component can be evaluated in terms of the process taking place at the interface of the two phases [2]. During the active stage of metal leaching when the kinetics of component transition from the solid phase into the leaching solutions is primarily governed by diffusion, the dissolving process is limited by the diffusion of solution into the mass of rock body; this process is described by the first Fick's law (in one-dimensional space along the x-axis):

$$Q = -DA\frac{dc}{dx},$$

where Q — amount of the component diffusing through surface A in a unit time,
D — diffusion coefficient.

If concentration gradient is a function of all three space coordinates, $c = c(x,y,z)$, a similar equation can be written for a 3-dimensional space.

If the concentration field varies in time we apply the second Fick's law, a one-dimentional form of which runs as follows:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

Since generally the leaching flows are not laminar, the convective mass transfer formula should be used:

$$Q = -D_t A\frac{dc}{dx},$$

where D_t — coefficient of turbulent diffusion which is similar to the one in the first Fick's law.

As was mentioned earlier, the description of the solid phase as a granular material leads to substantial errors in output estimates of the leaching process. Recent development of measuring techniques brought about considerable changes in the models estimating the state of the rock body [6]. The following physical principles of the measurement techniques have produced a great effect on the concepts involved in models for solid phase description in dumps:

1. Measurements of natural gamma radiation of the rock body in question. The natural gamma radiation of the mining zone provides a quantitative estimate of argillaceous content of the zone, because K^{40}, the most frequently found natural isotope decays emitting easily identifiable gamma quants. K^{40} is a component of argillaceous materials, the most frequent filler of natural and/or dump-contained cavities. The accumulation of argillaceous minerals in a given zone lowers its

water permeability, therefore the intensity of natural gamma radiation gives a quantitative measure of potential impermeability for the zone in question. Thus, it has become possible to identify filled cavities in the rock body.

2. Water content measurements. Practically the only obstacle for the flux of accelerated neutrons (10^4 km per s) is provided by the atoms of hydrogen which cause in the impact a 50 % loss of neutron energy. Thus, in a given medium the fraction of fast neutrons transformed into slow ones (2 km per s) is related to the amount of hydrogen atoms. Therefore, in a given medium under the conditions of constant distance between the source and the detector of neutrons, a constant power of the neutron emitter and constant geometry of its opening the intensity of accelerated neutron flux is inversely proportional to the amount of hydrogen atoms. In any mineral entity the number of hydrogen atoms per unit volume is negligibly small for all minerals except water. Therefore, the aforesaid correlation serves to estimate moisture content in the zone.

3. With a known moisture content, the intensity of reflected gamma-ray flux is related to the volume density of the rock body reflecting the gamma-rays.

The application of new instrumentation designed to record the above-mentioned phenomena in measurements of dump parameters has brought conceptual changes in the model of granular rock body undergoing leaching. It has become possible to take in the actual state of the rock at the moment of measuring. Size distribution of rock pieces was found to produce no effect on the temporal dependence of metal extraction during leaching of fresh rock, e.g. in new dumps. In the course of the process, however, when the diffusion of solutions into internal parts of solid phase becomes more pronounced, concentration gradient of the active agent in the solid phase increases making the depth of solution penetration the main parameter of the system. The size of particles and its distribution in the rock should, therefore, be accurately determined alongside with variations in flow direction of solutions during leaching in dumps. It is also necessary to find and take into account the cavities between rock pieces filled with argillaceous material and the compressing of the rock body by higher rock pressure in the deeper parts of the dump. It is further required to know the amount of solution in contact with rock pieces throughout the zone. The combined measurement of these three parameters allows to evaluate the factors that characterize the granular composition of the rock body. The solid phase parameter is no longer represented by the grain diameter which in the previous model provided a numerical characteristic of maximum distance for infiltration of the leaching agent, but by the value of maximum distance which is not related to any form of the elements of the rock body [5].

In conclusion we shall try to specify our concept more precisely.

Fixing a certain direction, say a direction along the vertical of the dump, we assume that the dump height is divided by horizontal planes

located sufficiently close to each other. In each such section the distribution of one half of a maximum distance between the cavities in the rock mass of that section is found. Summing up the integral values of the mass along the dump height we obtain the total mass of solid phase. Let the one half of a maximum distance between cavities be a distance parameter d. The evaluation of a distance parameter distribution is a task similar to the granulometric analysis in mineral processing. If for some reason no tabulated data are available for our computation methods, the distribution of this parameter may be described by one of the familiar distribution functions [2]:

(a) **normal distribution:**

$$F_N(d) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u} e^{-\frac{t^2}{2}}\, dt,$$

where $u = \frac{d-d_a}{\sigma}$

σ — standard deviation,

d — distance parameter, and d_a is its average value

(b) **Schuhmann's distribution:**

$$F_{sh}(d) = 100 \left(\frac{d}{d_o}\right) k,$$

where d_o — Schuhmann's dimension modulus,

k — Schuhmann's distribution modulus.

(c) **distribution of Rosin-Rammler:**

$$R(d) = 100 \cdot e^{-\left(\frac{d}{d'}\right)^n},$$

where d' — parameter value such that $R(d) = \frac{100}{e}$,

e — the base of natural logarithms,

n — the parameter of the distribution

These distributions of a distance parameter are based on graphic and computational evaluation of experimental results.

REFERENCES

1. Schubert, H., Aufbereitung fester mineralischer Rohstoffe, *III*, Leipzig (1972).
2. Schubert, H., Aufbereitung fester mineralischer Rohstoffe, *I*, Leipzig (1974).
3. Karlin, S., A First Course in Stochastic Processes, Mir, Moscow (1971) (in Russian).
4. Rosental, A.K., Sedel'nikov, V.A., and Sukhodrev, V.M., *Phiziko-tekhn. problemy razrab. polezn. iskopayemykh*, No. 3, 100 (1977).
5. Roman, R.J., *Trans. Soc. Min. Eng. AIME, 262*, 73 (1977).
6. Howard, E.V., *Trans. Soc. Min. Eng. AIME*, 70 (1968).

APPLICATION OF MICROBIOLOGICAL METHODS TO UNDERGROUND LEACHING OF URANIUM ORES

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INTRODUCTION

Natural leaching of sulphide minerals, exposed to the atmosphere, has been occurring for geological time. For centuries, man has been aware of the acid drainage originating from coal workings, copper mines and waste dumps. With the advent of the technical sciences, the processes involved in acid mine drainage production were considered to be solely chemical in nature. However, in 1947, it was shown that a specific microorganism, called *Thiobacillus ferrooxidans* [1], was responsible for acid mine drainage from abandoned coal workings; by action on the pyrite and marcasite minerals in the coal — oxidizing the sulphide part of the mineral to sulphuric acid, and the ferrous iron part to ferric iron, the latter being subsequently precipitated out. In 1954 [2] it was shown that the same bacterium was responsible for the oxidation of exposed copper sulphide mineralizations, producing sulphuric acid and solubilizing copper. Numerous studies [3, 4, 5] have subsequently revealed that *T. ferrooxidans* under suitable conditions can attack most sulphide minerals, producing weak sulphuric acid, oxidizing ferrous to ferric iron and solubilizing the other metals present as their sulphate salt. Notable exceptions are the mercury and lead sulphides.

Many situations in which *T. ferrooxidans* play a role were of commercial significance, long before it was realized that the controlling processes were bacterial in nature. The problem of acid mine drainage produced from abandoned coal workings was and still is substantial. The acid mine drainage produced from base metal mines carries in solution a variety of metals. Techniques were developed early [6], albeit without an understanding of the processes involved, for the recovery of copper on a commercial scale. At the present time, the microbiological leaching of low grade ore dumps for copper recovery is an essential adjunct of many conventional mining operations. Uranium is not produced directly by the action of *T. ferrooxidans* on uranium minerals. However, where these minerals are present in association with iron sulphides, the bacteria produce acidic ferric solutions, which in turn oxidize 4-valent uranium into its acid soluble 6-valent form. Uranium produced in this manner is an important feature of certain uranium mining operations [7, 8]. Thus, this method of uranium recovery is designated as bacterially-assisted ferric iron leaching. Other metals, which may be solubilized by microbial action on their respective mineralizations, such as nickel and zinc, are not recovered commercially at the present time.

The literature on microbiological leaching is extensive. Some of the latest review articles are by Beck [9], Ito [10], Zajic [11], Watanabe [12], Fletcher [13, 14], Gupta and Sant [15], Karavaiko [16], Imai [17], Malouf [18], Trudinger [19], and Brierley [20].

This paper will describe the theoretical aspects of microbiological leaching, the constraints to its practical use and the application of the microbiological leaching process to uranium exploration and mining. The paper will document its present application in the leaching of waste material from conventional underground mining practices such as was done at Elliot Lake [7, 8] and for leaching "in place" of low grade ores, as is practiced at Agnew Lake Mines [21]. The microbiological leaching process is also used, but indirectly, to extract uranium from gold tailings at Buffelsfontein in South Africa [22]. Any potential replacement of existing processes for processing conventional mining grades of uranium ore, is highly speculative. It is expected that, once a number of variables affecting the microbiological leaching of uranium ores have been elucidated, the process may find wide application in the leaching of low grade ores, either "in place" or after some processing of the low grade materials. An extensive multi-year research program to study these variables is currently underway at B.C. Research.

Before addressing the underground leaching process for uranium ores, it is necessary to first briefly summarize the various factors affecting bacterial activity in general as well as the leaching mechanisms.

FACTORS AFFECTING BACTERIAL ACTIVITY

Insofar as we know, only one bacterial species, named *T. ferrooxidans*, is responsible for microbiological leaching of sulphide minerals. Although other *Thiobacilli* spp. may be present in leaching situations, the biochemical reactions which are mediated microbiologically, can all be attributed to the single species, *T. ferrooxidans*. During early investigations, researchers reported a number of similar species, assigned a variety of names, such as *Ferrobacillus ferrooxidans* and *F. sulfooxidans*. However, subsequent studies [23] of the biochemical reactions of these reportedly different organisms indicated that they were all of a single species.

Even to the microbiologist, *T. ferrooxidans* almost appears to be "something new under the sun". It is a chemoautotroph, i.e., it derives the necessary energy for metabolic processes and growth by the chemical oxidation of inorganic substances, in this case principally ferrous iron and the sulphides of heavy metals (some other reduced forms of sulphur can be utilized, e.g. elemental sulphur, thionates, etc.). The organism does not utilize organic compounds, at least in any natural situation, and many organic compounds are inhibitory.

1. **Ferrous Iron.** *T. ferrooxidans* can oxidize soluble ferrous iron to the ferric form, as represented by the following equation:

$$2Fe^{2+} + \frac{1}{2}O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O.$$

Although this reaction consumes acid, the ferric iron may precipitate out as the hydroxide, producing a new excess of acid as follows:

$$2Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3 + 6H^+.$$

The latter reaction is pH dependent, particularly at or near the pH values most conducive to the activity of *T. ferrooxidans*, i.e. about 2. The precipitation reaction tends to buffer the microbiological system at this value, so that the ferric iron is present both as the soluble ionic form, or as ferric hydroxide (or as described subsequently, as jarosite). The net effect of the oxidation of soluble ferrous iron is the production of acidic ferric solutions containing some iron precipitated in the ferric form. In field situations, the oxidation of ferrous iron in both the soluble and insoluble form is of consequence to this brief.

2. **Sulphides.** Considerable information is available concerning the action of *T. ferrooxidans* on the sulphide minerals, because of their industrial importance. Our experience is that most iron, nickel and zinc sulphides leach well. Among the copper sulphides, chalcocite, covellite, bornite and tetrahedrite leach well, but chalcopyrite with much more difficulty, as well as unpredictably. Pentlandite usually leaches better than chalcopyrite, although not all samples leach well. Galena (lead sulphide) and molybdenite do not leach to any significant degree.

The principal insoluble iron sulphides, occurring naturally as minerals are pyrite, pyrrhotite, marcasite, and the copper-bearing minerals, chalcopyrite and bornite . *T. ferrooxidans* is the only organism known with the capability of attacking these insoluble minerals, while oxidizing the ferrous part of the mineral to the ferric form, and the sulphide portion to sulphate. Attack is directly on the solid mineral, without first solubilizing the mineral; a relatively unique microbiological phenomenon. The intracellular mechanisms involved are not completely understood. However, the energy produced for the organism by oxidation of the ferrous portion of the molecule is considerably less than that derived from the sulphide portion.

The following two equations serve to illustrate the microbiological oxidation of pyrite.

$$4FeS_2 + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_3 + 8H_2SO_4$$

The second reaction involves the formation of a basic iron sulphate.

$$6FeS_2 + 22\frac{1}{2}O_2 + 15H_2O \rightarrow 2AFe_3(SO_4)_2(OH)_6 + 8H_2SO_4$$

— where A is a monovalent cation such as ammonium (NH_4^+), sodium (Na^+), potassium (K^+) or hydronium (H_3O^+).

The net effect of the biological oxidation of soluble ferrous iron as well as of the insoluble iron-bearing sulphides such as pyrite is the production of acidic ferric solutions containing some iron precipitated in the ferric form. The precipitation reaction appears to favour formation of jarosite as the more stable form than the ferric hydroxide. Complete conversion of pyrite to ferric hydroxide produces 50 % more sulphuric acid per unit of pyrite than does the corresponding conversion to jarosite. In practice, acid production is intermediate between the two values. Although final pH may depend on the nature of the host rock, the potential can exist for production of pH values which will limit bacterial activity, i.e. below 1.6.

Uranium oxide can be leached as a chemical consequence of the biological leaching of iron sulphides, usually pyrite. The leach solutions produced microbiologically from the action on pyrite are acidic ferric iron lixiviants, similar to those utilized in the hydrometallurgical extraction of uranium from its pulverized high-grade ores. The hexavalent uranium is acid-soluble and the 4-valent form is converted to the 6-valent form through chemical oxidation by the acidic ferric leach solution. Moreover, bacterial regeneration of the soluble ferric iron occurs. The chemical leach reaction is as follows:

$$UO_2 + Fe_2(SO_4)_3 + 2H_2SO_4 \rightarrow UO_2(SO_4)_3^{4-} + 2FeSO_4 + 4H^+$$

As discussed subsequently, the uranyl sulphate ion may be mildly inhibitory to the bacteria, as are most anions other than sulphate.

3. **Other factors.** To enhance the microbiologically associated solubilization of uranium, it is necessary to promote the microbiological oxidation of ferrous iron and, if present, pyrite. This is particularly so, if the uranium minerals are associated with pyrite. Thus, optimization of conditions affecting growth and activity of *T. ferrooxidans*, and those relating to the release of uranium, are virtually congruent.

Providing the appropriate substrate is available as an energy source, the important factors in the microbiological oxidation of sulphide minerals are: adequate moisture; the correct pH; an adequate supply of essential nutrients; and the absence of any inhibitors.

Bacteria require a moist environment. Although this moisture value may vary modestly, between site-specific locations, the best gauge of "free" water is the relative humidity in the microenvironment encompassing the bacterial-mineral contact. When the relative humidity drops appreciably below 80 %, bacterial activity terminates. Between 80 and 100 % relative humidity, available water limits the level of bacterial activity. In actual leaching conditions, "free" water is essential if the motile bacteria are to move from one surface to another. An additional requirement for water under leaching conditions is to permit transport of essential nutrients to the bacteria, to permit the dissolution

of the dissolved heavy metals and the concurrent chemical release of uranium; and to remove the dissolved metals.

T. ferrooxidans functions in an extremely acid environment, and is insensitive to the presence of most cations, which is attributed to the biophysical nature of the cell wall. This bacterium is also relatively insensitive to changes in hydrogen ion concentration, which is normally expressed on the pH scale. It has been our experience that *T. ferrooxidans* will oxidize ferrous iron over the pH range of 1.1 to 3.5, with an optimum value below 2.4. Other workers indicate optimum activity over the range 2.0 to 2.4 [24, 25], with 1.3 as a lower limit for activity. To appreciate the insensitivity of this organism to hydrogen ion, it tolerates a pH change from 1.8 to 3.5; equivalent to a change in sulphuric acid concentration of 0.76 g/l. Most bacteria will only tolerate a change from pH 5 to 8, equivalent to sulphuric acid concentration change of only 4.9×10^{-4} g/l.

T. ferrooxidans is an aerobe, i.e., oxygen is the ultimate electron acceptor for the electrons lost by the substrate during the bio-oxidation process. Oxygen is limiting only when the availability, or rate of replenishment is less than the rate of utilization as an electron acceptor. The transfer of oxygen into the water surrounding the bacteria may be rate limiting, particularly in natural leaching (quiescent) situations; as compared to a stirred reactor. Under quiescent conditions, a thin water film surrounding the bacteria-mineral contact permits access of gaseous oxygen, with transfer rates proportional to the air-water surface.

Like all other life forms, *T. ferrooxidans* requires certain nutrients for its growth and metabolic activities. Water and oxygen are essential cellular constituents, as well as being required for substrate oxidation. Other requirements are for carbon, nitrogen and phosphorus. Carbon dioxide is the sole source of carbon and the energy derived from substrate oxidation is used to fix this carbon source into the various biochemical molecules contained in bacterial protoplasm. The 0.03 % carbon dioxide contained in the atmosphere is normally adequate for all purposes, except some high-rate reactions which go forward only in the laboratory. Nitrogen is required for protein anabolism, and must be present as an inorganic source. Frequently nitrogen residues, sufficient for optimum bacterial action, are present in mining situations as a result of blasting operations; otherwise they must be provided. Phosphorus in the inorganic form is required for the biochemical molecules used in energy storage and transfer. Usually adequate phosphate is present in the host rock or in the water supplies, but must be provided where this is not the case. Undoubtedly the bacteria utilize other trace nutrients. However, we have been unable to show that they are limiting in any natural or laboratory situation.

Some heavy metals, including uranium, appear inhibitory to *T. ferrooxidans*. It has been postulated that formation of the uranyl sulphate anion, at the pH levels conducive to activity of *T. ferrooxidans*, is responsible for this inhibition [26]. Similarly it has been con-

sidered that the inability of *T. ferrooxidans* to utilize molybdenum sulphides is associated with the inhibitory action of the molybdate ions, which form at these pH levels. With regard to uranium, *T. ferrooxidans* can be acclimated to function effectively in the presence of substantial uranium concentrations. This acclimation can be demonstrated in the laboratory, however, the gradual continual acclimation which can occur under field conditions is more effective, and we have encountered *T. ferrooxidans* in mine drainage with uranium concentrations of up to 1.6 g/l.

Certain organic compounds can inhibit most forms of microbial life but a review of same is not appropriate to this brief. However, no evidence is available to indicate that those organic compounds and materials which normally are to be found in association with microbial leaching situations are in any way toxic or inhibitory to the leaching bacteria.

Although *T. ferrooxidans* will eventually appear in all natural conditions which are conducive to its growth and activity, it is feasible to initiate the organism's activities by deliberate inoculation of large numbers of the bacteria, either from natural sources, or as specifically cultured for this purpose. The time saving cannot be predetermined, because once the organism appears, it is usually only a matter of weeks at most, until populations increase to a size capable of effecting substantial changes. Inoculation may have more intrinsic value in situations where an ore body or dump is being deliberately acidified with the intent of assisting the initiation of bacterial activity.

An important feature of *T. ferrooxidans*, relevant to the mechanism of establishing its activity in natural situations, is its propensity to attach itself to solid surfaces. Although this might be expected of a microorganism which oxidizes insoluble substrates, most microorganisms are freely suspended in the aqueous fluid in which they are contained. In the presence of solid phase, however, in excess of 99 % of *T. ferrooxidans* are normally attached to the available solid surfaces, inert surfaces such as jarosite precipitated during the oxidation of ferrous iron, or the mineral surface represented by the insoluble sulphides. The paramount result is that *T. ferrooxidans* are not subject to "washout" under these conditions. Furthermore, as inocula, solid residues are more effective than the supernatant liquids derived from the same systems.

PRACTICAL CONSTRAINTS TO THE UNDERGROUND BIOLOGICAL LEACHING OF URANIUM ORES

In most cases, the more important variables which are constraints on biological leaching processes are:

1. Presence of sulphide minerals.
2. Amount of mineral surface exposed to air and water.

3. Oxygen availability of the mineral surfaces.
4. Water-moisture availability of the mineral surfaces.
5. Acid consuming character of the ore/gangue.
6. Ferric iron salt precipitation.

1. **Presence of sulphide minerals.** At the present time, microbiological leaching processes only occur in nature when metal sulphides are present. Bacterially-assisted leaching of uranium only proceeds when an iron sulphide mineral is present in the ore, together with the uranium values. In practice, this form of leaching, either as manipulated for uranium recovery or resulting from a mineralization exposed to the atmosphere, is mainly of consequence where substantial amounts of pyrite occur congruently with the uranium oxides. It is also possible that outside sources of ferrous iron and/or some form of sulphur could be utilized in bacterially-assisted leaching of uranium, if economically feasible.

2. **Effective mineral surface.** Mineral values most frequently are distributed in relatively small amounts throughout the host gangue. The mineral surface exposed to the leaching agents, either chemical or bacterially-assisted, is a function of ore particle size; increasing as particle size decreases. Commercial chemical leach operations pulverize their ore as a means of enhancing the rate and extent of uranium recovery. In bacterially-assisted leaching, the degree of ore fragmentation which has resulted from mining operations, or in a limited number of cases has been done deliberately, also enhances leaching rates and extraction. By analogy with the copper mining industry, the biological leaching of surface dumps of low grade wastes is substantially affected by the fracturing occurring during mining and dump construction, and could be deliberately assisted by control of particle size if merited on a cost-benefit basis. At the present time, most leach dumps operated in conjunction with open pit mining operations, are constituted of run-of-mine ore.

3. **Oxygen availability.** Since most biological mineral leaching processes involve the oxidation of the sulphide minerals such as pyrite, bornite and chalcopyrite, large amounts of oxygen must be made available at the mineral surfaces, two molecules of oxygen being required for each molecule of sulphur. Mineralizations amenable to biological leaching frequently contain large quantities of pyrite, which aids the leaching process in that the resulting sulphuric acid acts as a solute for metal values and offsets the acid demand of the gangue materials. However, oxidation of this pyrite may be responsible for a substantial proportion of the oxygen requirements. In most practical applications, such as leach dumps, oxygen availability forms one of the major limitations to the leaching process. The method of leach solution application to the surface of the dump, the amount of fines present in the ore as well as the degree of compaction can greatly influence the amount of oxygen which is able to penetrate into the dump.

4. **Moisture.** Water is essential to the leaching process, not only for bacterial metabolic processes and as a carrier for the oxygen and CO_2

required by the bacteria, but also for carrying of the metal sulphates produced. Since oxygen is brought to the mineral surfaces by diffusional processes, it is of advantage to maintain the thinnest possible water layer around the mineral particles so as to create the least diffusional barrier for the transport of the oxygen. Leaching in heaps and dumps often progresses fastest while the dump is on rest, that is, while solution is not applied to the surface of the dump and minimal amounts of gravitational water exist in the dump. The length of the rest period is determined by the rate of evaporation and the concentration of salts in the remaining liquid phase around the particles. When these two factors become critical, the ore surfaces must be wetted again. This wetting usually consists of a washing cycle to remove the metal values produced during the preceding rest period.

5. **Acid consuming character of the ore/gangue.** Many ores and wastes contain alkaline gangue that reacts with the sulphuric acid produced by the oxidation of the contained sulphides or with the acid added to the leach solution. If total acid demand of the alkaline gangue content is higher than the acid that can be produced by microbiological oxidation of the contained sulphides, then this material will not leach unless externally-added acid is used.

However, if the acid demand of the gangue is less than the potential acid production by biological means, then leaching ultimately will produce acidic ferric solutions, subject to other applicable constraints. The actual pH of the drainage and the effectiveness in releasing uranium will be a function of the balance between the acid demand of the gangue and the biological production of acid. In ores where pyrite levels are high, and alkalinity of host rock is low, acid production may result in pH values which limit bacterial action. Where pyrite levels are less, or alkalinity of host rock is high, bacterial production may ensue, but drainage pH values may be greater than 2, albeit still in the biological range. At pH values of near the upper limit of bacterial activity (2.8—3.0), iron precipitation following solubilization, would be expected to be virtually complete. Under such circumstances, release of uranium would also be expected to be reduced.

Where acid production and host rock alkalinity are marginally in balance, it can be economically feasible in certain instances to utilize an external source of acid to control the pH values at desired levels. Certainly from the standpoint of hydrometallurgical uranium recovery from solution, pH values below 2.0 (actually 1.5) are desirable.

6. **Ferric iron salt precipitation.** As indicated previously, the solubility of ferric iron is greatly affected by the acidity (pH) of the solution. Where effective leaching is progressing and pH values are about 2.0 or lower, dissolved ferric iron concentrations can be up to 10 g/l. However, at pH 3.0 solubility decreases to less than 0.1 g/l. The iron precipitation that occurs at these high pH values can substantially affect leach rates by "blinding" of mineral surfaces, preventing access of bacteria and essential nutrients. Particularly above pH 2.8, ferric

iron tends to precipitate out as the gelatinous ferric hydroxide rather than as the crystalline basic sulphate. The finely crystalline basic sulphate does not significantly affect leach dump permeability, whereas the hydroxide coats mineral surfaces, interfering with oxygen transfer and leach solution penetration. In addition, the hydroxides, or more particularly, the ferric oxides which subsequently form, are extremely difficult to dissolve except with very strong acid, which is totally impractical in commercial dump operations. Dumps "blinded" in this manner are literally put out of operation.

APPLICATIONS RELATING TO URANIUM EXTRACTION

1. **Underground.** The potential for natural leaching of uranium ores, through bacterially-assisted processes, was recognized shortly after the discovery of bacterial involvement in the oxidation of iron sulphide and other sulphide minerals [27]. However, the economic recovery of uranium from acid drainage of mines at Elliot Lake was initiated in 1960 at the Stanrock Mine, some two years after the commencement of operations [27]. Actually, attention was first directed to the mine drainage because of corrosion problems originating as the acidity developed and pH values dropped to 2.5 or less. It was not until 1964 that the presence of *T. ferrooxidans* in the acidic effluents was confirmed [28]. At that time, conventional operations at many of the Elliot Lake mines were minimal, and considerable attention was directed to bacterially-assisted leaching of uranium and its recovery [7].

Leaching techniques involved various procedures for applying water to the walls of the mined out stopes and the muck piles left in the underground workings. Literally by trial and error it was determined that a periodic wetting (or washing) provided superior results. Some attempts were made to enhance uranium release by the use of iron salts and nutrients to stimulate bacterial activity [8]. In particular, high pressure hosing was necessary to remove basic sulphate salts coating stope walls. Subsequently, it was shown that uranium release was enhanced by nitrogen additions, but more particularly by ensuring that muck piles and stope walls were kept damp between the three-monthly washings used to recover the solubilized uranium. Solution grades of up to 2.5 g U_3O_8/l were common [29]. Conventional hydrometallurgical techniques were utilized for uranium recovery. Underground leaching remains a part of uranium recovery operations at Elliot Lake, although the major part of the operations is represented by conventional mining activities.

The relevance of endeavors as practiced at Elliot Lake, as a technique for uranium recovery from underground workings, principally abandoned stopes, must be assessed on a site-specific basis, according to the constraints placed on the bacterially-assisted process and other related factors. Assuming sulphides are present and acid production

exceeds or equals demand of the gangue, moisture most frequently will be the limiting constraint. By examining the constraints applicable to a site-specific situation, uranium recovery can normally be enhanced by instituting appropriate measures, where economically justified. This assumes that the underground workings are part of a continuing mining operation, where collection of drainage and retrieval of uranium can be incorporated into existing operations.

At the same time, other related problems require examination: such as size distribution of mine residues, the best method of applying and distributing the leach solutions, the efficient collection of the pregnant solutions, as well as the mechanics for recycling the barren leach solutions. Grimes [30] has developed a mathematical model, confirmed by experimental data, based on the hypothesis that each ore piece is penetrated radially the same distance over the same time period: consequently uranium extraction is a direct function of ore particle size. However, Manchee and Garrett [31] have indicated differential release of uranium from ore pieces the same size, but of differing uranium content; interpreted to be associated with the distribution of pyrite within the ore. This confirms our own experience that each mineralization must be examined on an individual basis. Predictions of performance are feasible on the basis of laboratory and pilot-scale tests.

Small-scale tests can determine reliably that an exposed mineralization will not leach, when constraints inherent to the mineralization will prevent leaching. The same tests will also indicate when leaching may occur; albeit proven routine tests to indicate whether uranium will be solubilized in economically recoverable amounts and concentrations, are not yet available. However, current research programs can be expected to progress in this direction over the next 1—2 years.

2. **In Place.** When uranium grades are too low to justify conventional mining operations, it may be feasible to break the low grade ore in place, remove the swell to the surface and leach the broken ore remaining in the stopes, in place.

Canada's Agnew Lake Mines is an example of such a bacterial leaching operation and Rowswell [21] describes that at Agnew Lake Mines extremely heavy blasting substitutes for commercial crushing. It was thought that the rock is reduced in size to less than six inches (< 30 mm) and that advantage is taken of the naturally inclined placement of the mineralization as well as of the tectonic forces which have acted on the rock, creating the massive fracturing necessary for the transport of the leach solutions to the uranium minerals within the rock mass. It was furthermore thought that the uranium minerals were associated with pyrite and pyrrhotite in the matrix of the Agnew Lake conglomerate. Thus, rapid bacterial breakdown of these sulphides would enhance the uranium extraction process.

Extensive experience has been gained at Agnew Lake Mines in both in-place leaching and surface leaching of run-of-mine and crushed low grade ores. The waste products produced are the solids left under-

ground in the stopes, the leached-out heaps of ore on the surface and the iron, thorium and rare earths which were leached with the uranium. The radium will generally remain with the solids, 2/3 of which remain underground. Leach solutions are recycled but any bleedoff is neutralized with lime and aerated to assure all contained iron is oxidized and precipitated. Barium chloride is added to the waste solution to precipitate residual amounts of radium 226.

Critical factors in this type of operation are the degree of size reduction that can be obtained and the relative amount of mineralization that is available at the particle surfaces. Self-evidently, the biological production of acid and the net demand of the gangue must be in approximate balance. Otherwise excess acid production will limit biological activity, with a resultant depletion of oxidizing capability, whereas minimal acid production may render the leaching process uneconomic. Although a low acid demand by the gangue precludes the presence of appreciable carbonate minerals, the alkaline earths associated therewith must be absent in order to avoid formation of the insoluble sulphates inside the rock capillaries.

Important constraints to this form of *in place* leaching are associated with physical access. A high proportion of the ore must be wetted by the leach solutions, to permit uranium dissolution and transport. Further, the nature of the surrounding ore body must be such that the leach solutions can be contained, collected and brought to the surface for uranium retrieval and recycling.

In place leaching can be carried out under either flooded or trickle leach conditions. In order to affect rapid biological pyrite oxidation, large quantities of oxygen are required at the mineral surfaces. Without question, the problem of supplying this oxygen ranks high on the list of constraints to the leaching process. Trickle leaching provides by far the most efficient method for uranium extraction since relatively small volumes of leach solution are circulated per unit volume of ore. The slowly downward percolating solution carries large pockets of air with it into the broken orebody, thus supplying ideal conditions for rapid and continuous oxygen diffusion through thin layers of water to the mineral surfaces. The incorporation of rest cycles will produce not only significant energy savings in the solution handling circuit, but will also result in significant increases in pregnant solution grades.

Obviously, an underground, in place, leaching operation should be carefully planned, keeping in mind that wetting efficiency reduces drastically for every 3 meters of height, in excess of 10 m, of the broken orebody.

In some cases it may not be possible to obtain access to the top of the broken ore, such as in small stopes filled with ore. In such cases floodleaching, that is leaching by alternately flooding and emptying the stope, may provide an acceptable method of uranium extraction. The use of rest cycles here is even more important as is the use of multiple stopes, since the unit volumes of leach solution are very high resulting in potentially low pregnant solution grades.

REFERENCES

1. Colmer, A.R., and Hinkle, M.E., *Science, 106*, 253 (1947).
2. Bryner, I.C., Beck, J.V., Davis, D.B., and Wilson, D.G., *Ind. Eng. Chem., 46*, 2587 (1954).
3. Trussell, P.C., Walden, C.C., and Duncan, D.W., *Western Mines, 37*, 26 (1964).
4. Duncan, D.W., Walden, C.C., and Trussell, P.C., *Trans. Soc. Min. Eng., 238*, 122 (1967).
5. Bruynesteyn, A., and Duncan, D.W., In: Recent Advances in Mining and Processing Low Grade and Submarginal Mineral Deposits, Resources and Transport Division of the United Nations Secretariat (Eds.), Pergamon Press, New York, N.Y., 46 (1976).
6. Taylor, J.H., and Whelan, P.F., *Trans. Inst. Min. Met., 52*, 35 (1943).
7. Fisher, J.R., *Trans. Can. Inst. Min. Met., 69*, 167 (1966).
8. Fisher, J.R., Lendrum, F.G., and MacDermid, B.G., Paper presented at the Annual Meeting of the American Institute of Mining and Engineering (1966).
9. Beck, J.V., In: Fermentation Advances, Academic Press, New York (1969).
10. Ito, I., *Kagaku* (Tokyo), *24*, 818 (1969).
11. Zajic, J., Microbial Biogeochemistry, Academic Press, New York (1969).
12. Watanabe, S., *Hakko Kyokaishi, 27*, 459 (1969).
13. Fletcher, A.W., *Trans. Inst. Min. Met.*, Section C, *79*, 247 (1970).
14. Fletcher, A.W., In: Microbial Aspects of Metallurgy, J.D.A. Miller (Ed.), American Elsevier Publishing Co., New York (1970).
15. Gupta, R.C., and Sant, B.R., *J. Sci. Ind. Res., 29*, 372 (1970).
16. Karavaiko, G.I., *Uspekhi mikrobiologii, 6*, 174 (1970).
17. Imai, K., In: Proceedings of a Symposium on Biochemical and Industrial Aspects of Fermentation, 329 (1971).
18. Malouf, E.E., *Min. Eng., 23*, 13 (1971).
19. Trudinger, P.A., *Mineral Sci. Eng., 23*, 43 (1971).
20. Brierley, C.L., *Crit. Rev. Microbiol., 6*, 207 (1978).
21. Rowswell, M.D., Presentation to the Canadian Nuclear Association, Montreal, June 1977.
22. Matthew Hall Ortech Ltd., *Quarry Mine, 18*, 17 (1979).
23. Hutchinson, M., Johnstone, K.I., and White, D., *J. Gen. Microbiol., 44*, 373 (1966).
24. Malouf, E.E., and Prater, J.D., *J. Metals, 13*, 353 (1961).
25. Tomizuka, N., Yagisawa, M., Someya, J., and Takahara, Y., *Agri. Biol. Chem., 40(5)*, 1019 (1976).
26. Tuovinen, O.H., and Kelly, D.P., *Arch. Microbiol., 98*, 167 (1974).
27. MacGregor, R., Paper presented at the Annual Meeting of the Canadian Institute of Mining and Metallurgy, Montreal, P.Q. (1964).
28. Harrison, V.F., Gow, W.A., and Ivarson, K.C., *Can. Min. J., 87*, 64 (1966).
29. Duncan, D.W., and Bruynesteyn, A., *Trans. Can. Inst. Min. Met., 74*, 116 (1971).
30. Grimes, M.E., In: Proc. Solution Mining Symposium, American Institute of Mining and Engineering, 338 (1974).
31. Manchee, R.J., and Garrett, K.H., *Trans. Inst. Min. Met., 83*(811), C115 (1974).

PROCESSING OF METALLURGICAL SLAGS AND ORE-DRESSING TAILINGS

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INTRODUCTION

The increasing requirements for maximum utilization of mineral raw materials are principally met in two ways: firstly, by preventing losses of useful constituents in the main process and, secondly, by developing advanced methods and techniques for utilizing vast resources predominantly classified as dressing, metallurgical and chemical industry wastes. However, until today in processing of 1 t of copper porphyry ore as much as 1 kg Cu is usually lost in tails. Characteristically, while the lower limit of copper content of amenable ores has already declined to 0.3 %, the copper content of waste copper slags amounts to 0.4—0.6 %. Traditional concentration methods have already attained the limit of their potentialities and the degree of utilization of useful constituents only can be increased by the use of radically new complex techniques, with the view of organizing low-waste and non-waste processes in metallurgy and in concentration of minerals.

This report presents results of a study on the utilization of waste copper slag and tailings from flotation of polymetallic sulphide ore using a combination of physical/mechanical and microbiological processes.

MATERIALS AND METHODS

Investigations with the above wastes involved the use of schemes based on the existing methods of physico-chemical dressing [1, 2], and extractive hydrometallurgy [3] and biohydrometallurgy [4]. A characteristic element of processing schemes dealing with such material is the pretreatment stage. This stage stems from the essence of the basic technological concept, i.e. recycling of waste mineral products. It involves application of various processes to the substrate to secure a successful selective extraction of useful constituents in subsequent main operations. In the instances discussed herein pretreatment involves: grinding, activation of the partly oxidized sulphides, modification of the mineral composition of the slag, a combination of repulping and leaching processes.

Principal constituents of copper slag used in this work were as follows (%): SiO_2 — 31.88, Al_2O_3 — 9.20, CaO — 0.83, MgO — 4.35, K_2O — 1.40, Na_2O — 1.43, S — 2.43, Fe_{total} — 41.25, Fe_3O_4 — 9.56, Cu — 0.58.

The product is a vitrous, nearly homogeneous mass, with the specific weight of 2.93 g/cm^3. The consumption of H_2SO_4 for neutralizing the —0.1 mm coarse sample was 115 kg/t. The principal mineral constituents of the slag are: fayalite, glass, and magnetite in the 16:3:1 relationship. What we have is a skeletal-parallel structure, copper (chalcocite, chalcopyrite, bornite, neodigenite) being contained in the form of aggregates and impregnations, particularly in the vitrous mass. Valuable constituents of the product are: non-ferrous metals, iron and glass.

Flotation tailings, apart from the main aluminosilicate part, contained 0.22 % Cu, 4.5 % S, 0.418 g/t Au.

RESULTS

We may assert that utilization of even copper alone from this type of slags is quite problematic notwithstanding diverse approaches [5, 6]. Leaching of copper from the slag using ferric sulphate solutions obtained microbiologically under the effect of *Thiobacillus ferrooxidans* proved impossible, since this raw material is characterized by a high content of alkaline components. The leaching solution was rapidly neutralized, the Fe^{2+} concentration increased, and there occurred precipitation of ferric hydroxides, oxide copper minerals only being dissolved (total recovery of Cu in the 30-day cycle was not above 8 %).

Unsatisfactory results were also obtained in the processing of the product using traditional dressing methods (flotation, magnetic separation) [4, 6]. The main reason for this was the unfavourable mineralogical composition of waste copper slags. It was necessary, therefore, to find a method for modifying the mineralogical composition. As seen from Fig. 1, the melted slag was reoxidized by blowing through either air or O_2 as a result of which fayalite was destroyed ($FeO - Fe_2O_3 - SiO_2$) and the reoxidized slag contained separate phases of glass, magnetite, limited amounts of fayalite, metallic and oxide copper (Fig. 2).

Following this operation the Fe_3O_4 content would rise from 9.5 to 40 %, a complete combustion of sulphur was achieved, and after rapid cooling a product was obtained with a low mechanical strength despite a high magnetite content. A selective recovery of components from such a product was in principle already possible: magnetite was extracted by means of magnetic separation (field intensity — 85 kA/m), copper, of which 90 % is represented by the —0.1 mm fraction, was leached from the non-magnetic fraction using ferric sulphate solution obtained microbiologically under the effect of *T. ferrooxidans* strains endowed with a high iron-oxidizing activity. The solid residue from leaching represents a silicate product satisfying the requirements of certain industries.

Typical results obtained in the concentration of reoxidized slag are shown in Table 1. The qualitative characteristics of final products

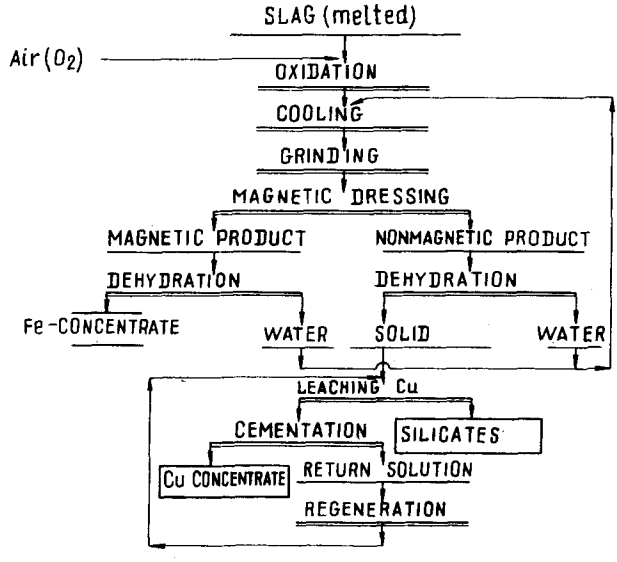

Fig. 1. Basic flowsheet for copper slag processing

permit them to be further utilized by the industry. Furthermore, a preliminary technico-economic assessment points to the profitability of the technological process involved.

The losses of useful constituents in ore-dressing tailings are due to a number of reasons, but in this case we are interested in the analysis of possibilities for reclamation of valuable constituents. The use of physico-mechanical methods for dressing of low-grade material is de-

Table 1

Qualitative and quantitative indices in concentration of reoxidized copper slag

Products	Recovery, %	Components					
		Copper		Iron		Quartz	
		β, %	ϵ, %	β, %	ϵ, %	β, %	ϵ, %
Iron concentrate	65.50	0.11	13.00	50.00	82.00	11.20	22.75
Cement copper	0.58	72.00	76.00	15.70	0.25	—	—
Silicate product	33.00	0.15	9.00	20.75	17.24	75.00	77.25
Spent solution after cementation	—	0.20*	2.00	8.00*	0.48	—	—
Slag	100.00	0.55	92.00	40.00	100.00	32.00	100.00

* — Indicated concentrations are measured in g/l.

void of economic feasibility even if the product is amenable to dressing. The techniques of extractive metallurgy and, particularly, of biometallurgy provide for a more complete recovery of a number of constituents from such wastes [6, 7, 8]. However, profitability is inherent only in technologies based on a combination of traditional concentration methods with those of biohydrometallurgy (Fig. 3).

As seen from Fig. 3, the waste is repulped with the leaching solution which is 80 % recycled in the technological scheme. Thus, already during the pretreatment of the initial material for flotation easily-soluble copper minerals are partially leached, oxidized sulphidic surfaces refreshed and floatability of FeS_2 increased under the effect of copper ions contained in the solution. As a result of flotation in acid

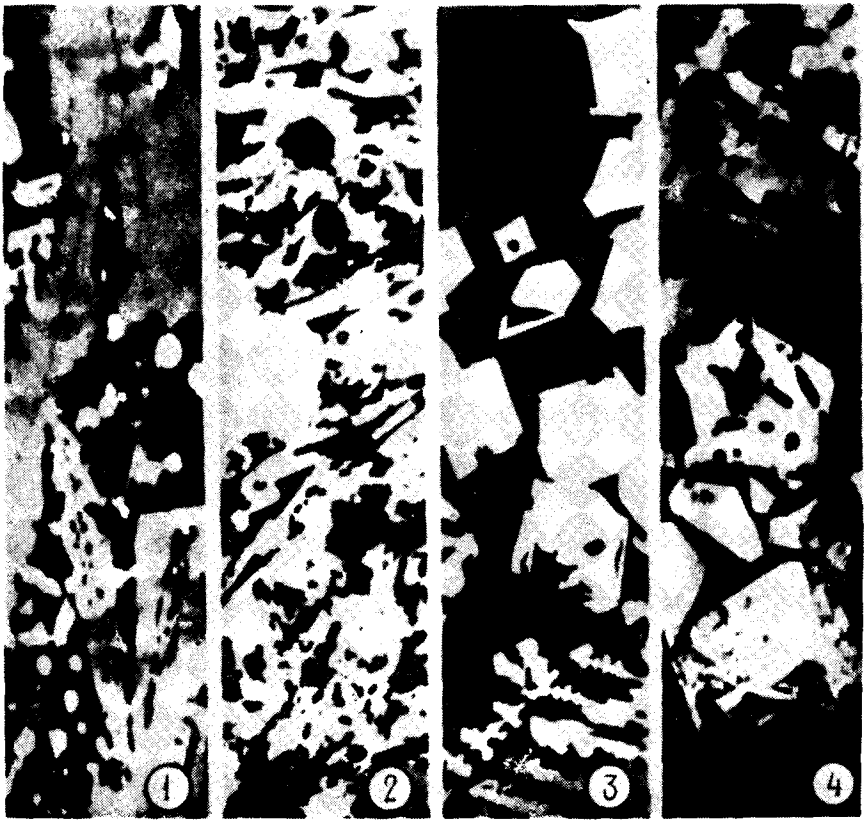

Fig. 2. Micrography of polished sections of waste slags from copper pyrometallurgy:

1 — waste slag; 2 — partly oxidized slag; 3 — oxidized slag; 4 — reoxidized copper slag

medium (pH = 2.75) the collecting aerofloat recovers a collective sulphide concentrate containing Cu, S and Au. The waste is stored at the dumping ground, waste waters are partly (averagely 20 %) fed to the cementator to remove dissolved copper, and after neutralization are brought out of the circuit to keep the concentration of total iron down to 12—15 g/l.

As in the case of slag, in this case the role of biocatalytic processes effected by *T. ferrooxidans* is to produce ferric sulphate solution.

For the purpose of environment protection efforts should be made in the realization of the proposed scheme to achieve a closed water circulation, decontamination of floating agents, neutralization and refreshment of sulphate solutions.

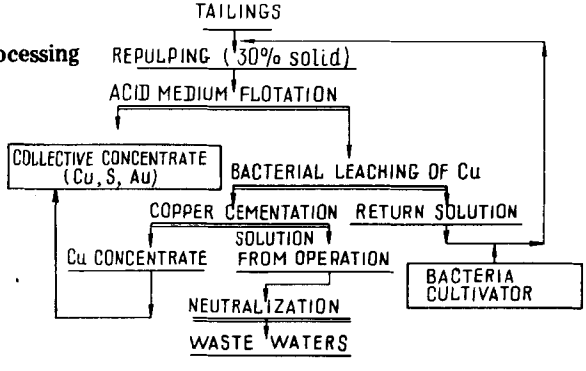

Fig. 3. Basic flowsheet for processing of flotation tailings

An experimental trial of the technological solution in question has pointed to the possibility of maintaining a stable process characterized by the indices shown in Table 2.

Table 2

Qualitative and quantitative indices in concentration of flotation tailings

Processes and products	Recovery, %	Components					
		Copper		Sulphur		Gold	
		β, %	ϵ, %	β, %	ϵ, %	β, %	ϵ, %
Leaching							
Cement copper	0.04	18.00*	19.80	—	—	—	—
Flotation							
Collective concentrate	6.00	2.37	80.00	45.00	60.25	4.18	59.75
Tailings	93.96	0.04	20.00	1.91	39.75	0.178	39.25
Repulped tailings	100.00	0.18	100.00	4.49	100.00	0.42	100.00

* — The indicated low copper content is due to the use of precipitator, i.e. clinker produced by the rolling of zinc cakes.

DISCUSSION

A preliminary technico-economic analysis of the indices obtained in reclamation of the products concerned points to the possibility of organizing profitable processes based on using a specific complex of processing methods and setting up low-waste and non-waste technological schemes. Particularly promising in this respect is the use of waste slag. Following the modification of its mineralogical composition, as proposed by us, it is possible to effect the separation and extraction of useful slag components in the form of three final products. Furthermore, it can be definitely asserted that, following the mineralogical transformation, the most rational aspect of the technology is the microbiological part of the scheme. Finely-dispersed copper contained in slags largely in the metallic form cannot be recovered by means of flotation (β_{Cu} = 4.98 %; ϵ_{Cu} = 53.24 %). By using ferric sulphate solution this is successfully realized. What is more, the leaching solution obtained microbiologically is of low cost; on the one hand, dissolution of iron in residual fayalite improves the quality of the silicate product, on the other hand, a higher iron content of the solution is a prerequisite of a high oxidizing activity of the solutions and their acidification due to a partial hydrolysis of iron.

It can be presumed that the utilization of valuable constituents that are today lost with the wastes is impeded principally due both to their low concentrations and to unfavourable mineralogical and physicochemical properties of the wastes, resulting from the processing (melting, regrinding, oxidation, reaction with residual reagents). A promising trend for bringing down losses of metallic and non-metallic mineral components in wastes is the use of technological schemes combining physico-mechanical and dressing methods and those of biohydrometallurgy, in certain cases possibilities being open, moreover, for setting up non-waste technologies and for expansion of the raw-material basis.

REFERENCES

1. Manual of Ore Dressing, Nedra, Moscow (1972) (in Russian).
2. Chernyak, A.S., Chemical Concentration of Ores, Nedra, Moscow (1976) (in Russian).
3. Holms, J., Denchar, A., Stewart, L., and Parker, J., In: Extractive Metallurgy of Copper, *11*, AIME, New York, 907 (1976).
4. Genchev, F., Groudev, S., Gaidarzhiev, S., Gushcherov, G., and Todorov, Ts., In: 5th Congress of Microbiology, Varna, *3*, 209 (1981) (in Bulgarian).
5. Babachev, G., and Petrov, S., Utilization of Metallurgical Slags, Tekhnika, Sofia (1980) (in Bulgarian).
6. Genchev, F., and Groudev, S., Development of Technology for Extraction of Copper and Iron from Waste Metallurgical Slags, Report of Investigation, Higher Institute of Mining and Geology, Sofia (1976) (in Bulgarian).
7. Chadwick, J.R., *World Mining*, *33*, 6, 48 (1980).
8. Dasher, John, *Min. Eng.*, *23*, 4, 48 (1971).

PART V

ENVIRONMENTAL PROTECTION IN MICROBIOLOGICAL HYDROMETALLURGY

EVALUATION OF ACID PRODUCTION POTENTIAL OF MINING WASTE MATERIALS

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INTRODUCTION

The oxidation of reduced sulfur compounds and ferrous iron by the leaching bacterium *Thiobacillus ferrooxidans*, resulting in acid generation and metals solubilization, has long been recognized as a naturally occurring process that can be exploited in mining operations [1, 2]. Indeed, methods of promotion and acceleration of biological activity have been so successful that bioleaching of copper sulfide concentrates is now considered technically feasible [3—5] and less polluting than smelting methods.

However, acidic effluents emanating from many coal and sulfide mines, often attributed to biological activity, is recognized as an environmental hazard. Inhibiting these naturally occurring organisms on a practical scale is much more difficult than accelerating their activity.

This paper details the chemistry of biologically assisted acid production and describes methods developed to determine whether or not a mining waste material has the capability to become acid producing when left exposed to the atmosphere. If initial tests show a material to be a potential acid producer, then scale-up testing, in the form of column leach tests on large core or −20 cm material, will elucidate effluent characteristics and what effect climatic conditions have upon these characteristics. Such information is essential for the physical design of waste piles so that acid production can be minimized, and for the conceptual design of effluent treatment facilities.

BACKGROUND AND THEORY

The production of acid mine waters arises from the oxidation of metallic sulfide minerals, particularly those containing iron. In theory, this oxidation can occur either chemically or biologically, but in

practice the bacterium *T. ferrooxidans* is always present in acid mine waters, suggesting that the organism plays a major role in the formation of acid mine waters.

T. ferrooxidans is a unique bacterium; its energy for growth is obtained from the oxidation of reduced sulfur compounds (e.g. sulfides) and ferrous iron. It is the only organism known that has the capability of oxidizing the sulfide portion of insoluble metallic sulfides. The bacterium requires an aquatic environment but air is the source of the oxygen and carbon dioxide required. The bacterium also requires a source of ammonia nitrogen as well as small amounts of phosphate, calcium and magnesium, which are usually present in natural waters.

Numerous evidence exists which suggests that *T. ferrooxidans* can attack sulfide minerals by direct oxidation of the sulfide moiety [6—10]. An enzyme containing a sulfhydryl group is postulated to attack the sulfide ion, and a polysulfide chain is built up [11]. The sulfur atoms on this chain are ultimately oxidized through to the sulfate ion form which is released into solution. Ferrous iron, if present, is oxidized simultaneously to the ferric form by a different enzyme system.

Microbiological acid production from sulfide minerals can be illustrated using pyrite as an example.

$$4FeS_2 + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_3 + 8H_2SO_4 \quad (1)$$

This equation represents the complete hydrolysis of all the ferric iron and the production of two moles of sulfuric acid per mole of pyrite. In practice, we find that in an acidic environment, the iron does not precipitate as ferric hydroxide, but rather as a basic ferric sulfate or jarosite type mineral, represented by the formula:

$$A\ Fe_3(SO_4)_2(OH)_6, \text{ where A can be } H_3O^+, NH_4^+, K^+, Na^+, \text{ etc.}$$

Assuming all the iron precipitates as hydronium-jarosite, the following equation explains the oxidation of pyrite.

$$12FeS_2 + 45O_2 + 34H_2O \rightarrow 4H_3OFe_3(SO_4)_2(OH)_6 + 16H_2SO_4 \quad (2)$$

In this case, 1.33 moles of sulfuric acid are produced per mole of pyrite. In practice, neither equation (1) nor equation (2) applies completely and the actual amount of sulfuric acid produced in a natural situation will be dependent upon a combination of reaction (1) and (2) and does vary between 0.67 and 1 mole of acid per mole of sulfide present.

If the mineralization contains a copper sulfide such as chalcopyrite, acid can be produced according to either reactions (3) or (4), thus producing 0.5 moles or 0.17 moles of acid per mole of sulfide.

$$4CuFeS_2 + 17O_2 + 10H_2O \rightarrow 4CuSO_4 + 4Fe(OH)_3 + 4H_2SO_4 \quad (3)$$

$$12CuFeS_2 + 51O_2 + 22H_2O \rightarrow 12CuSO_4 + 4H_3OFe_3(SO_4)_2(OH)_6 + 4H_2SO_4 \quad (4)$$

However, some sulfides such as bornite (Cu_5FeS_4) will be net acid consumers when oxidized, as shown by the following reaction.

$$12Cu_5FeS_4 + 111O_2 + 20H_2SO_4 \rightarrow 60CuSO_4 + 4H_3OFe_3(SO_4)_2(OH)_6 + 2H_2O \quad (5)$$

Other non-ferrous sulfides such as millerite (NiS) and sphalerite (ZnS) also undergo direct biochemical oxidation, which can be represented as follows:

$$MS + 2O_2 \rightarrow MSO_4 \quad (6)$$

where M = Zn, Ni, Pb, Co, etc.

From the foregoing discussion, it is evident that pyrite is the major contributor to acid production, and that the maximum possible amount of acid generation is one mole per mole of sulfide present. In practice, the amount of free acid produced is usually considerably less due to incomplete sulfur oxidation. If one assumes a one-to-one ratio, a safety factor will ensue.

T. ferrooxidans is also capable of producing acid by the oxidation of dissolved components in waters emanating from mining and milling operations. In this paper we will concern ourselves only with the formation of strong acid, that is, sulfuric, and not with the formation of weak organic acids resulting from heterotrophic growth on available organic matter.

Two possible sources of strong acid arise from soluble components; the oxidation of ferrous iron and the oxidation of reduced sulfur compounds such as thiosulfate or polythionates. If ferrous iron is present, it will oxidize slowly as follows:

$$2\,Fe^{2+} + 1/2\,O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (7)$$

However, if *T. ferrooxidans* is present, the rate of this reaction can be increased by up to 500,000 times [12].

Although reaction (7) consumes acid, the ferric iron produced is less soluble than the ferrous iron and it tends to hydrolize, releasing its acid content.

$$2Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3 + 6H^+ \quad (8)$$

Thus, a net gain of 2 moles of hydrogen ions per mole of ferrous iron is obtained if the hydroxide product is formed.

With reduced sulfur compounds, either chemical or biological oxidation can take place, depending on conditions. The amount of acid released would depend on the ionic species present, the nature of the associated cations and the mode of oxidation. Three possible situations are given by equations (9), (10), and (11).

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ \quad (9)$$

$$S_3O_6^{2-} + 2O_2 + 2H_2O \rightarrow 3SO_4^{2-} + 4H^+ \quad (10)$$

$$S_4O_6^{2-} + 7/2\ O_2 + 3H_2O \rightarrow 4SO_4^{2-} + 6H^+ \quad (11)$$

These three equations assume complete oxidation of all the reduced sulfur compounds, a result normally occurring only in the presence of sulfur oxidizing bacteria. Chemical oxidation in the acidic environment is usually incomplete.

PRINCIPLE OF ACID PRODUCTION POTENTIAL TEST PROCEDURE

A small-scale test procedure has been developed to determine whether a waste material may become acid producing [13]. For the purpose of this paper, we will briefly discuss the principle of the method.

To determine whether a waste material has the potential to become acid producing, the acid consuming capability of the material, expressed as kg H_2SO_4/tonne waste, is determined by chemical titration of a finely ballmilled sample (—400 mesh). This number is compared with the maximum theoretical amount of sulfuric acid which could be produced, calculated stoichiometrically from the sample's total sulfur content. If the alkaline content of the sample consumes more acid than could theoretically be produced, there is no danger that the waste, in run-of-mine size, will become a source of acidic effluents. The sample is classified as a non-acid producer.

However, if the opposite is true, that is, the waste material could theoretically produce more acid than it can consume, a biological leach test must be performed to determine how much of the contained sulfur can be converted into sulfuric acid. This biological test consists of mixing into a 250 ml baffle-bottom Erlenmeyer flask, 15—30 grams of —400 mesh sample with 70 ml of a nutrient medium [14]. The test pH is stabilized at 2.2—2.5 over a period of a few days, followed by inoculation with an active culture of *T. ferrooxidans*. The flask is loosely stoppered and put onto a gyratory shaker in an incubation room, which has a CO_2-enriched atmosphere and is temperature controlled at 35 °C. This procedure ensures conditions ideal for bacterial growth. An active bacterial population will be indicated by a steadily decreasing sample pH as biochemical sulfide oxidation occurs. At this

point, the flask receives further incremental additions of sample and the effect on pH is closely monitored. If the test pH rises and approaches the sample's natural pH, then the waste material is confirmed to be a non-acid producer, because any acid produced biologically is consumed by alkaline components in the material. However, if the test pH remains low, indicative of a steady net acid production, then the waste material is confirmed to be a potential acid producer.

SCALE-UP TESTING PRINCIPLE AND PROCEDURE

Since the acid production potential tests are performed on a small scale on finely ground material, under conditions ideal for biological growth, scale-up effects must be considered in order to realistically evaluate a waste material. Factors that will affect the biological conversion of sulfide in a run-of-mine waste are numerous and extremely difficult to assess. Major factors are:

1) Ratio of exposed sulfide material to alkaline gangue.
2) Distribution of the sulfide minerals in the waste.
3) Depth of oxygen penetration into the waste pile.
4) Amount and depth of moisture penetration into the waste pile and effective mineral surface wetting.
5) Length of dry periods.
6) Presence of inhibiting soluble metals.
7) Temperature.

Preventing water and/or air from entering the waste pile will eliminate the danger of acid production. However, on a practical scale, such preventative measures may not be feasible. Thus, provisions must be made to minimize acid production and to provide treatment facilities for the acidic effluents produced. A scale-up test procedure has been developed, which is performed on either core material or run-of-mine waste, that will assess the acid-producing character of run-of-mine waste and characterize the effluents produced from such wastes as a result of natural leaching processes.

Appropriately sized sample material is placed in 2 to 6 meter high columns and leached with re-circulating neutral pH distilled water to which an active culture of *T. ferrooxidans* has been added. Such recirculation keeps any free acid produced in the leach circuit and thus enhances the rapid establishment of an environment amenable to microbiological sulfide oxidation processes. Samples of the recirculating solutions are assayed at appropriate intervals to determine the rate of increase of selected metals and chemicals of environmental concern. A list of the typical water quality parameters of concern to British Columbian coal and metal mine operators (Table 1 and 2, respectively) is quite comprehensive, and shows that analytical costs associated with

frequent solution assays can be a significant financial factor in a major testing programme.

As would be expected, the pH of the effluents produced from waste materials plays an important role in solution quality. Not only does the pH control the solubility of the various metals but, in the case of the sulfide wastes, a low pH encourages biological breakdown of metal sulfides, thus increasing the rate of solubilization of corresponding metal sulfides as well as production of ferric sulfate lixiviant. The onset of rapid biological sulfide oxidation commences when the pH of the solution in contact with the waste, through natural causes, has reached

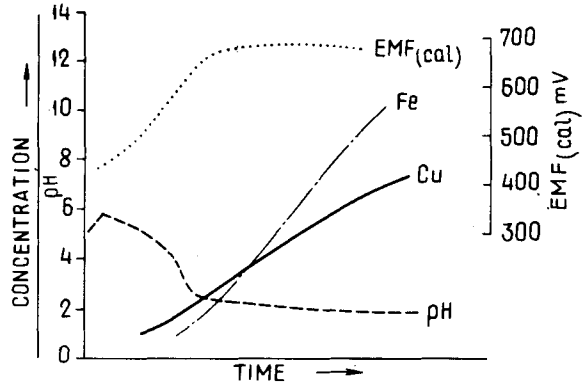

Fig. 1. Effect of pH upon metal concentration in effluents from acid producing waste material

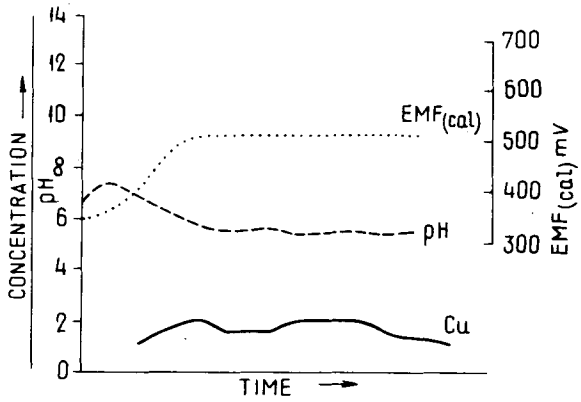

Fig. 2. Effect of pH upon metal concentration in effluents from non acid producing waste material

a value between 5.0 and 4.5 (Fig. 1). Rapid increases in copper and iron concentrations coincide with the establishment of a high oxidation potential, normally above 600 mV (relative to calomel).

If the pH does not decrease below 5 (Fig. 2), rapid biological activity does not occur, the oxidation potential remains below 600 mV, and dissolved metal concentrations remain low. In some tests carried out in our laboratories, copper and iron concentrations remained below 0.2 mg/l and 0.1 mg/l, respectively, when 100 kg of waste was leached with 20 litres of water.

If a material proves to become acid producing under the continuous recirculating leaching method employed, it is important to determine if such material will remain acid producing when submitted to single pass leaching with simulated rain water, since such leaching will remove acid. During a long dry spell, the leaching bacteria will continue to oxidize sulfides, produce acid, and cause high concentrations of metal values and salts in the interstitial waters. During a rain period, the percolating rain will wash the waste materials and remove a portion of

Table 1

Water quality parameters. Coal mine development

Temperature
pH
Dissolved Oxygen
Dissolved Solids
Suspended Solids
Volatile Suspended Solids
Turbidity
Alkalinity
Specific Conductance
Total Organic Carbon
Hardness (calculated)
Sulfate
Total Phosphate
Nitrate and Nitrite
Ammonia
Fluoride
Total Calcium
Total Magnesium
Total Mercury
Dissolved Zinc
Dissolved Copper
Dissolved Iron
Dissolved Mercury
Dissolved Silver
Dissolved Lead
Dissolved Arsenic
Dissolved Cadmium

Table 2

Water quality parameters. Metal mine development

Temperature
pH
Dissolved Oxygen
Dissolved Solids
Suspended Solids
Volatile Suspended Solids
Turbidity (NTU)
Alkalinity
Dissolved Magnesium, Calcium
Specific Conductance
Sulfate
Ammonia
Total Carbon
Total Organic Carbon
Phenol
Total Mercury
Total Phosphorus
Ortho Phosphate
Dissolved Phosphorus
Nitrite
Nitrate
Dissolved Arsenic
Dissolved Iron
Dissolved Copper
Dissolved Manganese
Dissolved Zinc
Dissolved Lead

the dissolved species, thus producing their relatively high concentration in the effluent emanating from the waste. It is of great importance to determine if, after washing by rain, the remaining interstitial water will again become acidic. This is very much a function of the acid-producing characteristics of the wastes and the amount of oxygen that can penetrate into the waste. Unless the waste is moderately acid producing, the neutralizing effect of the rain water may remove sufficient acidity from the interstitial waters to prevent subsequent biological activity. In cases where the waste material remains acidic, the characteristics of the effluents to be produced from a commercial-sized dump of such waste can be determined reasonably accurately, providing information of great assistance to the designer of the effluent treatment facilities.

We prefer to make such a determination in 6 metre high columns on −20 cm material. By subjecting the waste material to rest cycles with a duration similar to the length of dry spells at mine site, followed by washing-leaching with quantities of neutral pH water, equivalent to, for example a 10 year − 4 hour maximum rainfall, a reasonably accurate assessment can be made of the maximum amount of the various metals and salts that can be extracted per thousand tonnes of waste. Only when the information obtained from such an assessment is available can a practical assessment be made of the need to minimize effluent production by making the waste dump impermeable. If treatment facilities are unavoidable, advantage can sometimes be taken of the biological acid production by recovering some of the metal values of economic importance from the effluents.

CONCLUSION

From the foregoing description of waste material testing, it is apparent that any such testing programme should be carefully designed to incorporate variations in waste characteristics and climatic conditions, as well as waste dump configuration and dump construction methods. The results of such a properly executed test programme can have a significant effect on the cost of the necessary effluent treatment facilities by preventing over-design.

REFERENCES

1. Malouf, E.E., and Prater, J.D., *J. Metals*, *13*, 353 (1961).
2. Malouf, E.E., *Mining Eng.*, 43 (1977).
3. Chen, C.S., Huddleston, R.L., Johnson, M.A., In. *4th Joint A.I.Ch.E. −C.S.Ch.E. Chem. Eng. Conf.*, Vancouver, Sept. 9−12 (1973).

4. Torma, A.E., Ashman, P.R., Olsen, T.M., and Bosecker, K., *Metall.*, *33*, 479 (1979).
5. McElroy, R.O., and Bruynesteyn, A., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, Murr, L.E., Torma, A.E., and Brierley, J.A. (Eds.), Academic Press Publishers, New York (1978).
6. Duncan, D.W., Landesman, J., and Walden, C.C., *Can. J. Microbiol.*, *13*, 397 (1967).
7. Beck, J.V., and Brown, D.G., *J. Bacteriol.*, *96* (4), 1433 (1968).
8. Duncan, D.W., and Walden, C.C., *Dev. I. Microbiol.*, *13*, 66 (1972).
9. Sakaguchi, H., Torma, A.E., and Silver, M., *Appl. Environm. Microbiol.*, *31* (1), 7 (1967).
10. Torma, A.E., *Rev. Can. Biol.*, *30*, 209 (1971).
11. Silver, M., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, Murr, L.E., Torma, A.E., and Brierley, J.A. (Eds.), Academic Press Publishers, New York (1978).
12. Lacey, D.T., and Lawson, F., *Biotech. Bioeng.*, *12* (1), 29 (1970).
13. Bruynesteyn, A., and Duncan, D.W., In: *AIME Annual Meeting*, paper (A-79-29), New Orleans, Feb. 19–21 (1979).
14. Silverman, M.P., and Lundgren, D.G., *J. Bacteriol.*, *77* (5), 642 (1959).

ENVIRONMENTAL PROTECTION AND USE OF MICROORGANISMS FOR INDUSTRIAL WASTE WATER TREATMENT

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INTRODUCTION

Microbiological methods can be effectively used for the removal of organic and mineral pollutants from industrial waste waters. It is important that the removal of organic compounds from water should utilize not only the traditional means of intensifying oxidation reactions by controlled aeration and temperature, but also take into account the specific aspects of microbial metabolism: cometabolism, transformation of individual functional groups of xenobiotics, etc. Metal ions are removed through immobilization by the products of microorganism life activity and as a result of reactions of oxidation and reduction of variable-valency elements. Cyanides are destroyed by heterotrophic microorganisms which utilize the nitrogen of the cyanides in the presence of a nitrogen-free source of carbon.

Toxic pollution of the planet constitutes the worst form of natural ecosystems' depletion and degradation and the main adverse consequence of human impact on the environment. Stresses of ecological systems are caused by the contamination of the natural environment with both organic pollutants, natural and man-made, and a large group of mineral substances, the worst pollutants among them being heavy metals — mercury, lead, copper, cadmium and zinc. The ever mounting influx of industrial organic and mineral pollutants into the ecosystems severely overtaxes the self-purification capacity of the biosphere, which is based on the life activity of microorganisms. About 30—40 years ago when the main pollutants comprised municipal and household wastes, the problem of purification was successfully resolved, since naturally occurring substances were easily oxidized and decomposed through intensification of spontaneous microflora activity by aeration and temperature control.

Initially, waste waters were purified by passage through filtration and sewage irrigation fields where organic substances were oxidized by the spontaneous microflora of the soil. Later, oxidation ponds and aeration tanks appeared, where the process of purification was carried out by an association of microorganisms forming what is known as activated sludge.

It is not our purpose to analyze the state of the problem of microbiological treatment of industrial waste waters in the individual branches of the national economy; in this lecture we believe it more

appropriate to demonstrate the problems that arise and how microbiologists deal with them in the attempt to protect the environment against pollution. Nor shall we dwell on the technical devices or technological features of the processes of waste water treatment. We would like to draw attention to two aspects:

1. Specific traits of decomposition of organic compounds in industrial wastes by microorganisms.

2. Microbiological removal of metal ions and cyanides present in the waste waters of mining and metallurgical productions.

MICROBIOLOGICAL DECOMPOSITION OF ORGANIC COMPOUNDS IN INDUSTRIAL WASTE WATERS

The advent of chemical industry brought into the waste waters organic compounds that had not existed in nature; many of these are difficult to decompose microbiologically.

Protection of the environment against pollutants will be only successful if the chemical compounds carried with waste waters are amenable to microbiological decomposition and, when decomposed, enter the circulation of substances in the biosphere.

Despite its urgency, the problem of elaborating microbiological techniques for the treatment of industrial waste waters is still at the stage of initial research. This is partly due to the fact that for a long time this problem was overlooked by scientists working in the field of theoretical microbiology and was mainly studied by experts in industrial laboratories where more attention was given to technology than to the scientific foundations of the "microorganism-substrate" interaction.

Owing to the products of chemical synthesis and of coal coking, and wastes of iron and non-ferrous metallurgy and of other industries, that are present in waste waters, the latter's composition has become highly complex. The spontaneous microflora of activated sludge is not always capable of purifying water to a satisfactory quality, thus necessitating more energetic scientific research aimed at the intensification of microbiological treatment processes.

Naturally occurring organic compounds carried with waste waters are mineralized by microorganisms to the end products, whereas far from all man-made compounds can be decomposed by microorganisms. Hence the necessity to find microorganisms capable of transforming man-made chemical compounds and utilizing them through ordinary microbial metabolism. These reactions take place at the very initial stages of foreign compound transformation by microorganisms, i.e., at the stage of peripheral metabolism.

A convincing example of the reactions of preliminary metabolism, carried out by microorganisms in order to involve chemical compounds into the processes of central metabolism, is provided by the microbio-

logical transformation of nitro compounds, which are highly toxic to the majority of saprophytic microorganisms. The microbial destruction of nitrobenzenes, nitrophenols, and nitrobenzoic acids begins with the reduction of the nitro group by denitrifying bacteria, producing corresponding amino derivatives which are non-toxic and can be easily metabolized by many microorganisms [1, 2].

A two-stage process is suggested for the removal of these compounds from waste waters. At the first stage aromatic compounds are reduced by anaerobic microflora of the thermophile-fermented sediments of municipal treatment facilities. As a result of nitro group reduction and partial detoxification of waste waters, the latter's components can be subsequently oxidized at the second stage in aeration tanks.

Many chemical compounds present in waste waters cannot be decomposed, or used as a source of carbon and energy, by microorganisms, and therefore pass intact through the treatment facilities. It was observed that many compounds that were hard to decompose as a sole source of carbon, were metabolized by microorganisms in the presence of easily accessible sources of carbon under conditions of cometabolism. In the USSR the problem of cometabolism is being successfully studied by Skryabin and Golovleva [3]. Under conditions of cometabolism, both transformation and complete degradation of foreign compounds are possible.

We studied the effect of organic additives on the rate of microbial destruction of organic flotation reagents: oxidized kerosene (OK) and oxidized recycle (OR-100) [4]. Glucose (2 g/l) was introduced as an additional source of carbon into the culture *Pseudomonas* sp. The results of the study indicated that oxidized recycle was completely degraded during the experiment (24 days) following addition of glucose to the medium while many components of kerosene in the medium with glucose remained unaffected by the microorganisms.

Because of their toxicity, many synthetic organic substances which are difficult to decompose microbiologically, when passing through treatment facilities, produce a destructive effect on the microflora of the activated sludge, necessitating a preliminary decomposition at the local pre-treatment installations. As a result of selection, researchers isolated microorganisms capable of destroying resistant toxic compounds, such as hexamethylenediamine, formaldehyde and dimethyl dioxane [5], α-methylstyrene [6] and others.

Industrial growth increases the volumes of waste waters to be treated, and raises the concentrations of toxic organic and mineral pollutants, accentuating the urgent call for intensification of treatment processes.

An effective way to intensify the treatment of industrial waste waters may be to increase the oxidative capacity of activated sludge through addition of chemical mutagens [7, 8, 9, 10, 11]. Under the effect of chemical mutagens, nitrosomethylurea or dinitrosoethylurea,

the composition of the microbial population of the sludge is transformed, changing its fermentative properties. The treated sludge precludes development of filamentous bacteria, and settles better. As a result of the increase in the oxidative capacity of the sludge, the rate of oxidation of organic substances increases, the expenditure of air for aeration and the time of aeration are reduced.

A method was found to intensify the removal from waste waters of 2-methyl-5-vinylpyridine (MVP), a toxic product which is hard to decompose by microorganisms. To raise the efficiency of MVP removal the activated sludge was treated with nitrosomethylurea. This drastically increased the effective capacity of the aeration tank, making it possible to raise the MVP content of waste waters from 20 to 175 mg/l.

Thus, the presence of products of chemical synthesis in waste waters calls for a new approach to the problem of microbiological treatment, an approach taking into account both the specific features of the compounds to be degraded and the peculiarities of the microorganisms' metabolism. Only basic research in the field of physiology, biochemistry and genetics of microorganisms will make it possible to find effective methods for destruction of organic compounds which are to be removed from waste waters.

MICROBIOLOGICAL REMOVAL OF METAL IONS AND OF CYANIDES

Another important scientific and practical task is the elaboration of a microbiological technology for the removal of metal ions from water, which ultimately means converting them to an immobilized, insoluble state. Following chemical and physical processes, also important for determining the dynamics of the metal content of water is the biological factor. Apart from higher aquatic plants, metal ions are sorbed by water-dwelling algae and microorganisms.

Sorption of metals by microorganisms and by the products of their life activity is of a physico-chemical nature, i.e., what takes place is the adsorption of metals on substrates of biological origin. Neufeld and Hermann [12] observed the sorption of mercury by activated sludge during a three-hour contact period. This metal, as well as zinc and cadmium, was sorbed very rapidly by activated sludge. Though the end equilibrium in regard to the metals tested was achieved after two weeks, the three-hour contact was quite sufficient for ensuring nearly complete equilibrium between the concentration of the metals in the solid and liquid phases. We observed mercury precipitation on the surface of activated sludge in waste waters of a synthetic rubber factory. The maximal mercury saturation took place during the first 6 to 12 hours of contact followed by a phase of slow sorption.

The ability of products of mineralization of vegetable remnants to precipitate metals was used as the basis of a technology for the removal of metal ions from waste waters of dressing plants [13—16]. For this purpose, a secondary settling pond was redesigned: reeds were cut down on an area of 34.6 ha. This stimulated the life activity of heterotrophic microorganisms and enhanced the decomposition of the vegetable mass, resulting in a drastic drop in the concentration of heavy metals in the water.

Of great importance for the immobilization of metals is the biogenic formation of hydrogen sulfide. This process is very important also in the formation of sulfide ore deposits, since in the presence of hydrogen sulfide all metals precipitate.

Usually, the conditions in the water are not optimal for the development of sulfate-reducing bacteria, but in the sludge and bottom layer of water, in the presence of organic matter and sulfates, sulfate-reducing bacteria may be important for the removal of metal ions from water. In our experiments we observed a direct relationship between the number of sulfate-reducing bacteria, the presence in the medium of organic matter accessible to them, and a drop in the concentration of copper in the solution to zero values.

In cases when the conditions in purification facilities are unfavourable for the life activity of sulfate-reducing bacteria, these organisms may be cultivated in containers made of semipermeable membrane, for example, cellophane, that are placed in water, in the path of heavy metal ions [17]. The hydrogen sulfide produced by the bacteria diffuses through the cellophane and interacts with the metals, precipitating them in the form of sulfides.

The oxidation and reduction of metals by microorganisms is an important link in the chain of geochemical transformations of elements on the globe. Their oxidation by autotrophic microorganisms provides the latter with energy; however, a large contribution is made by heterotrophic microorganisms, for which the transformation of metals is an auxiliary process, and is not significant energetically. In accordance with their chemical properties, metals frequently act as electron acceptors in the chain of anaerobic processes, with a decrease in valency as a result of their reduction by microorganisms.

The transformation of metals with variable valency by microorganisms considerably affects their solubility in water. This circumstance is taken into account in the development of microbiological technology for the removal of arsenic, chromium and other elements from waste waters.

Chemical removal of arsenic from waste waters is based on the oxidation of As (III) to As (V) utilizing pyrolusite. The method's disadvantage is that it requires multiple dilution of the influent waste

waters. This oxidation is necessary for obtaining a form of arsenic which produces practically insoluble precipitates with phosphates and calcium. Compared to chemical oxidation, microbiological oxidation of As (III) to As (V) appears more economical. Cultures of heterotrophic arsenic-oxidizing bacteria were isolated — *Pseudomonas putida*, *Alcaligenes eutrophus*, which oxidized up to 2 g/l of arsenite [18]. Waste waters containing arsenic are passed through oxidation columns in which the bacteria have been adsorbed on the surface of plastics or wood shavings. In the latter case it becomes unnecessary to supply the solutions with organic matter, since the organic carbon required for the nutrition of the bacteria is obtained from the wood.

While removal of arsenic from waste waters requires stimulation of microbiological oxidation of this element, the elaboration of a technology for the removal of chromium followed the path of microbiological reduction [19, 20] because, of the two forms of chromium, Cr (VI) and Cr (III), the trivalent form is the less toxic, and the reduction of this element serves therefore as a means of its detoxification. First an enrichment, and then pure culture of *Bacterium dechromaticans*, which uses chromates as electron acceptors under anaerobic conditions, have been obtained [19]. This microorganism utilizes the chemically bound oxygen of chromates for oxidizing organic substances in household sewage. A continuous-flow biological installation was designed for working out the technological process of removal of Cr (VI) compounds from industrial waste waters. The resulting technological process for the purification of chromium-containing waste waters by microorganisms has passed pilot trials and is now being introduced at a number of industrial enterprises around the country.

We have given some examples of metal immobilization as a result of the life activity of microorganisms, demonstrating how metals are converted to a low-mobile insoluble form through interaction with the products of microbiological transformation of organic matter and with hydrogen sulfide of biogenic origin. Such biological neutralization of harmful or toxic substances may be ecologically significant since the immobilized elements may be permanently or temporarily rendered incapable of actively affecting the environment.

The waste waters of non-ferrous metallurgical and chemical industries contain considerable quantities of cyanides, which are biological poisons, to be destroyed by all available technological means. Modern chemical methods of removing cyanides from waste waters rely on oxidation to cyanates, completely destroying the CN-group and precluding the secondary contamination of water with cyanides. Most frequently used for this purpose are chlorination and ozonization. These oxidation methods, however, have a serious shortcoming; they fail to destroy complexes of cyanides with metals widely used for technological purposes. To remove these from the water, ion-exchange methods may be effectively used.

Observations show that the microbiological process of cyanide decomposition is considerably accelerated upon the introduction into the medium of nitrogen-free sources of carbon, for example, carbohydrates [21, 22]. Thus, treatment of waste waters of a lead concentration plant destroyed 24 to 70 % of thiocyanates in 5 days with the addition of carbohydrates (sucrose) and only 3.7 to 28 % without an additional carbon source. Under industrial conditions it is recommended, to enhance the development of non-specific cyanide-destroying microflora, to enrich the waste waters with plant remnants or plant hydrolysate.

A study of how nitrogen sources influence the destruction of cyanides by microorganisms has revealed that the addition of nitrogen compounds to the medium inhibits the process of cyanide decomposition. Sometimes in practical treatment industrial and municipal-household effluents are mixed. Our observations show that in such cases the presence of compounds rich in organic nitrogen slows down the process of cyanide decomposition.

Under industrial conditions the addition of ammonium sulfate to the waste waters for the purpose of stimulating the life activity of the microorganisms oxidizing the organic flotation agents, slowed down the rate of cyanide destruction.

The inhibition of cyanide destruction in the presence of readily accessible nitrogen apparently indicates that microorganisms switch to nitrogen nutrition from the latter source. In the case when cyanides are the only source of nitrogen in the medium, there is a close relationship between the propagation of heterotrophic microorganisms and the drop in cyanide concentration within a wide range of C/N ratio.

Thus, to remove cyanides from waste waters, it is necessary to create favourable conditions for the propagation of heterotrophic microorganisms, primarily by providing them with a nitrogen-free source of carbon at optimal temperature and aeration. At iron-and-steel plants the two-stage treatment of waste waters of the coking process has been found inappropriate because this technology envisages separate stages for removal of phenols and of thiocyanates. Treatment intensity sharply increases when phenol-thiocyanate-containing waters are treated in a single aeration tank-mixer according to a single-stage scheme [23]. In this system, heterotrophic bacteria consume phenol as the source of carbon, assimilating the nitrogen of thiocyanates and cyanides.

CONCLUSION

The aforementioned data point to the great potential of microbiology for resolving the problems of optimal control and intensification

of industrial waste water treatment processes. Diversity of chemical transformations carried out, completeness of reactions and efficiency of the solutions found make the microbiological method of waste water treatment easy to realise, profitable and able to compete with its chemical counterpart.

REFERENCES

1. Rotmistrov, M.N., Gvozdyak, P.I., and Stavskaya, S.S., Microbial Destruction of Synthetic Organic Substances, Science Literature, Kiev (1975) (in Russian).
2. Naumova, R.P., Kulikov, N.I., Amerkhanova, N.M., Okrushko, V.E., Belousova, G.O., and Zolotukhin, A.M., In: Microbiological Methods of Environmental Pollution Control, V.A. Lambina (Ed.), Pushchino, 79 (1979) (in Russian).
3. Skryabin, G.K., and Golovleva, L.A., *Izvestiya AN SSSR*, ser. biol., No. 2, 232 (1972).
4. Ilyaletdinov, A.N., and Mendeshev, A., *Mikrobiologiya*, *48*, 3, 534 (1979).
5. Tul'chinskaya, V.P., Zhitetskaya, L.L., Moiseyeva, L.V., and Krivitskaya, T.N., In: Microbiological Methods of Environmental Pollution Control, V.A. Lambina (Ed.), Pushchino, 72 (1979) (in Russian).
6. Aliyeva, R.M., Dzhusupova, D.B., and Ilyaletdinov, A.N., *Ibid.*, 86.
7. Rapoport, I.A., and Vasilyeva, S.V., *Ibid.*, 32.
8. Romadina, E.S., and Ksenovich, G.F., *Ibid.*, 38.
9. Silantyeva, N.S., *Ibid.*, 40.
10. Graiver, N.A., and Dulepova, A.S., *Ibid.*, 47.
11. Vanifatyeva, M.G., Filippov, N.A., and Sergiyenko, Z.M., *Ibid.*, 42.
12. Neufeld, D., and Hermann, E.R., *J. Wat. Poll. Control Federation*, *47*, 2, 310 (1975).
13. Ilyaletdinov, A.N., Enker, P.B., and Yakubovsky, S.E., *Mikrobiologiya*, *45*, 6, 1092 (1976).
14. Ilyaletdinov, A.N., *Vodnye resursy*, No. 2, 158 (1980).
15. Ilyaletdinov, A.N., *Vestnik AN Kaz. SSR*, No. 7, 33 (1980).
16. Ilyaletdinov, A.N., Enker, P.B., and Loginova, L.V., *Mikrobiologiya*, *46*, 1, 113 (1977).
17. Ilyaletdinov, A.N., and Glazunov, V.D., Author's Certificate No. 812762, *Bulleten' izobretenii i otrkytii*, No. 10 (1981).
18. Abdrashitova, S.A., Ilyaletdinov, A.N., Mynbayeva, B.N., and Abdullina, G.G., *Prikladnaya biokhimiya i mikrobiologiya*, *18*, 2, 248 (1982).
19. Koren'kov, V.N., *Trudy Kazmekhanobr.*, Yu.N. Svidosch (Ed.), Alma-Ata, Collection No. 8, *1*, 231 (1972).

20. Koren'kov, V.N., and Vorobyov, L.F., In: Microbiological Methods of Environmental Pollution Control, V.A. Lambina (Ed.), Pushchino, 50 (1979) (in Russian).

21. Ilyaletdinov, A.N., Enker, P.B., and Vlasova, Z.G., *Mikrobiologiya*, *46*, 2, 358 (1977).

22. Ilyaletdinov, A.N., Enker, P.B., and Vlasova, Z.G., *Trudy instituta mikrobiologii i virusologii AN Kaz. SSR*, A.N. Ilyaletdinov (Ed.), Alma-Ata, *26*, 9 (1980).

23. Papkov, G.I., Mgen, V.A., and Malaya, I.A., In: Microbiological Methods of Environmental Pollution Control, V.A. Lambina (Ed.), Pushchino, 59 (1979) (in Russian).

PART VI

NEW TRENDS IN BIOGEOTECHNOLOGY

MICROBIAL BIOGEOTECHNOLOGY

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Intensive development of various branches of microbiology during the passed 10—15 years was accompanied not only by tremendous progress of fundamental knowledge but also by persistent attempts of varied uses of microorganisms and their metabolites in diverse industries. Big successes in the practical application of microorganisms have been scored in those spheres of man's industrial activity which have traditional ties with microbiology of long standing. First and foremost this pertains to various food industries, biosynthesis of medicines and other physiologically active compounds as well as to agricultural practices involving microorganisms.

The present report has for an object to review the investigations which contributed to scientific foundations and concrete microbial technologies employed in various branches of mining industry. This recent field of knowledge may be called Microbial Biogeotechnology and considered as rather a specific component of general biotechnology. Specific features of microorganisms used in biogeotechnology, originality of the chemical composition and physico-chemical properties of the raw materials processed by microbes and, finally, peculiarities of ecological conditions in which microbial processes occur in mineral deposits — all this stands to reason that the microbial biogeotechnology may be considered as an independent field of human research and industrial activity.

One of the best investigated biogeotechnological processes is the use of thionic bacteria for the leaching of iron, non-ferrous metals and uranium from sulfidized rocks. Particular features of the microbial leaching of metals are treated in sufficient detail in other sections of this book. Therefore, the present paper will concentrate upon the possible uses of microorganisms during extraction and processing of other minerals.

Table 1 shows some examples of the geochemically important microbial processes occurring in exploited oil-fields, coal basins, and native sulfur deposits and which are of high technological significance during mining and processing of these minerals. This table indicates also the basic microorganisms which play the key role in specific processes brought about during extraction and processing of the tabulated minerals. In fact, these processes involve an essentially more varied score of microorganisms: generally the whole complex of the natural microflora populating waters and rocks of a deposit. Activities of the microbes shown in Table 1 solely crown the entire complexity of interrelations existing in biocenosis and have a notable impact on technological techniques and, consequently, should be subject to primary consideration and regulation.

Before going into details of specific examples of microbial technological techniques of extraction of minerals, one should dwell upon some theoretical bases of modern biogeotechnology.

The first constituent part of such fundamental bases is the physiology of microorganisms, primarily, the particular physiology of those specific groups of microorganisms which are presented in Table 1 and often found under ecological conditions close to the extremal ones.

Table 1

Use of microorganisms in mining industry, some examples

Natural or technological process	Microorganisms
Oil production and processing	
Prospecting of oil and gas fields	Hydrocarbon oxidizing microorganisms
Drilling using microbial polymers	Heterotrophic mucoid bacteria
Transformation of stratal water composition and H_2S corrosion of equipment	Sulfatereducing bacteria
Use of surfactants to enhance oil recovery	Heterotrophic microorganisms
Use of polymers to enhance oil recovery	Heterotrophic microorganisms
Use of gas generation to enhance oil recovery	Fermentative anaerobes and methanogenic bacteria
Plugging of pores in oil-bearing ores	Hydrocarbon oxidizing and heterotrophic bacteria
Purification of the environment from oil pollution	Hydrocarbon oxidizing microorganisms
SCP production	Hydrocarbon oxidizing microorganisms
Coal mining	
Gas content decrease in coal strata	Methaneoxidizing bacteria
Decrease in methane release from worked-out areas	Methaneoxidizing bacteria
Acid drainage formation	Thiobacteria
SCP production from casing-head gas	Methaneoxidizing bacteria

Table 1, continued

Natural or technological process	Microorganisms
Production and processing of native sulfur	
Prospecting of non-carbonate sulfur ores	Thiobacteria
Transformation of ground water composition and H_2S corrosion	Sulfatereducing bacteria
Sulphuric acid weathering of deposits proper, ore stocks and products	Thiobacteria

The second most important component is the modern dynamic ecology of microorganisms providing for a quantitative assessment of microbial metabolic pathways under natural conditions.

The third constituent part of modern biogeotechnology is the theory and practice of limiting and stimulating microbial growth in conditions of a flow culture both of pure and mixed populations.

The first of these theses does not seem to a biologist to need special evidences. It is obvious, for example, that without isolation of pure cultures of acidophilic thiobacilli or methanotrophic bacteria and detailed investigation of their physiology and biochemistry, the very concept of biological oxidation of sulfides in acidic drainage water or methane oxidation under low-temperature conditions would not have been rigorously substantiated. Experience points, however, to the fact that with isolation of new species of microorganisms from the natural microflora and their thorough investigation our ideas about peculiarities of specific physiological species of microorganisms may evolve essentially and this, in turn, will influence an assessment of the geochemical role of microorganisms.

In this context, an instructive example is given by recent investigations conducted by N. Pfennig and his associates on physiological aspects of sulfate-reducing microorganisms. Up to the recent time, scientific literature was dominated by Campbell and Postgate's standpoint according to which there exist two types of such bacteria — a non-sporous genus *Desulfovibrio* and a sporous one *Desulfotomaculum* [1, 2]. It was also assumed that all bacteria referred to this group utilize for sulfate reduction quite a limited set of organic acids, alcohols, and hydrogen.

As studies expanded on mud deposits and stratal H_2S-containing waters in oil-fields, numerous bacterial cultures were isolated which differed sensibly by a number of their characters from Campbell and Postgate's type cultures [3, 4]; part of the new cultures have been referred to the third genus — *Desulfomonas.* Quite recently, after publication of a paper by Widdel [5], it became evident that the existing taxonomy and physiological peculiarities of the group of sulfate-reducing bacteria should be entirely revised, since Widdel succeeded in isolating a vast collection of cultures fairly unusual as to their morphology and a spectrum of utilized organic compounds.

In the course of the aforementioned studies it was revealed that some sulfate-reducing bacteria, in particular, *Desulfotomaculum acetooxidans* [6] were capable of performing sulfate reduction using acetate. These data have strongly shaken a harmonious concept of anaerobic decomposition of organic matter according to which acetate is utilized under natural conditions exclusively by methane-producing bacteria and, consequently, it was precisely the acetate mechanism of methane genesis that was considered as the basic route in various anaerobic ecosystems [7, 8].

Another example of a cardinal revision of the geochemical significance of microbial activities with regard to defining more precisely their physiological peculiarities may be given by the hypothesis of peroxide mechanism of ferrous iron and manganese oxidations by the so-called iron- and manganese-oxidizing microorganisms [9, 10]. Within the framework of this hypothesis the geochemical role of these microbes has closer similarity to the activities of common heterotrophs and consists, above all, in mineralization of organic compounds, while the function of iron and manganese oxidation and accumulation of their oxides is portrayed as secondary and not rigorously obligatory.

Of paramount importance for the theory of microbial biogeotechnology as well as for its industrial applications is the second thesis — need for investigation of geochemical activity of microorganisms in situ, i.e. directly in deposits of minerals. And here, the problem is not conditioned exclusively by the fact that the conditions of microbial existence in deposits approximate frequently to the extreme ones by such parameters as high acidity and raised contents of heavy metals in the zones of oxidation of sulfur and sulfide deposits or differ by extremely high temperature and salinity, as often observed in oil-fields.

The core of the matter resides in the fact that for a correct interpretation of microbial biogeochemical processes and especially for a due elaboration of biogeotechnological techniques, it would be a principally erroneous practice if one is guided only by results obtained with pure cultures of microorganisms in bench conditions. Need is felt for elaborating methodological approaches and concrete methods for determination of the physiological activity of microbes directly in natural conditions.

It is noteworthy that from the standpoint of setting, this problem appears important not only for geomicrobiology but also for other fields of ecological microbiology. An eminent Soviet scientist Winogradsky was a consistent adherent of such methods to be used in soil microbiology and wrote as early as in 1926: "the methods for studying real processes brought about by microbes in nature should be based upon investigation of microbial communities on the whole under natural conditions" [11].

Nowadays, geomicrobiologists have at their disposal a comparatively large arsenal of methods for conducting research of the kind (Table 2). Data condensed in this table show that for many microbial

Table 2

Some techniques for quantitative determination of geochemically important processes performed by microorganisms

Process under study	Technique employed	Studied objects in nature	Basic references
Dissimilatory sulfate reduction	Use of $^{35}SO_4^{2-}$ and analysis of label distribution in sulfate reduction products (H_2S sulfides)	Sulfur and oil fields, bottom sediments of water basins	12—14
Chemical and biological hydrogen sulfide oxidation	Use of $H_2{}^{35}S$ and analysis of label distribution in oxidation products (S^0, SO_4^{2-}, intracellular S)	Sulfur deposits, water basins	15, 16
Carbon dioxide assimilation	Use of $^{14}CO_2$ to study rates of photosynthesis, chemosynthesis and heterotrophic assimilation by analysing radioactivity of biomass and exometabolites	Water and bottom sediments of seas, lakes and water reservoirs	17—19
Microbial methanogenesis	Use of $^{14}CO_2$ and ^{14}C acetate, and analysis of radioactivity of newly formed methane	Oil, gas and sulfur fields, bottom sediments of water basins	20, 21
Microbial methane oxidation	Use of $^{14}CH_4$, and analysis of label distribution in oxidation products and biomass of methanotrophic microorganisms	Coalfields, water basins	22, 23
Microbial nitrogen fixation	Use of $^{15}N_2$ Acetylene technique	Soils, water and sediments of water basins	24
Denitrification	Inhibition of denitrification by acetylene followed by the analysis of nitrous oxide accumulation using gas-chromatography technique	Bottom sediments, soils	25

processes involved in the sulfur cycle as well as in the cycles of carbon and nitrogen quantitative approaches have been worked out based upon the use of labelling techniques and high-sensitive gas chromatography.

Application of these methods provided for quantitative estimates of a number of biogeochemical processes both at ecosystem and global levels. Factual data cited in Table 3 characterize the rates of microbial methane production in various ecosystems including such important for biogeotechnology objects as underground waters of gas and oil fields.

The third column of this table deserves thorough consideration as it points to the fact that the natural mechanism of methane production reposes upon carbon dioxide reduction, and not acetate fermentation, as this is generally assumed in the microbiological and geochemical literature.

Autotrophic fixation of carbon dioxide which occurs in the oceanic water column as well as sulfate reduction in oceanic sediments were investigated in greater detail that enables already to make their quantitative assessment at the global level. Table 4 gives well-known magnitudes of the primary production of organic matter in the ocean, and Table 5 shows recent results which characterize the scope of vital activity of sulfate-reducing bacteria in oceanic sediments of various regions. Based on the data cited in Table 5, it was calculated that sulfate-reducing bacteria metabolize annually in oceanic sediments about 450—500 Tg of sulfate sulfur which is as much as nearly three times the annual world output of sulfur-containing raw materials.

Thus, the latest data of dynamic geomicrobiology and biochemistry make it possible to assess quantitatively certain natural processes conditioned by microorganisms that, on the one hand, is of great importance for the general biological and biogeochemical concepts and, on

Table 3

Methanogenesis intensity (μl CH_4 per day per kg wet silt or stratal water) and part of methane generated from CO_2 in various anaerobic ecosystems

Sampling site	Intensity	Methane from CO_2, %
Kichier Lake, fresh [26]	20—520	46—92
Gulf of Riga [27]	16.5—27.3	—
Baltic Sea [27]	0.10—7.30	55—99
Persian Gulf and Gulf of Oman [26]	0.01—0.10	74—98
Gulf of California [26]	0.001—0.006	86—99
Arabian Sea [26]	0.004—0.01	37—99
Stratal waters of gas bearing fields of the Subcaspian depression [26]	0.06—0.64	80—99
Freshened stratal waters of oil field [28]	0.10—0.30	to 95
Mineralized waters of oil field [28]	0.015—0.029	to 95

Table 4

Organic matter primary production in the World Ocean according to different authors (million tons C per year)

Organic matter primary production	Reference
23110	29
44000	30
33100	31
43500	32

the other hand, induces practical workers to the search for routes of practical application of a useful geochemical activity of microorganisms and curbing their destructive activity as agents of biological corrosion and other deleterious effects.

While assessing results obtained on the rates of microbial processes in natural conditions from the technological point of view, one should bear in mind two basic circumstances which prove to be hazardous for the vital activity of microorganisms in most ecosystems and, particularly, in deposits of minerals. First, the growth of microorganisms

Table 5

Results of studies on the sulfate reduction intensity in the World Ocean sediments using radioactive isotopes

Sampling site	Intensity, $\mu g \cdot kg^{-1} \cdot day^{-1}$
Continental seas	
Black Sea deep sediments	645*
Baltic Sea sediments	1310
Azov Sea sediments	1800**
World ocean sediments	
Upper part of shelf (up to 100 m)	94.0
Lower part of shelf (100—200 m)	32.0
Upper part of slope (500—1000 m)	77.6
Lower part of slope (1000—3000 m): Arabian Sea	46.3
Lower part of slope (1000—3000 m): Gulf of California	41.0
Lower part of slope (1000—3000 m): Open ocean	3.4
Deepwater sediments (deeper than 3000 m)	2.1

* — Data of [33].

** — Data of [34].

The other results are from the Laboratory for biogeochemistry, Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences.

under natural conditions, in contrast to that in fermenters, occurs in the situation of a rigid limitation. Generally, the principal limiting factors are the following: low concentration of a readily accessible organic matter indispensable for all heterotrophs, reduced concentration of oxygen required by all aerobes, and limitation in phosphorus and nitrogen. Besides, except for aquatic ecosystems which approximate to the conditions of flow cultivation, all natural ecosystems are characterized by utterly unfavourable mass-exchange conditions.

As suggest data shown in Table 6, in most deposits of minerals microorganisms are acting under the stress of, as a minimum, two-three unfavourable environmental factors approximating by their values to the extreme ones. In most deposits such unfavourable factors are anaerobic environment often combinated with toxic levels of hydrogen sulfide produced by sulfate reduction and enhanced mineralization of stratal waters (see Table 6).

Analysis of ecological factors unfavourable for the development of microbial processes in mineral deposits (Table 6) may be used as a guideline for elaborating technological techniques aimed at repression of deleterious microbial processes and activation of useful ones.

Aeration is one of the potent and fairly simple technological means of promoting aerobic microbial processes. This technique is successfully used, in particular, in all types of microbial metal leaching from ores. It proves to be equally efficient in the vat, heap and underground leachings using aerobic thionic and iron-oxidizing bacteria.

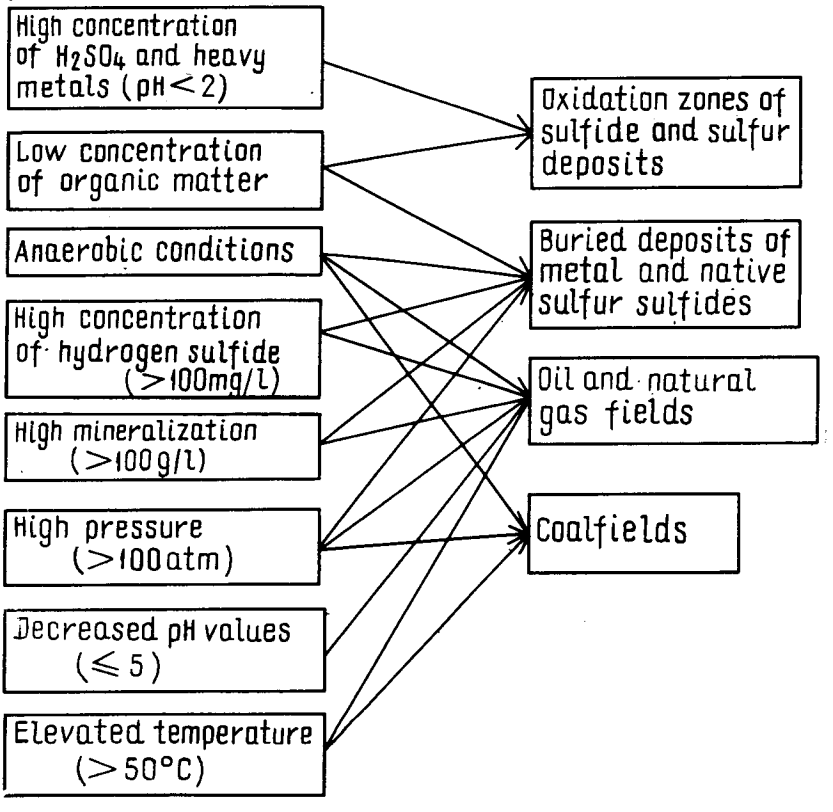
To give other examples of the efficiency of stratal water aeration, for activating microbial processes in rocks and mineral deposits, a mention should be made about certain works of American microbiologists from the Sun Oil Company [35, 36]. During a break of the underground oil pipe-line belonging to this company happened in the Philadelphia region in 1971, over 500 tonnes of the high-octane oil infiltrated into porous and fissured dolomites which were used as water-bearing horizons for drinking water supply to this large and densely populated area.

No more than one third of the oil spilt has been pumped out through the specially drilled wells. It was calculated that natural processes of the oil wash-out and oxidation would reduce its content to a level of tolerated concentrations in drinking water not earlier than after one century. Therefore, an attemp was made to induce the activity of hydrocarbon-oxidizing bacteria populating ground water. Nitrate and phosphate solutions were pumped down through the previously drilled wells followed by forced aeration of stratal water.

Data shown in Fig. 1a indicate that immediately after aeration had been started, the total level of microorganisms (dark spots) and the numbers of hydrocarbon-oxidizing bacteria, in particular (fair spots), increased drastically. In control wells without oil pollution and aeration the numbers of microorganisms remained at a much lower level during the whole period of observations (Fig. 1b).

Table 6

List of basic ecological factors limiting the microflora development in mineral deposits



During one and a half year of an intensive treatment of the polluted area some 58 tonnes of ammonium sulfate and 39 tonnes of sodium phosphate were spent for activating microbial processes. This resulted in oxidation of about 150 tonnes of oil, and after the 18-month period the hydrocarbon concentration in ground water dropped to 5 mg/l. In other words, by virtue of fairly simple biogeotechnological techniques based on the knowledge of the physiology of hydrocarbon-oxidizing bacteria, a 75-fold increase of the rate of an aerobic microbial process in ground water has been reached.

In this connection it should be pointed out that aeration of underground water through a system of wells can be applied for activation of not only aerobic but also of some anaerobic microbiological processes in mineral deposits. All oil-producing countries are known to employ pumping of surface water to enhance oil recovery. Use of such

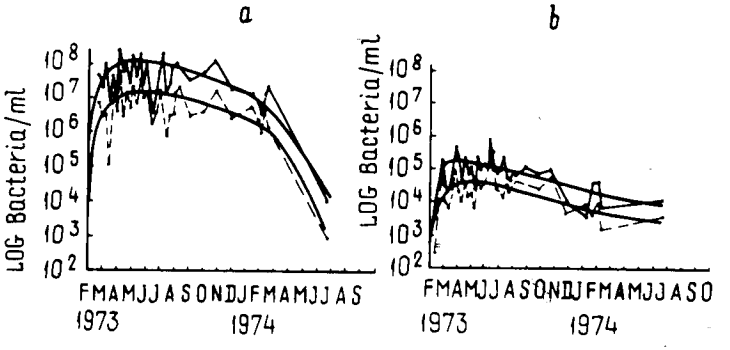


Fig. 1. Variation in numbers of hydrocarbon-oxidizing bacteria (lower curve) and bacteria counted directly (upper curve) in underground water of a zone of oil pollution (a) and in the wells of a non-polluted area (b) from [35]

techniques results very often in an intensive growth of anaerobic sulfate-reducing bacteria in oil-bearing strata, the phenomenon leading to a pollution of oil and oil gas with microbial hydrogen sulfide and further to a corrosion of mining equipment [3].

First attempts to interpret an inducement of sulfate reduction along with water pumping into oil wells came to a statement that additional sulfate was introduced in oil-bearing strata. Sulfate reducers, however, require for their vital activity not only sulfate but also some low molecular weight fatty acids which cannot be practically produced from oil under anaerobic conditions.

Elucidation of interrelationships between aerobic hydrocarbon-oxidizing bacteria and anaerobic sulfate-reducing bacteria in the zone close to the flood water — petroliferous stratum interface revealed (Fig. 2) that dissolved oxygen is introduced with flood water into the oil-bearing stratum bordering with a well [37—40]. The magnitude of redox potential in this zone is rather high and favours development of aerobes. Oxidized hydrocarbon derivatives are produced in this zone at the expense of partial oxidation of oil hydrocarbons by hydrocarbon-oxidizing bacteria. These derivatives are transported with water flow into the anaerobic zone of the stratum where they are used by substrates for the growth of sulfate-reducing and other anaerobic bacteria.

Similar technique for inducement of an anaerobic microbial process by activation of the aerobic hydrocarbon-oxidizing microflora was used by researchers from the Laboratory of Biogeochemistry (Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences) in oil-fields of the Bondyuzhskoe deposit [41, 42]. The purpose of these experiments was, however, somewhat different; they were

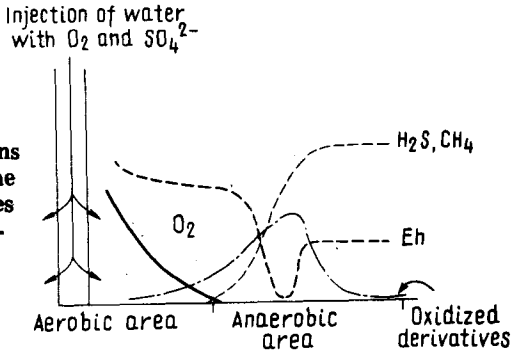


Fig. 2. Variation of Eh, concentrations of oxygen, hydrogen sulfide, methane and oxidized hydrocarbon derivatives in the face-adjoining zones of flood-water pumping wells (Scheme)

aimed at activating methanogenic bacteria instead of inducement of sulfate reduction, as it happened.

Prior to experiments, we studied the distribution of aerobic and anaerobic bacteria in underground water of the oil-fields discussed. Data on distribution of aerobes and the rates of methane genesis are shown in Table 7. The number of anaerobic bacteria was higher in strongly freshened water and might be as great as several thousands cells per litre. Pure cultures of methanogenic bacteria were isolated from the flood-water and highly freshened stratal water. The study of the morphology, and physiological and biochemical properties of these organisms allowed us to identify them as *Methanobacterium bryantii*, strain *Omeljanskii* and *M. formicicum*, strain *Kuznetsovii*, since they differed from typical strains.

Using radioactive isotopes we showed that methanogenic bacteria were active in conditions of stratal waters. The total rate of methanogenesis, resulting of the sum of CO_2 reduction by hydrogen and reduction of methyl groups of acetate, was from 15 to $300 \cdot 10^{-6}$ ml $CH_4 \cdot litre^{-1} \cdot day^{-1}$ (Table 7). The bulk of biogenic methane was formed by CO_2 reduction. As seen from Fig. 3, the highest rate of methane production was observed in highly freshened waters with mineraliza-

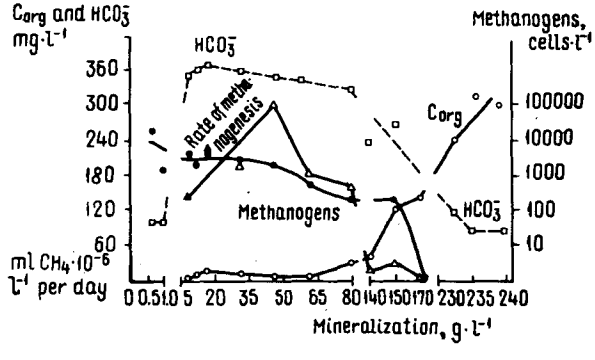


Fig. 3. Physico-chemical and microbiological characteristics of underground waters with different salinity in the Bondyuzhskoe oil field

Table 7

Physico-chemical conditions, distribution and geochemical activity of anaerobic microorganisms in stratal waters of the Bondyuzhskoe oil field [41]

No. of well	Mineralization, g/l	pH	Eh, mV	Content, mg · l⁻¹			Bacterial number, cells · l⁻¹		Methanogenesis intensity, ml CH_4 · l⁻¹
				ΣC_{org}	CH_3COOH	HCO_3^-	sulfate reducing	methanogens	
Injected surface water									
428	0.7	7.3	+405	*	0.7	102.0	4000	25000	0
28	0.8	7.8	+330	*	0.3	97.6	6000	1300	0
Highly freshened water									
348	8.0	6.9	+80	5.0	3.0	348	9000	6000	145.7
265	10.0	7.0	+65	12.0	2.9	358	8000	2500	106.0
295	15.0	7.0	+100	16.1	1.0	373	250	6000	227.0
47	30.0	6.9	+80	12.0	1.4	361	1600	2500	195.0
303	45.0	6.9	+100	5.0	1.4	348	4000	2500	305.0
94	58.0	6.8	+75	6.0	0.6	347	250	600	181.0
254	80.0	6.7	+65	31.5	1.3	326	1500	250	159.6
Weakly freshened water									
371	140.0	6.6	+130	38.0	0.3	232	3000	*	14.8
370	150.0	6.4	+115	124.0	2.4	272	0	250	28.9
323	170.0	6.5	+150	145.4	1.7	159	0	0	0
Highly mineralized water (brines)									
316	233.2	5.8	+140	244.0	12.0	110	*	0	0
306	234.4	5.7	+150	315.0	27.3	85	0	0	0
66	239.6	5.9	+150	290.5	29.1	85	0	0	0

* — Means the lack of data.

tion from 15 to 45 mg/l. The intensity of methanogenesis dropped in stratal waters with higher mineralization level. Methanogens were not found in the water with the total salinity of 170 g/l and higher. High concentrations of dissolved organic matter including acetate and an appreciable decrease of bicarbonate content in brines point to a subsidence of all microbial processes in this zone.

Studies of the carbon stable isotope composition of methane and bicarbonate in stratal waters yielded additional information on recent methanogenesis (Fig. 4). Methane from regions of recent methanogene-

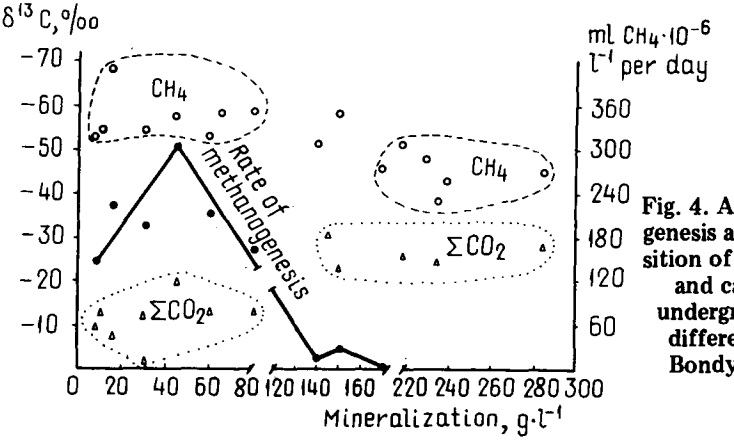


Fig. 4. Activity of methanogenesis and isotopic composition of carbon in methane and carbon dioxide in underground waters with different salinity in the Bondyuzhskoe oil field

sis is significantly enriched with ^{12}C compared to methane from highly mineralized waters. On the other hand, carbon of bicarbonate in freshened water is heavier by its isotopic composition compared to bicarbonate carbon in brines.

The major source of mineral carbon in stratal waters is microbial oxidation of oil with the carbon isotope composition in the range of −25 to −28 ‰. So, the enrichment of carbonate carbon with heavy isotopes occurs only during microbial CO_2 and HCO_3^- reduction to methane. A light isotope composition of methane in freshened stratal waters is an additional evidence of an active modern microbial methanogenesis in this part of the oil-field.

Special attention was paid to the studies of microorganisms in the zone close to the flood-water — oil stratum interface in the Bondyuzhskoe oil-field. Fig. 5 shows that during the flooding of the oil-field, some amount of oxygen is introduced in the oil-bearing stratum with injected fresh water. In the zone of contact oxygen is, however, rapidly consumed and redox potential drops rather quickly. At the same time, the total number of bacteria increases significantly. The content of dissolved organic carbon and bicarbonate also increases.

Emergence of anaerobic conditions in the zone of contact and enrichment of water with soluble organic matter and CO_2 activate microbial processes, in particular, the rate of methanogenesis (Fig. 5).

Comparison of the results of field experiments in the Bondyuzhskoe oil-field (Fig. 5) with a theoretical scheme, emerged from works of V.A. Kuznetsova on interaction of aerobic and anaerobic processes in the zone close to the flood-water/oil stratum interface of flooded oil-fields using example of sulfate reduction (Fig. 2), exhibits good correlation.

Substantial activation of microbial methane production by the products of aerobic microbial oil decomposition performed by cultures of aerobes isolated from the oil-field in question was also demonstrated using samples of underground water supplemented with cultural liquid containing products of partial oxidation of oil (Fig. 6).

Preliminary studies were followed by an experiment on activation of methane production by flooding the oil-bearing stratum with aerated water supplemented with phosphate and nitrate. For this purpose, the stratum was flooded with 35 m^3 of aerated water with some 2,000 m^3 of air; after this the well was plugged for two months. After such an incubation period, experimental ejection of stratal water started and

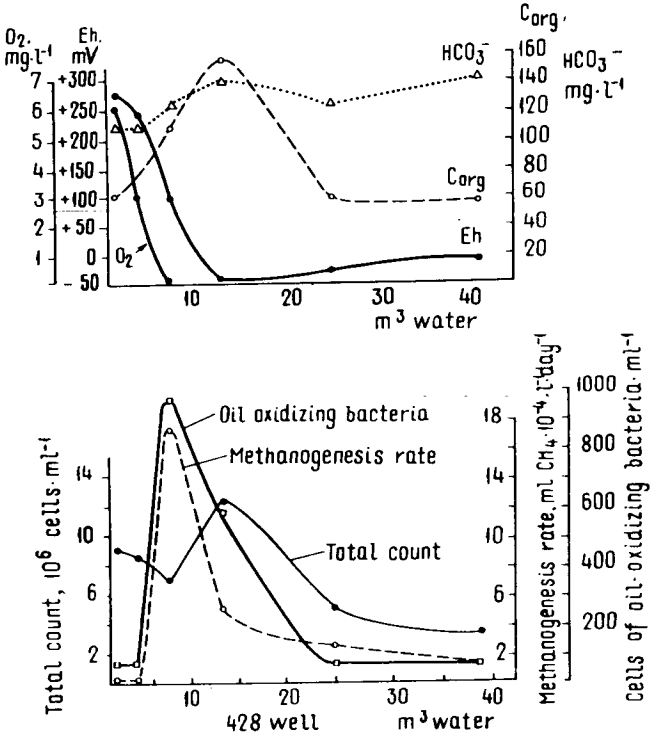


Fig. 5. Variation of microbiological and hydrochemical parameters of the stratal water in the zone of flood-water pumping wells of the Romashkinskoe (upper part) and Bondyuzhskoe (lower part) oil fields

followed by measurement of a set of microbiological and geochemical parameters. Control data had been obtained during water ejection from the same well prior to its flooding with aerated water (see Table 7).

Table 8 presents basic data on the increase of geochemical activity of methanogens. For the discharge of 115 m^3 of water, the amount of methane generated in experimental conditions was 24 times as great as that produced in control conditions. Altogether, 16.5 m^3 of methane was generated during the experimental discharge.

The ratio between the amount of methane and the sum of heavy hydrocarbons in the gas composition is an important characteristic of the nature of methane. In the experiment this ratio was 14.6, while it did not exceed 1.7 for the gas from the production wells of the oil-field, thus pointing to an essential difference in their genesis.

The carbon isotope composition of methane changed significantly during the experimental discharge. Its $\delta^{13}C$ values, especially in the first samples were —88.2 ‰. The average $\delta^{13}C$ values of methane released during the experiment made up —64.1 ‰. It might be compared with the average value of $\delta^{13}C$ of methane sampled from the nearest production wells (with mineralization level of 80 g/l) which was —55.7 ‰. Such a significant lightening of methane released during the experimental discharge indicates that at least major part of methane is of a microbial origin. This is also confirmed by the analysis of the carbon isotope composition in carbonates from stratal liquids. Carbonate from stratal water of the experimental discharge has the average $\delta^{13}C$ value of about —10.1 ‰. It is substantially heavier than that of the control discharge (the average $\delta^{13}C$ value is —24.9 ‰) and carbonates from the nearest production wells (the average $\delta^{13}C$ is

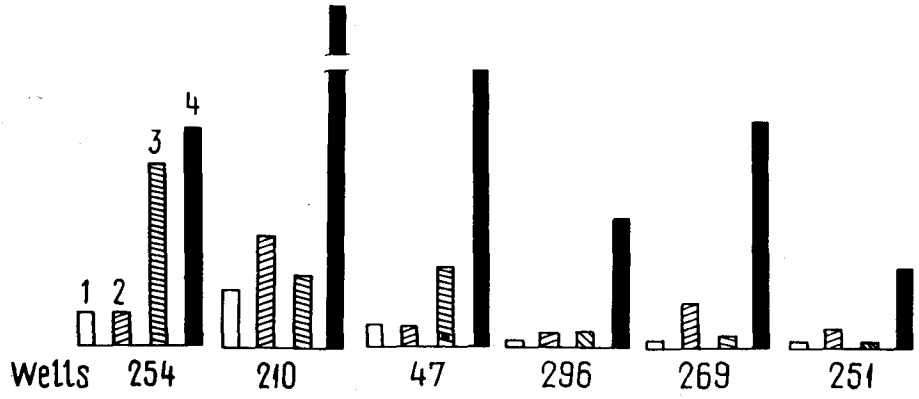


Fig. 6. Influence of various additives on the rate of methanogenesis in the stratal waters of the Bondyuzhskoe oil field:

1 — without additives; 2 — supplemented with 200 mg/l NH_4^+; 3 — supplemented with 92 mg/l PO_4^{3-}; 4 — supplemented with a culture liquid of petroleum-oxidizing bacteria

Table 8

Variations in the methane content and the sum of carbon mineral forms and their carbon isotope composition in water of well 428 during control and experimental ejections

Volume of ejected water, m^3	Volume of methane released, l		CH_4	$\delta^{13}C$ of methane, ‰	$\Sigma CO_2 + HCO_3^- + CO_3^{2-}$ in mg HCO_3^-/l		$\delta^{13}C$ of carbonates, ‰	
	control	experiment	C_2—C_5		control	experiment	control	experiment
4.5	0.5	1.1	17.3	*	138	172	−15.5	−12.9
12.3	2.3	*	*	*	180	*	−26.8	*
25.8	10.4	*	*	*	198	*	−30.5	*
54	37.7	596	16.3	−88.2	300	336	−30.3	−10.2
99	67.0	1531	14.0	−68.9	348	420	−25.8	−11.0
115	81.3	1984	14.0	−55.5	348	420	−20.3	−9.1
150	*	3314	11.3	−87.3	*	540	*	−14.5
204	*	5016	1[illegible]9	−58.7	*	528	*	−11.9
282	*	6831	1[illegible].9	−56.7	*	516	*	−8.6
355	*	8877	13.5	−66.9	*	528	*	−9.8
397	*	10471	15.0	−62.1	*	540	*	−6.6
472	*	12921	16.7	−58.5	*	528	*	−11.8
547	*	14742	16.7	−59.0	*	480	*	−8.1
619	*	16523	18.1	−59.5	*	480	*	−6.8
	Average		14.6	−64.1			−24.9	−10.1

* — Means the lack of data.

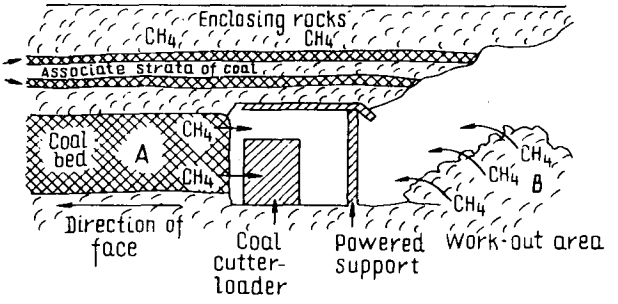


Fig. 7. Sources of methane emission into the atmosphere of a coal mine (scheme):

A — coal stratum under mining; B — broken rocks in the worked-out area

—15.4 ‰). Such a heavy isotope composition of carbonates also points to an active bacterial methanogenesis due to CO_2 reduction.

Hence, the proposed activation of the stratal microflora leads to an inducement of microbial processes resulting in raised concentrations of organic compounds and of bacterial methane newly formed in the stratal waters and, consequently, contributing to an enhancement of oil recovery.

The three examples from the current practice of different branches of industrial biogeotechnology, we have just considered, seem to suggest that with proper knowledge of the physiology of microorganisms and thorough investigation of ecological conditions existing in a deposit, even a fairly simple technological technique — increased aeration — may lead to a desirable and quite important effect.

However, the potentials of biogeotechnology in resolving important problems, the mining industry is faced with, are not confined to the examples cited and methodological approaches described.

In scientific and patent literature on biotechnology and, particularly, on petroleum microbiology and problems of the environment protection one may find some suggestions, and their number goes increasing, for, e.g. introduction in various ecosystems of new strains of microorganisms isolated earlier from natural sources and then thoroughly studied and selected in laboratory conditions. In most cases the authors of such proposals believe that simple inoculation of a more productive or more acid-tolerant or more psychrophilic strain to the natural association should result in activation of a desired process.

Such a simplistic approach to the use of microorganisms in natural conditions for activation of microbial processes seems to have no future and might only compromise the very idea about regulation of vital activity of microorganisms in complex conditions arising in their natural habitats.

We believe that should need be for introducing in a natural ecosystem of a "foreign" microorganism, care must be taken also about a complex of biogeotechnological means providing for an active survival of such microorganisms.

Results of research on application of methane-oxidizing bacteria for reducing the methane level in coal mines may be cited as an example of a more or less successfull solution of such a problem.

Fig. 7 portrays a section of a mechanized drift with a coal cutter-loader. Basic sources of methane are the coal stratum itself (A) and fragmented rocks from the rock breakage zone behind the coal mining complex (B). Both sources release methane into the mine atmosphere, and the moment its concentration approximates to the explosive one the automatic alarm devices switch off all the operating mechanisms. Work in the mine is resumed only after the methane level has been considerably reduced by aeration.

Thus, the faster advances the coal face and more coal is extracted per unit of time, the greater is the rate of methane release and explosion risk and, consequently, the coal mining process should be frequently interrupted. One of the methods enabling to reduce methane concentration in a coal stratum prior to its mining and thus to decrease the methane release into the atmosphere of a mine consists in microbial treatment of a coal block.

The ability of methane-oxidizing bacteria to oxidize methane in aerobic conditions is well known to microbiologists. However, the problem is complicated by the fact that such microflora does not occur in coal strata and, furthermore, anaerobic conditions prevail in coal blocks. Therefore the technology of microbial lowering of methane content in coal should provide for both introduction of microbial cells in a coal stratum and establishment of favourable conditions for reproduction of such aerobic bacteria.

According to one version of such technology (Fig. 8) tested in some Donbass mines [44], suspension of methane-oxidizing bacteria is injected into a coal stratum through the middle borehole with a high-pressure pump. Then air is pumped into the coal block for 2—3 weeks thus supplying oxygen for the microflora. Products of microbial oxidation of methane and excess air are removed through discharge holes. After such a treatment during 15—20 days, the gas content in coal is reduced by 55—60 %. Of this amount one half of methane is discharged with the air flux while the other is oxidized by microbes to carbon dioxide [44]. Even higher effects may be obtained by an extention of the period of aeration of coal treated with bacterial suspension, especially in the case when the treatment of a coal block is performed from the surface prior to the mining.

As mentioned above, important quantities of methane, sometimes up to 90 % of its total amount entering the mine atmosphere may be emitted from the rock breakage zone. In this zone, especially in its part adjoining the operating face, the crashed rocks are well aerated, and hence favourable conditions are created for methane-oxidizing bacteria.

Introduction of biomass of active methane-oxidizing bacteria in the form of specific live filter along the border of the rock breakage zone (Fig. 9) results sometimes in a substantial drop of methane concentration in the mine [45]. Contents of methane and carbon dioxide generated by its oxidation in the atmosphere of a real mine may vary

sensibly depending upon the rates of coal mining and aeration. Therefore for detection of the microbial oxidation in this experiment we resorted also to the method of measuring carbon isotope ratios in methane and carbon dioxide available in the mine atmosphere [22].

Studies of the microbial oxidation of methane in laboratory conditions revealed that the product of its oxidation — carbon dioxide — was enriched with the light isotope ^{12}C whereas the heavy isotope ^{13}C was accumulated in residual methane [46]. Data shown in Fig. 10 indicate

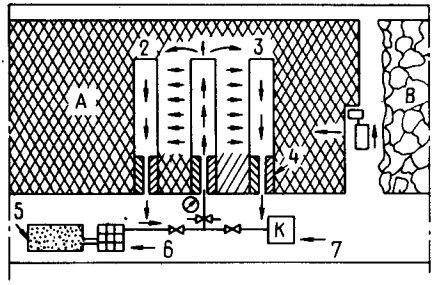


Fig. 8. Diagram of the microbial treatment of a coal stratum to decrease its methane content:

1 — culture injection and stratum aeration well; 2, 3 — degassing holes; 4 — zone of holes sealing; 5 — tank with culture of methane-oxidizing bacteria; 6 — pump; 7 — high-pressure compressor; A — coal stratum; B — worked-out area

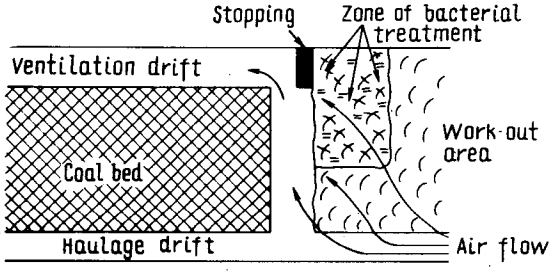


Fig. 9. Diagram of the use of microbial method for reducing the content of methane released from the worked-out area

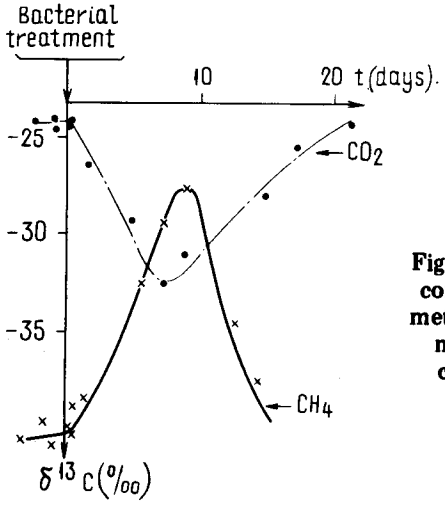


Fig. 10. Variation of carbon isotopic composition in carbon dioxide and methane in the atmosphere of a coal mine using microbial method of combating methane level in the worked-out area

that the isotopic composition of carbon dioxide and methane is notably changed in the mine atmosphere with the development of an active microbial oxidation of methane in the rock breakage zone; similarly to the laboratory experiments, carbon dioxide is enriched with the light isotope and residual methane with the heavy one. It may be calculated from the data on variation of isotopic composition that the concentration of methane in the mine air decreased by 40—45 % due to the activity of methane-oxidizing microflora introduced in the form of artificial filter into the rock breakage zone.

In conclusion it should be noted that further progress in applied microbial biogeotechnology will require above all broad fundamental investigations of the distribution and geochemical activity of microorganisms under natural conditions. A great deal is known about physiological peculiarities of geochemically important microorganisms as well as about the areals of their distribution in various ecosystems. Recent years have seen a notable activation of works on modelling of natural microbial processes in laboratory conditions. However, quantitative aspects of the activity of microorganisms in natural conditions, investigation of factors capable of stimulating or inhibiting the functioning of particular parts of complex microbial cenoses have not yet been given sufficient effort.

Still, the examples we have discussed indicate that the setting of such problems is far from being doomed to a failure, and in a number of cases the regulation of microbial processes in natural conditions is not only feasible but also appropriate and economical from the point of view of man's industrial activity. In other terms, it may be said that microbial biogeotechnology has proven its right to existence not only as an interesting field of fundamental knowledge but also as a science capable of real contribution to the technological progress in various branches of mining industry.

REFERENCE

1. Campbell, L.L., and Postgate, J.R., *Bacteriol. Rev.*, *29*, 359 (1966).
2. Postgate, J.R., and Campbell, L.L., *Bacteriol. Rev.*, *30*, 732 (1966).
3. Rozanova, E.P., and Kuznetsov, S.I., Microflora of Oil Fields, Nauka, Moscow (1974) (in Russian).
4. Rozanova, E.P., *Uspekhi mikrobiologii*, 10, 27 (1978).
5. Widdel, F., Anaerober Abbau von Fettsäuren und Benzoesäure durch neue isolierte Arten Sulfat-reduzierender Bakterien, Dissertation zur Erlang. des Doktorgrades, Göttingen, 445 (1980).
6. Widdel, F., and Pfennig, N., *Arch. Microbiol.*, *122*, 119 (1977).
7. Zavarzin, G.A., Lithotrophic Microorganisms, Nauka, Moscow (1972) (in Russian).

8. Bonch-Osmolovskaya, E.A., *Uspekhi mikrobiologii*, 14, 106 (1979).
9. Dubinina, G.A., *Mikrobiologiya*, *47*, 4, 591 (1978).
10. Dubinina, G.A., *Mikrobiologiya*, *47*, 5, 783 (1978).
11. Winogradsky, S.N., Principles of ecological microbiology, Conclusion, Microbiology of Soil, Publ. USSR Acad. Sci., Moscow, 789 (1952) (in Russian).
12. Ivanov, M.V., *Mikrobiologiya*, *25*, 3, 305 (1956).
13. Ivanov, M.V., Lein, A.Yu., and Kashparova, E.V., In: Biogeochemistry of Diagenesis of Ocean Deposits, Nauka, Moscow, 171 (1976) (in Russian).
14. Jørgensen, B.B., *Geomicrobiol. J.*, *1*, 1, 11 (1978).
15. Ivanov, M.V., *Mikrobiologiya*, *26*, 3, 338 (1957).
16. Sorokin, Yu.I., Transactions of the Institute of Inland Waters of the USSR Academy of Sciences, iss. 12(15), 75 (1966) (in Russian).
17. Steeman-Nielsen, E., *J. Conseil pour l'explorat de la mer*, *18*, No. 2(1952).
18. Kuznetsov, S.I., Utilization of radioactive carbon dioxide for determination of relative magnitude of photosynthesis and chemosynthesis in some lakes of various types, Isotopes in Microbiology, Publ. USSR Acad. Sci., Moscow (1955) (in Russian).
19. Romanenko, V.I., *Mikrobiologiya*, *33*, 134 (1964).
20. Belyaev, S.S., Finkel'shtein, Z.I., and Ivanov, M.V., *Mikrobiologiya*, *44*, 309 (1975).
21. Ivanov, M.V., Belyaev, S.S., and Laurinavichus, K.S., Methods of Quantitative Investigation of Microbiological Production and Utilization of Methane, Microbial Product and Utilization of Gases, Göttingen, 63 (1975).
22. Belyaev, S.S., Laurinavichus, K.K., and Ivanov, M.V., *Mikrobiologiya*, *44*, 542 (1975).
23. Ivanov, M.V., Ziakun, A.M., Bondar', V.A., Litvinov, P.S., Petukh, A.P., Miaken'ky, V.I., and Miagky, B.I., Reports of the USSR Academy of Sciences, *257*, 6, 1470 (1981).
24. Dugdale, R., Dugdale, V., Nees, J., and Goering, J., *Science*, *130*, 859 (1959).
25. Sorensen, J., Jørgensen, B.B., and Revsbech, *Microbiol. Ecol.*, *5*, 105 (1979).
26. Belyaev, S.S., In: Role of Microorganisms in the Turnover of Gases in Nature, Nauka, Moscow, 241 (1979) (in Russian).
27. Lein, A.Yu., Namsaraev, B.B., Trotsyuk, V.Ya., and Ivanov, M.V., *Geomicrobiol. J.*, *2*, 4, 299 (1981).
28. Ivanov, M.V., and Belyaev, S.S., Proceedings of 1982 International Conference on Microbial Enhancement of Oil Recovery, United States Department of Energy, 48 (1983).
29. Koblentz-Mishke, O.J., Valkowinsky, V.V., and Kabanova, J.G., In: Scientific Exploration of South Pacific, W.S. Wooster (Ed.), Nat. Acad. Sci., Washington (1970).
30. Bruevich, S.V., and Ivanenkov, V.N., *Okeanologiya*, *11*, 835 (1971).
31. Platt, T., and Subba Rao, D.V., In: Photosynthesis and Productivity in Different Environments, J.R. Cooper (Ed.), Cambridge University Press, 249 (1975).
32. De Vooys, C.G.N., In: The Global Carbon Cycle, SCOPE Report No. 13, John Wiley and Sons, Chapt. 10, 259 (1979).
33. Sorokin, Yu.I., *Mikrobiologiya*, *31*, 5 (1962).
34. Tolokonnikova, L.I., *Mikrobiologiya*, *46*, 2, 352 (1977).
35. Jamison, V.W., Raymond, R.L., and Hudson, J.O., *Dev. Ind. Microbiol.*, *16*, 305 (1975).

36. Raymond, R.L., Jamison, V.W., and Hudson, J.O., Beneficial stimulation of bacterial activity in ground waters containing petroleum products, Alche Symposium series, *73*, 106, 390 (1976).
37. Kuznetsova, V.A., and Li, A.D., *Mikrobiologiya*, *32*, 683 (1964).
38. Kuznetsova, V.A., Role of bacteria in sulfate reduction in oil fields, Abstract of MSc Thesis, Moscow (1965) (in Russian).
39. Gorlenko, V.M., and Kuznetsova, V.A., *Prikladnaya biokhimiya i mikrobiologiya*, *2*, 264 (1966).
40. Li, A.D., and Kuznetsov, V.A., *Neftepromyslovoye delo*, 9, 9 (1963).
41. Ivanov, M.V., Belyaev, S.S., Laurinavichus, K.K., Obraztsova, A.Ya., and Gorlatov, S.M., *Mikrobiologiya*, *51*, 2, 336 (1982).
42. Ivanov, M.V., and Belyaev, S.S., Microflora and Modern Methanogenesis in Flooded Oil Fields, Abst. Intern. Conf. Microb. Enhancement Oil Recov. Spenngri-La, Oklahoma, 25 (1982).
43. Belyaev, S.S., Obraztsova, A.Ya., Laurinavichus, K.S., and Ivanov, M.V., Reports of the USSR Academy of Sciences, *226*, 6, 1483 (1982).
44. Moskalenko, E.M., Ivanov, M.V., Nesterov, A.I., Smolianinov, N.G., Perminov, B.N., and Nazarenko, A.V., In: Ecology and Geochemical Activity of Microorganisms, Centre of Biological Research, Pushchino, 172 (1976) (in Russian).
45. Miaken'ky, V.I., Petukh, A.P., Litvinov, P.S., and Miagky, B.I., *Tekhnika bezopasnosti, okhrana truda i gorno-spasatel'noye delo*, 12 (1979).
46. Bondar', V.A., Namsaraev, B.B., Ziakun, A.M., Nesterov, A.I., and Ivanov, M.V., Variation in the isotope content of methane carbon in experiments simulating microbiological oxidation of methane in coal mines, Abstracts of the VIII All-Union Symposium on Stable Isotopes in Geochemistry, Moscow (1980) (in Russian).

MICROBIOLOGICAL METHODS OF MANGANESE LEACHING IN INDIA

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INTRODUCTION

India, with a population of over 650 million and a geographical area of more than 3.2 million sq.km, has abundant mineral wealth, which despite continuous search by various agencies has large unexplored areas. The gross production of mineral commodities in 1971 amounted to Rs. 4,800 million which corresponded to Rs. 9 per capita. Compared to the world average, the per capita mineral production or consumption is less than 3 %, which is exceedingly low and almost unbelievable. Needless to say, our very large population always gives us the disadvantage in per capita statistics.

In general, India has good reserves of iron ore, coal, mica, kyanite and sillimanite and reasonably good reserves of manganese and chrome ores. The export of the latter two ores is earning valuable foreign exchange at present. As the nation marches ahead in achieving self-reliance and growth through industrialization, it is possible that our domestic demands would limit the export of these materials. As in the case of other non-ferrous metals it is possible to experience a shortfall of these commodities. However, in case of manganese ores in particular the present problem seems to be the deteriorating situation in the production of this mineral.

Manganese ores enjoy high order of strategic importance because no quality steel can be produced without the addition of small amount of manganese. The production of manganese ore is closely related to that of steel and it is aptly called the 'Achilles heel' of the iron and steel industry, on the fabric of which the economy of a nation rests. Before we deal with the problem in particular we will summarize the existing information on occurrence of manganese in nature.

The earth's crust contains approximately 0.1 % manganese. Manganese possesses many properties similar to iron and is found closely associated with this element in nature. The widespread and simultaneous occurrence of these two elements and the complimentarity of properties of some of their compounds suggests the biogeochemical formation of these two elements in sedimentary deposits. However, sulfides of manganese (Alabandite) are not as common as pyrite and their formation by biogeochemical means is doubtful [1]. Manganese is less than 1/50 as abundant as iron [2] and its distribution in the crust is by no means uniform. For instance, in soils its concentration can range from 0.02 to 10.0 % [3]. From our point of view, the most important property is that the element shows variable valency cha-

racter and can exist in the oxidation states of 0, +2, +3, +4, +6 and +7. In nature, however, +2 and +4 oxidation states are common, whereas the +3 state occurs to some extent and +2 state can occur as a free ion in solution.

Among the various forms of manganese that occur in nature, the common ones are oxides, hydroxides, silicates and carbonates of manganese (siderite). The carbonates are found in sedimentary deposits and are reported from hydrothermal deposits, which is also true of oxides of manganese. Biogeochemical formation of sedimentary and authigenic deposits has been widely claimed, partly on the basis of findings of fossils or live bacteria and algae in iron and manganese deposits and partly on the basis of laboratory demonstrations of formation of oxides by microorganisms. These deposits usually contain the tetravalent form of manganese; however, trivalent manganese is also found in manganite, braunite and hausmanite. The most important ore of manganese from the point of view of abundance as well as economy is pyrolusite. Table 1 presents a source-wise listing of important manganese minerals and their ability to interact with microorganisms.

The most important manganese deposits in the world are those in the Chiatura District of Caucasia in the USSR, which consist essentially of pyrolusite mineral. It is suggested that manganese oxidizing bacteria participated in the formation of Karce-Alma (Fergana) deposit, which is the largest manganese deposit in the USSR. It is postulated that in the late Cretacean period favourable conditions existed for manganese oxidizing type of flora in the Tethys basin, which resulted in the

Table 1

Important manganese minerals and their leachability

Mineral	Formula	Source	Microbial leachability
Rhodochrosite	$MnCO_3$	Sedimentary	+
Pyrolusite	MnO_2		+
Birnessite	MnO_2		
Manganite	$MnOOH$		+
Hausmanite	Mn_3O_4		—
Pyrochroite	$Mn(OH)_2$		+
Manganosite	MnO		+
Todorokite	Mn(II) Ca, Na, K, Mn(IV), Mn(II), $(Mg)_6O_{12}\cdot 3H_2O$		—
Braunite	$3Mn_2O_3\cdot MnSiO_2$	Igneous	—
Pyromanganite	$(Mn, Fe)(SiO_3)$	Hydrothermal	—
Rhodonite	$(Mn, Fe, Ca)(SiO_3)$	Sedimentary	—
Jacobsite	$MnFeO_4$		+
Alabandite	MnS		+

formation of manganese deposits of Fergana [4]. Similar palaeobiological rationale has been used to explain the formation of manganese deposits in the Urals, Chiatura, Nikopol and the Varna region (Bulgaria) and in Asia minor. One of the single largest sources of manganese is the Nsutu mine on the Gold Coast of Africa, which has yielded 7.5 million tons of manganese by open cast mining since its opening in 1914.

Historically, India has been recognized as one of the leading manganese producing countries in the world. Since 1892, the year of inception of manganese mining in the country, India has produced more than 60 million tons of marketable manganese ore. In 1970—71, it was established that out of the world production of manganese (about 20 million tons) India's contribution was approximately 8.4 % on an average. It might be remembered that India was the top exporter of this ore and since 1906, when India began to export ore to the developed countries, by about 1923, India had occupied an important position by contributing nearly 44 % of the total world production of the manganese ores. Eventhough, the manganese production has reached a figure of 1.8 million tons in 1976, a picture has started emerging now that the Indian ore is slowly loosing ground in the world market, where the prices are highly competitive and the cost of production in the country is showing a rising trend. In addition to the emergence of countries producing massive quantities of these ores, such as the USSR and Ghana, there are other reasons in the change of pattern in the export trade. This happens many times in countries that have very little or no domestic use for the mineral and the growth of the industry is totally dependent on the export market. The export of manganese is decreasing in recent years, as exports have been adversely affected due to a stiff competition from the larger and highly mechanized mines of Brazil, Ghana, South Africa and Australia. This has resulted in closure of several mines, so much so that UNDP had to appoint a commission to go into the details of the worsening situation in manganese industry.

The Indian manganese ore reserves have been placed at 180 million tons. Indian manganese ores are characterized by the presence of a remarkable assemblage of ore minerals and silicate. The principal ore minerals in the commercial grade are pyrolusite, psilomelane, cryptomelane and braunite with or without the presence of minor minerals like jacobsite, hausmanite, etc.

Ore deposits are distributed throughout peninsular region of India and the principal deposits are located in Madhya Pradesh, Maharashtra, Orissa, Bihar, Karnataka, Andhra Pradesh, Gujarat and Goa as illustrated in Fig. 1. Among the metallurgical and non-metallurgical uses of manganese, the use in ferrous metallurgy is the greatest, wherein 95 % of the world production is utilized. Use in dry-battery manufacture or use in glass, ceramic or chemical industry are some of the non-metallurgical uses of manganese.

Although the export situation depends largely on the balance of trade with the importing country, one can hardly find a fault if the

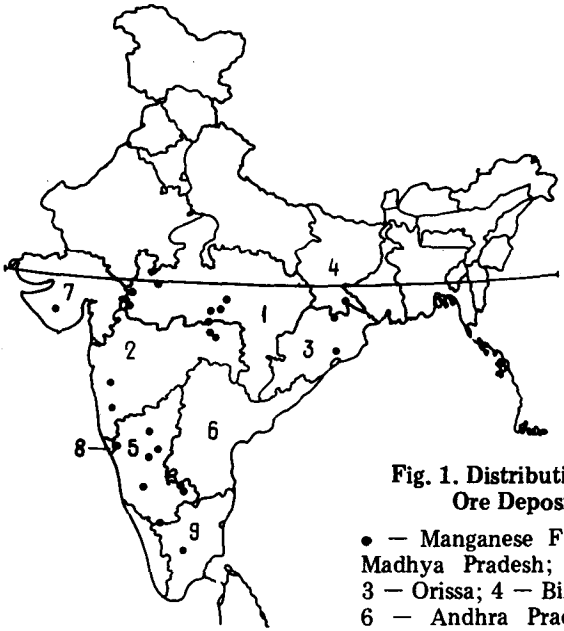

Fig. 1. Distribution of Manganese Ore Deposits in India:

• — Manganese Fields in India; 1 — Madhya Pradesh; 2 — Maharashtra; 3 — Orissa; 4 — Bihar; 5 — Karnataka; 6 — Andhra Pradesh; 7 — Gujarat; 8 — Goa; 9 — Tamilnadu

importing country buys the required minerals from a country with which it has a positive balance of trade and low freight or transport expenses. Therefore, we must try to seek solutions to the existing situation by way of:

a) seeking new and better sources of manganese;

b) trying to improve the quality of manganese ores.

Regarding finding new sources, it must be said that the terrestrial or land deposits are of limited nature and therefore exhaustible due to continuous removal of manganese from rich mining areas. At least for such well-known ores, it is very difficult to find new or better sources as most of them are already detected. Therefore, we must go in for marine or sea deposits, which are now being exploited in the form of manganese nodules at the bottom of the ocean. This new source of manganese is estimated to be accumulating at rates greater than current world consumption and it has been estimated that in nodules from Atlantic ocean, manganese in the form of pyrolusite is found up to 20 % level. The largest nodule field in the world is on the Pacific ocean floor on the US west coast. Recently, India has located manganese nodules in the Indian ocean. However, the technology to reap this harvest has to be still perfected.

Regarding improving the quality of ores two or three problems have been referred to us from time to time by the manganese industry

and we have tried to seek solutions to the problems using geomicrobiological techniques. The problems are:

1) Conversion of Mn_3O_4 form of manganese (hausmanite) to MnO_2 form (pyrolusite) — for use in dry-cell batteries.
2) Removal of impurities such as phosphorus from ores — for use in ferro-manganese alloys industry.
3) Reduction in iron content from the ore to conform with the desired Mn:Fe ratio — for use in supply to ferro-manganese industry.

There are other problems in the industry, but our selection of the problems was entirely based on the fact that it was possible to tackle these through microbiological approach. For instance, it is known that there are manganese oxidizing bacteria which have the power not only to convert manganese biologically, but also have the power to concentrate manganese from dilute solutions. Similarly, there are microorganisms which have the ability to remove or solubilize phosphorus or reduce iron. Using these known phenomena, it was decided to seek solutions to the problems mentioned above.

Microorganisms have been implicated in interacting with manganese and cause transformations in several ecosystems, such as soils, aquatic environments and even manganese deposits. The range of different microorganisms involved can be seen from Table 2, which includes many diverse groups of microorganisms from bacteria to fungi, algae and their various associations.

Among the different mechanisms proposed for transformation of manganese the important ones are as follows [2]:

A) **Indirect** — Alterations to microenvironment by:
   i) Eh modification;
   ii) pH modification;
   iii) adsorption to cell surface.

These alterations affect the manganese interaction in a non-specific way and are largely responsible for mechanical accretions or depositions or concentrations from dilute solutions.

B) **Direct** — The enzymatic oxidation and reduction of manganese

i) Enzymatic oxidation of manganese: — It is restricted to bacteria only. It proceeds by no less than three different mechanisms. The oxidation of free Mn^{2+} ions may be catalyzed by a manganese oxidase that conveys electrons to oxygen by a cytochrome pathway:

$$Mn^{2+} + 1/2\ O_2 + H_2O \rightarrow MnO_2 + 2H^+$$

This oxidase may be constitutive or inducible. Free Mn^{2+} ions may also be oxidized by catalase in a reaction with metabolically produced H_2O_2:

$$Mn^{2+} + H_2O_2 \rightarrow MnO_2 + 2H^+$$

While the oxidation of Mn^{2+} prebound to Mn^{4+} is catalyzed by a manganese oxidase that shunts electrons to oxygen by a cytochrome pathway:

$$MnMnO_3 + 1/2\ O_2 + 2H_2O \rightarrow 2H_2MnO_3$$

This manganese oxidase is constitutive and is found to be unable to oxidize free Mn^{2+}. Oxidation catalyzed by either of these enzymes may generate ATP as is the case with manganese oxidizing *Leptothrix discophora*, a facultative autotroph.

Table 2

Microorganisms implicated in manganese transformations

Microorganisms	Reference No.
A) **Oxidation**	
Bacteria:	
Arthrobacter	15, 29, 30
Bacillus manganicus	31
Bacillus spores	29
Clonothrix	32
Hyphomicrobium	8
Marine bacteria	15
Metallogenium personatum	33
Nocardia	34
Naumanniella polymorpha	35
Pedomicrobium	36
Pseudomonas	29
Siderocapsa	37
Sphaerotilus discophorus	38, 39
Fungi:	
Cephalosporium	40, 41
Cladosporium	8
Coniothyrium fuckelli	8
Periconia	41
Algae:	
Chlorococcum humicola	42
Sinergistic combinations:	
Bacterial-bacterial	43
Bacterial-fungal	49
Bacterial-algal	43, 44
B) **Reduction**	
Bacteria:	
Bacillus	45
Micrococcus	46
Fungi:	
Aspergillus niger	47
Pichia guillermondii	46

A number of organisms promote non-enzymic oxidation of manganese. In some of these cases mechanism of oxidation is either unknown or presumed to be non-enzymatic.

ii) Enzymatic reduction of manganese:— Organisms of the genera *Bacillus*, *Micrococcus* and *Pseudomonas* among others are capable of reducing manganese enzymatically through following mechanisms, where reducing power is derived from the metabolism of organic compounds like glucose.

$$\text{glucose} \xrightarrow{\text{bacteria}} ne^- + nH^+ + \text{end products}$$

$$n/2\ MnO_2 + ne^- + nH^+ \xrightarrow[\text{or uninduced bacteria}]{\text{induced bacteria}} n/2\ Mn(OH)_2$$
$$+$$
$$\text{ferri- or ferrocyanide}$$

$$n/2\ Mn(OH)_2 + nH^+ \rightarrow n/2\ Mn^{2+} + nH_2O$$

Some of these organisms could reduce both manganese and iron oxides. Anaerobic conditions stimulated Mn^{4+} reduction by some organisms [2].

That the microorganisms possess manganese oxidizing as well as the reducing activity was revealed when inorganic environment was extensively studied. The manganese oxidizing bacteria were isolated from soils and fresh water pipelines, whereas both the manganese oxidizing and reducing bacteria were studied from manganese nodules. It was seen that the biologically formed oxides are more easily available to plants than the chemically formed ones and, hence, the biological transformations in soils are very important to plant life [5].

METHODOLOGY

With respect to the problems mentioned earlier it was decided to carry out a systematic study of the various aspects involved in the bioconversion of manganese. With this view it was decided to isolate:

a) Manganese reacting bacteria;
b) Phosphorus solubilizing microorganisms;
c) Iron reducing bacteria.

The ecosystems used for these studies were:

i) Fresh water pipeline deposits;
ii) Soils from mining regions;
iii) Manganese and iron ores.

a) MANGANESE REACTING BACTERIA

i) Oxidizers

Based on the earlier experience of encountering the *Arthrobacter* sp. for the first time in such deposits in Australia [6], the whole ecosystem of fresh-water pipeline deposit in the causeway of river Mutha in India was analyzed and the seasonal variations were noted over a period of three years, in that, in summer the brown deposits yielded *Arthrobacter* sp., while in the winter months, the black deposits yielded budding *Hyphomicrobium* sp. [7]. Both these types were predominant in the deposits and representative species were isolated by enrichment culture technique using PC and M-2 medium [8, 9]. In addition, baiting technique was also used to isolate the associated microflora [10].

The manganese oxidizers were recognized by the brownish-black encrustations on the colony and were confirmed as oxidizers, due to the blue color developed on treating the colonies with benzidene hydrochloride reagent [11]. In the ecosystem containing manganese, it was always observed that the manganese reacting organisms were in much higher proportions than the inactives and there was a rough gradation from more reactive organisms in pipe deposits than in the ores or the soils in that order (Table 3). The organisms were characterized by using standard methods and Bergey's manual [12, 13].

These manganese oxidizers belonged to the *Arthrobacter* and *Hyphomicrobium* species. That this phenomenon was biological in nature was proved by the fact that inoculation of a small sample of deposit started the manganese oxidation, which could be prevented if biological inhibitors, such as sodium azide or mercuric chloride were added to the reaction mixture. This was the same case, when these inhibitors were added to a system containing pure cultures of manganese oxidizers, such as *Hyphomicrobium* and *Arthrobacter* sp.

Among the various factors, which were critically examined with respect to their effect on manganese oxidation, such as temperature, salt tolerance and effect of metal ions concentration the latter effect was worth mentioning. It was shown that iron and aluminium had no effect on the manganese oxidation in trace quantities tested, while zinc appeared to be inhibitory to manganese oxidation even at a concentration of 0.001 % (Table 4). This strengthens the belief in the hypothesis that when iron and manganese deposits simultaneously occurred, aluminium was also found to be present in some deposits, but zinc was always found to be absent from biologically formed deposits in nature.

ii) Solubilizers

It was reported that bacterial cultures which were capable of reducing iron oxides were also capable of reducing manganese oxides. Species of *Micrococcus*, *Bacillus* and *Pseudomonas* were reported to reduce manganese preferentially when iron and manganese were present together [14]. It was proposed that enzyme systems were involved in

Table 3

Characteristics of some bacterial isolates from different ecosystems containing manganese

Characteristics	Pipeline deposits			Ores			Soils		
	1	2	3	1	2	3	1	2	3
pH	7.0	6.8	6.5	7.0	7.0	6.8	6.5	7.0	8.0
Gram reaction:									
Positive	11	19	38	11	15	28	4	9	17
Negative	0	1	1	0	0	0	0	0	0
Variable (a)	2	3	3	3	3	4	2	2	2
Morphology:									
Rods	11	20	39	10	16	29	5	9	18
Cocci (b)	2	3	3	2	2	3	1	2	1
Motility	1	2	2	1	1	2	1	1	0
Acid production from:									
Glucose	0	0	0	0	0	0	0	0	0
Sucrose	0	0	0	0	0	0	0	0	0
Lactose	0	0	0	0	0	0	0	0	0
Nitrate reduction	0	0	0	0	0	0	0	0	0

(a) — Due to the pleomorphic nature of the organisms.
(b) — Probably coccoidal stages of *Arthrobacter* sp.

the reduction process and it was proved in case of *Bacillus* sp., isolated from marine ferro-manganese nodules, which possesses a MnO_2-reductase system [15, 16]. The same system was detected later in case of *Micrococcus* sp. [17, 18].

Using the scraping from pipeline deposits collected in summer months as inocula, enrichment was set up in one litre flasks containing 50 g of manganese ores (—65 mesh) and 300 ml of water with 50 ml of nutrient broth (pH 6.6). The manganese solubilization from the ore was assayed by periodate method [19] and from the flasks showing increase in solubilization, samples were streaked on nutrient agar where two types of colonies predominated. These were purified and identified as belonging to *Bacillus* and *Pseudomonas* sp.

b) PHOSPHORUS SOLUBILIZERS

It is known that the fungi have definite role in solubilization of phosphates in soils, due to their chelating activity of humic acid and production of other organic acids formed during the decomposition of the organic matter. In the laboratory, the phosphorus solubilizers are isolated on tri-calcium phosphate medium, as natural substrates such as rock phosphates are difficult to solubilize.

Table 4

Effect of different ions on manganese oxidation by *Hyphomicrobium*-strain AA

PC broth + $FeSO_4 \cdot 7H_2O$	Manganese oxidation	PC broth + $ZnSO_4 \cdot 7H_2O$	Manganese oxidation	PC broth + $Al_2SO_4 \cdot 16H_2O$	Manganese oxidation
" + 0.001 %	++	" + 0.001 %	—	" + 0.001 %	+
" + 0.002 %	++	" + 0.002 %	—	" + 0.002 %	+
" + 0.003 %	+	" + 0.003 %	—	" + 0.003 %	+
" + 0.004 %	+	" + 0.004 %	—	" + 0.004 %	+
" + 0.005 %	+	" + 0.005 %	—	" + 0.005 %	+

+ manganese oxidation.
— no manganese oxidation.

Out of 25 promising strains of *Aspergillus* sp. isolated from soils around Poona area, 6 cultures were selected, which were able to produce a distinct acidic reaction shown by decrease in pH to 2.0—2.5 after five days growth in liquid Czapek's medium (initial pH 6.0). These strains were further tested in liquid Czapek's medium to which 0.5 % tri-calcium phosphate was added and the amount of phosphorus solubilized was assayed periodically by Taussky & Schorr's method [20]. A culture of *Aspergillus niger* showing 390 μg/ml phosphorus solubilization in 96 hours was selected for further studies (Table 5).

Table 5

Phosphorus solubilization by *Aspergillus* cultures

Culture No.		Time (hours)					
		0	24	48	72	96	120
103	pH	6.0	4.9	4.2	4.1	3.4	3.4
	P	0.0	2.0	150	270	390	160
118	pH	6.0	5.0	4.5	4.4	3.7	3.6
	P	0.0	2.0	160	220	300	160
119	pH	6.0	5.5	4.4	4.2	3.7	3.8
	P	0.0	0.0	180	240	340	140
120	pH	6.0	4.6	4.4	4.0	3.4	3.6
	P	0.0	4.0	195	250	330	110
121	pH	6.0	4.8	4.3	4.0	3.6	3.6
	P	0.0	2.0	150	210	300	150
122	pH	6.0	5.6	4.7	3.9	3.5	3.5
	P	0.0	0.0	145	200	290	160

P — Phosphorus solubilized in culture filtrate (μg/ml)

Coincidently it was found out, while carrying out manganese oxidation experiments on the ores containing phosphorus (0.6 %), that the phosphorus was being leached out into the leachate, when *Hyphomicrobium* sp. was used as an inoculum. This prompted us to use the *Hyphomicrobium* sp. isolated in the previous studies for phosphorus solubilization experiments.

c) IRON REDUCERS

Microbial iron reduction has been reported in the species of *Bacillus*, *Pseudomonas*, *Micrococcus* and *Clostridium* [21, 22]. In the present studies, enrichments were carried out using Bromfield's medium with ferric hydroxide as the source of iron at pH 7 [23]. After incubation of seven days at room temperature (27—30 °C), the supernatant was tested for the presence of soluble iron by *o*-phenanthroline reagent [24].

Samples were screened aerobically and anaerobically and pure cultures were isolated which mainly belonged to *Bacillus*, *Pseudomonas*, *Enterobacter* and *Micrococcus* spp.

Using the cultures obtained as above, the following experiments were carried out:

1) Bioconversion studies to upgrade the variety of manganese ores;
2) Leaching experiments to solubilize manganese from the ores;
3) Biological removal of phosphorus;
4) Microbial reduction of iron.

It may be mentioned that before using the cultures obtained for any of the processes that follow, the cultures were subjected to adaptation experiments to raise the tolerance level of these cultures to higher concentrations of manganese and other elements in the ore. This was done by gradual adaptation to increasing ore concentration of the medium. For instance, it was noted that manganese oxidizers like *Hyphomicrobium* and *Arthrobacter* tolerated 5 % and 20 % level respectively and the manganese solubilizers could withstand 20 % ore in the medium. The phosphorus solubilizers, *Aspergillus* sp. and *Hyphomicrobium* sp., were able to withstand 5 % concentration of ores. The iron reducers were trained to adapt to 7.5 % tolerance of iron ores.

1) **Bioconversion.** The conversion of Mn_3O_4 form to the γ-grade variety of MnO_2 is possible through the use of electrolytic process, which is rather expensive and is monopolized in India. Therefore, experiments were carried out using strong manganese oxidizing cultures of *Hyphomicrobium* and *Arthrobacter* spp. for this type of bioconversion:

a) Flask experiments were carried out in 500 ml Erlenmeyer flasks, which contained 100 ml of M-2 medium and 25 g of ore. The washed culture was added in 1 g quantity (wet weight) and aeration was carried out by means of a mini pump. Then it was noted that when the color

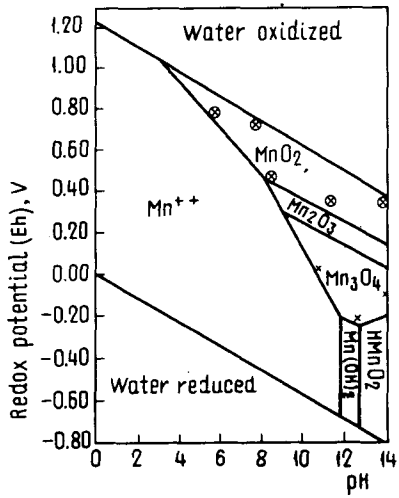

Fig. 2. pH-Eh Relationship between Manganese Species

Stability fields of manganese species in aqueous solution, free of bicarbonate and sulfate ions. Total dissolved-manganese activity of 0.01 ppm.
× — ore before treatment
⊗ — ore after treatment

was changed from brownish-black to dark black, it was taken as a visible indication of a change of state of oxidation of manganese. Slurry was then filtered through a Whatman filter paper (No. 4) and the ore particles on the paper were dried at 110 °C for three hours before analyzing them for the presence of MnO_2 form by comparing the standard pH and Eh relation between both manganese types [48]. The species of manganese in the ore, before and after treatment, is shown in Fig. 2. The definite shift in the oxidized state of ore to MnO_2 side was confirmed by X-ray crystallography, when it was shown that nearly 80 % of the crystals were of the γ-type.

b) Column experiments were set due to the initial success of flask experiments, when an air-lift percolator was filled with graded sand on which the culture film was stabilized. The ore was dispersed throughout the column on which a continuous flow of liquid medium was arranged in such a way that the microbial film was not disturbed to a great extent but it brought the culture and the ore particles in close contact.

c) Tank experiments: 100 kg of −65 mesh ground ore was placed in cement concrete leach tank, to which 2000 litres of water was added with the medium at pH 6.8 and each tank was inoculated with 4 litres of stock cultures of *Arthrobacter* and *Hyphomicrobium* sp. The pH was monitored and adjusted to 6.8. Samples were taken out after mechanical agitation for fifteen minutes each day and analyzed for presence of MnO_2 as described earlier. The bacterial leaching data is summarized in Table 6. The net cost of such an experiment was calculated and is presented in Table 7.

Table 6

Bacterial bioconversion data

Time (days)	pH (average)	Temperature (average), °C	% recovery using sp. of	
			Arthrobacter	*Hyphomicrobium*
15	6.8	28	78.4	85.2

Table 7

Cost of pilot plant experiment for conversion of manganese

Quantity of manganese ore used, kg	Cost of nutrients* (Rs)	Cost of other utilities** (Rs)	Total (Rs)
100	200	100	300

* — Nutrients used in the media were yeast extract, inorganic salts, etc.

** — Water, electricity, labour, etc.

2) **Leaching.** Using the isolates obtained during our studies on manganese solubilization, these experiments were carried out at all the three levels such as flasks, columns and tanks as described above. The only difference in these studies was that the solubilized manganese had to be precipitated by addition of lime for quantitative recovery. The results of a 90-day experiment are described in Table 8. The extraction efficiency based on the amount of manganese recovered was nearly 90 % for both the species.

Table 8

Leaching with pure bacterial isolates

Days	Manganese quantity in leachate, g		pH	
	Culture *Pseudomonas* sp.	Culture *Bacillus* sp.	Culture *Pseudomonas* sp.	Culture *Bacillus* sp.
0	0.32	0.32	6.8	6.8
10	1.80	3.44	6.7	6.6
20	3.44	9.56	6.6	6.5
30	5.52	12.34	6.6	6.5
40	11.36	16.76	6.5	6.5
90	20.85	20.12	6.5	6.5

Note. The system contained 50 g of manganese ore (44 % Mn content)

3) **Phosphorus solubilization.** Physical and chemical treatments or their combinations are not able to reduce the phosphorus content to an acceptable level of 0.1 %, as shown in Fig. 3. Therefore phosphorus removal was tried using *Aspergillus niger* strain 103 and *Hyphomicrobium* sp. The methodology was the same as described above and consisted of flasks and column experiments. The efficiency of phosphorus removal by *Aspergillus niger* strain 103 was 33.3 %, while that of *Hyphomicrobium* sp. was 90 %, rendering the ore acceptable for future use (Table 9).

Table 9

Comparison of microbial removal of phosphorus from manganese ore

Organism	% phosphorus		% extraction efficiency
	before treatment	after treatment	
Hyphomicrobium sp.	0.6	0.062	90
Aspergillus niger	0.6	0.400	33.3

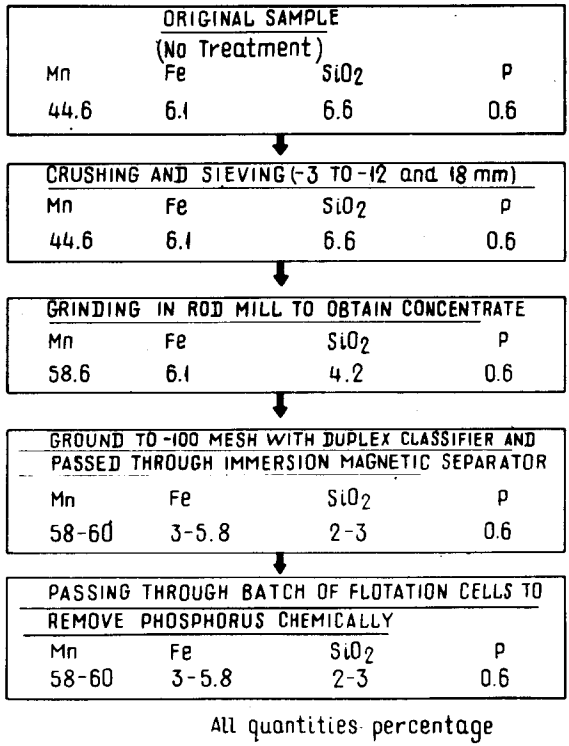

Fig. 3. Physical and Chemical Treatments Used for Removal of Phosphorus from Manganese Ores

4) **Iron reduction.** With a view to utilize iron reducing bacteria to solubilize iron, experiments were set up in flasks as well as columns using the methodology described above. The ores used were hematite, limonite and mixture of magnetite, limonite and bauxite. The level of soluble iron was estimated at regular intervals and it was found that amount of solubilized iron reached a maximum within 10—15 days, using *Pseudomonas* sp. No. 3. This culture was further used to remove iron from bauxite ore. In 30 days time the iron solubilized recorded a peak after 15 days and then fell off at lower levels probably due to precipitation or reoxidation of ferrous to ferric state (Table 10). To avoid this process a continuous five column system was tried, when the iron was completely removed in 40 days time. The mineralogy of the bauxite determined after this microbiological treatment proved that this method could be used in industry.

Although the above studies were of different nature than the problems connected with manganese industry, they could be applied profitably to similar situations in manganese industry. For instance,

the manganese ores in Goa region, which incidently is responsible for nearly 10 % of production of the ore in India, contained 58—68 % iron, 5—6 % alumina and good amount of silica. To bring down the Mn:Fe ratio to 7 to 8, the manganese percentage has to be brought up to 55 % with concomitant reduction in the quantity of iron to about the same level. Impurities such as alumina, phosphorus and silica should not be present in the ore. There is also another problem regarding the high grade ore, where the iron content is about 6—7 %, when it cannot be sold as battery grade ore. The reduction of iron from such manganese ores is also desirable and should be tried using microbiological techniques. Efforts are underway to carry out this type of work at our laboratory.

Table 10

Column leaching of bauxite

Incubation period, days	Column leachate	
	pH	Ferrous concentration (μg/ml)
0	7.2	—
7	6.0	6.335
10	5.55	14.450
15	5.55	25.875
25	6.05	20.725
30	6.50	16.550

PROSPECTS AND PERSPECTIVES

The above efforts describe the state-of-art which exists at present time in India. In fact, the large scale field trials are desirable to establish the value of these techniques to the mining industry. This is the suggested answer to the problems indicated in the initial discussion.

If we summarize the results obtained, the following highlights are visualized:

1) **Bioconversion.** The bioconversion of manganese has a definite industrial significance as it converts an inferior quality of ore to a better variety of manganese. There are only few countries in the world like Ghana which produce γ-variety of MnO_2, used for dry-cell battery manufacture directly from mines. In Western Australia, large reserves of low grade manganese ores exist, which are amenable to biological beneficiation as outlined above. In India, the existing electrochemical process is a patented foreign process. The chemical beneficiation process requires fuel oil, which is in short supply during these days of energy crisis. The industrialized countries with vast power resources

are reaping the dividends by importing our manganese ores and converting them into better variety of ores. We hope that our experiments would pave a way to develop such useful low-cost technology in India.

2) **Leaching.** The information on manganese reducing bacteria is of a fragmentary nature, especially their leaching or beneficiation capacity on manganese ores was never tested, event hough such a possibility was distinctly hinted at by many workers in the field [25]. The US Bureau of Mines has been keenly pursuing leaching of domestic low-grade manganese bearing materials with the aid of microorganisms for the last several years. The American interest in manganese stems from their low-grade manganese ore deposits, which necessitates import of high grade ores from countries like India. The work was carried out using *Bacillus* sp. isolated from manganese stock piles and tailing ponds from manganese beneficiation plants [26]. The amenability of manganese bearing material to bacterial leaching was proved with good extraction on a large scale. The action of microorganisms in leaching manganese without the presence of sulfide minerals is not yet defined. The Japanese and French investigators have shown that the presence of iron and zinc sulfides accelerates the solubilization of manganese and *Thiobacillus thiooxidans* can bring about the leaching at pH 3.4 at 30 ^{o}C. Although manganese dioxide is not soluble in sulfuric acid, it is solubilized in growing culture of *T. thiooxidans* [27, 28].

3) **Phosphorus removal.** The microbial removal of phosphorus from the ore is worth trying out on a large scale, as it has valuable industrial significance. It would be possible to arrange the manganese ore in heaps on site and the cultures could be allowed to percolate through the heaps to remove phosphorus (Heap Leaching Technique). The leachate could be gathered in a collection pond by arranging appropriate draining system and then it could be pumped back to the top of the heap to bring the phosphorus level of the ore to the desired level. Various parameters such as temperature, pH, etc. could be optimized for the commercial microbiological treatment.

The present studies also prove the value of obtaining indigenous microorganisms from the ecosystems concerned. Not only they tolerate high concentration of manganese present in the ore but they also carry out the process more efficiently. This may be the reason why the phosphorus solubilizing fungi were not as effective as bacteria from that ecosystem. The reason for removal of phosphorus by a manganese oxidizer could be that the phosphorus moiety might be tightly bound to the manganese in the crystal lattice structure of the ore and the change in the oxidation state of manganese might release the phosphorus moiety. If phosphorus is inaccessible to organic acids due to structural arrangement, it is obvious that the conventional phosphorus solubilizers may not work on these ores.

Present studies have indicated that rich manganese ores could be treated through microbial agencies successfully without any pre-treatment. This deserves the attention of the industry.

The ability of microorganisms to interact with the minerals can be used to solve many industrial problems including recovery of precious minerals from the low-grade ores or from industrial wastes. If the cost allows, as the present studies indicate, then such processes could be used to upgrade the variety of ores, replacing conventional expensive pyrometallurgical techniques with a comparatively inexpensive bio-hydrometallurgical process.

REFERENCES

1. Silverman, M.P., and H.L. Ehrlich, *Adv. Appl. Microbiol., 6*, 153 (1964).
2. Ehrlich, H.L., Geomicrobiology, Marcel Dekker, New York (1981).
3. Goldschmidt, V.M., Geochemistry, Clarendon Press, Oxford (1954).
4. Obut, A.M., In: Role of Biosphere in Geological Processes, Problems of Interrelation between Palaeontology and Tectonics, Proceedings of V and VI Sessions of All-Union Palaeontologic Society, A.M. Obut (Ed.), Gosgeoltekhizdat, Moscow, 26 (1962) (in Russian).
5. Bromfield, S.M., *Plant & Soil, 9*, 325 (1958).
6. Agate, A.D., Abstr. 13th Ann. Conf. Assoc. Microbiol. India, Ludhiana, 32 (1972).
7. Agate, A.D., and H.A. Deshpande, In: Conference Bacterial Leaching, GBF Monograph Series No. 4, W. Schwartz (Ed.), Verlag Chemie, New York, 243 (1977).
8. Tyler, P.A., and K.C. Marshall, *Antonie van Leeuwenhoek, 33*, 171 (1967).
9. Johnson, R.M., R.M. Schwent, and W. Press, *Limnol. Oceanogr., 13*, 658 (1968).
10. Hirsch, P., and G. Rheinheimer, *Arch. Mikrobiol., 62*, 289 (1968).
11. Bromfield, S.M., *Austr. J. Biol. Sci., 9*, 238 (1956).
12. Soc. American Bacteriologists, Manual of Microbiological Methods, McGraw-Hill, New York (1951).
13. Buchanan, R.E., and N.E. Gibbons (Eds.), Bergey's Manual of Determinative Bacteriology, 8th Ed., Williams and Wilkins, Baltimore (1974).
14. Troshanov, E.P., *Mikrobiologiya, 38*, 528 (1968).
15. Ehrlich, H.L., *Dev. Industrial Microbiol., 7*, 279 (1966).
16. Ehrlich, H.L., In: Ferromanganese Deposits of the Ocean Floor, D.R. Horn (Ed.), The Office of the Int. Decade of Ocean Exploration, National Science Foundation, Washington, D.C. (1972).
17. Ehrlich, H.L., W.C. Ghiorse, and G.L. Johnson, *Dev. Industrial Microbiol., 13*, 57 (1972).
18. Ehrlich, H.L., S.H. Yang, and J.D. Mainwaring, *Z. Allg. Mikrobiol. 13*, 39 (1973).
19. Vogel, A.I., Quantitative Inorganic Analysis, 3rd Ed., Longmans Green (1961).
20. Taussky, H.A., and E. Schorr, *J. Biol. Chem., 202*, 675 (1953).
21. Ottow, J.C.G., *Z. Allg. Mikrobiol., 8*, 441 (1968).
22. Ottow, J.C.G., and H. Glathe, *Soil Biol., Biochem., 3*, 43 (1971).
23. Bromfield, S.M., *J. Soil Sci., 5*, 128 (1954).
24. Sandell, E.B., Colorimetric Determination of Trace Metals, 3rd Ed., Interscience Publishers, Inc., New York (1959).

25. Zajic, J.E., Microbial Biogeochemistry, Academic Press, New York (1969).
26. Perkins, E.C., and F. Novielli, US Bur. Mines Rep., 6102 (1962).
27. Imai, K., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E. Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York, 275 (1978).
28. Imai, K., Tano, T., and H. Noro, US Patent No. 3433629 (1969).
29. Van Veen, W.L., *Antonie van Leeuwenhoek, 39*, 657 (1973).
30. Bromfield, S.M., *Soil Biol. Biochem., 6*, 383 (1974).
31. Beijerinck, M.W., *Folia Microbiol., 2*, 123 (1913).
32. Wolfe, R.S., *J. Am. Water Works Assoc., 52*, 335 (1960).
33. Perfil'ev, B.V., and D.R. Gabe, Capillary Methods of Investigating Microorganisms, Oliver & Boyd, Edinburgh (1969).
34. Schweisfurth, R., *Mitt. Int. Ver. Theor. Angew. Limnol., 14*, 179 (1968).
35. Ten Khak-Mun, *Dokl. Akad. Nauk USSR, 209*, 1203 (1973).
36. Aristovskaya, T.V., *Dokl. Akad. Nauk USSR, 136*, 954 (1961).
37. Dubinina, G.A., Gorlenko, V.M., and Ya.I. Suleimanov, *Mikrobiologiya, 42*, 817 (1974).
38. Johnson, A.H., and J.L. Stokes, *J. Bacteriol., 91*, 1543 (1966).
39. Mulder, E.G., and W.L. van Veen, *Antonie van Leeuwenhoek, 29*, 121 (1963).
40. Ivarson, K.C., and P.K. Heringa, *Can. J. Soil Sci., 52*, 401 (1972).
41. Timonin, M.I., W.I. Illman, and T. Hartgerink, *Can. J. Microbiol., 18*, 793 (1972).
42. Bromfield, S.M., *Aust. J. Soil Res., 14*, 95 (1976).
43. Bromfield, S.M., and V.B.D. Skerman, *Soil Sci., 69*, 337 (1950).
44. Kutuzova, R.S., *Mikrobiologiya, 44*, 129 (1975).
45. Trimble, R.B., and H.L. Ehrlich, *Appl. Microbiol., 16*, 695 (1968).
46. Bautista, E.M., and M. Alexander, *Soil Sci. Soc. Am. Proc., 36*, 918 (1972).
47. Barea, J.M., Olivares, J., Aguilar, A., and V. Callero, *Agrochimica, 15*, 472 (1971).
48. Hem, J.D., Chemical Equilibria and Rates of Manganese Oxidation, Geol. Survey Water Supply Paper 1667-A, US Government Printing Office, Washington, D.C. (1963)
49. Zavarzin, G.A., *Mikrobiologiya, 30*, 393 (1961).

ALUMINOSILICATES BIODEGRADATION: PROGRESS AND PERSPECTIVES

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INTRODUCTION

Aluminum and potassium are two vital elements for the economic balance of a modern country: the former for the variety of its industrial uses, the latter for its role in agriculture. The importance of aluminum in the world economy is illustrated by Table 1 [1], which shows the production of primary metal in the five continents. The data in Table 1 can, however, be deceptive, if incorrectly interpreted: in North America, which holds the first place in order of importance, the contribution of the United States to the world bauxite production actually only amounts to as little as 2 %, and the situation in Western Europe, even though it holds the second place as metal producer, is no different, if not worse. The situation appears particularly serious for Italy, whose bauxite reserves are far from sufficient to satisfy its needs.

As far as potassium is concerned, although the overall productions appear to be concentrated in America and Europe, as Table 2 shows, the major producers are confined to a few large countries, whereas other countries, such as Italy, depend entirely on imports, and Asia and Africa produce little or nothing. Up to the present time bauxite is the main run-of-mine ore from which aluminum is extracted, whereas nearly all potassium is derived from potassium salt mines. There exists, however, a wide range of minerals, the aluminosilicates, some of which are characterized by aluminum and potassium contents — sometimes together with other interesting elements like lithium and beryllium —

Table 1

Production of primary aluminum in five continents (000 short tons) [1]

Continental area	1979	1980	1981
Africa	442	482	532
North America	5976	6312	6176
Latin America	736	905	874
East Asia	1195	1287	901
South Asia	414	440	569
Western Europe	3968	4151	4110
Oceania	469	507	591
Total Production	13200	14084	13753

Table 2

World potash production (000 tons of K_2O) [2]

	1981	1982
North America	(10000)	(14400)
Canada	8200	12000
U.S.	2200	2400
West Europe	6400	6300
East Europe	12600	18000
East Germany	3400	3400
USSR	9200	14600
Asia	810	2000
Other	25	300
World total	30235	41000

which approach those presently considered economically acceptable for their extraction (Table 3). Amongst these, beryl and, for aluminum extraction, kyanite, spodumene and nepheline are already utilized in commercial operations.

In Italy, leucite occurs abundantly in the rocks of the volcanic belt of Central Italy, between Acquapendente in Upper Latium and the Alban Hills, and, further South, in the province of Caserta as well as in the Sessa Aurunca area and in the vicinity of the Vesuvius volcano (Fig. 1). On the basis of estimates made by Washington [3], who extensively investigated the Italian leucite occurrences, potash equivalent (K_2O) and alumina (Al_2O_3) reserves contained in the above mentioned deposits can be calculated in more than nine billion tons each [4]. However, all of the industrial attempts to exploit leucite for

Table 3

Composition of some silicates

Name	Chemical formula	Al_2O_3, %	Other important component, %
Andalusite	$Al_2O(SiO_4)$	62.92	
Beryl	$Be_3Al_2(Si_6O_{18})$	8.97	5.03 Beryllium
Jadeite	$NaAl(Si_2O_6)$	25.22	
Kaolinite	$H_4Al_2Si_2O_9$	39.49	
Kyanite	$Al_2(SiO_4)$	69.82	
Leucite	$KAl(SiO_3)_2$	23.36	17.91 Potassium
Microcline	$KAlSi_3O_8$	18.31	
Muscovite	$H_2KAl_3(SiO_4)_3$	38.40	
Nepheline	$Na_3K(Al_4Si_4O_{16})$	34.90	
Orthoclase	$KAlSi_3O_8$	18.31	
Petalite	$Li(AlSi_4O_{10})$	16.21	2.27 Lithium
Spodumene	$LiAl(Si_2O_6)$	27.40	3.73 Lithium

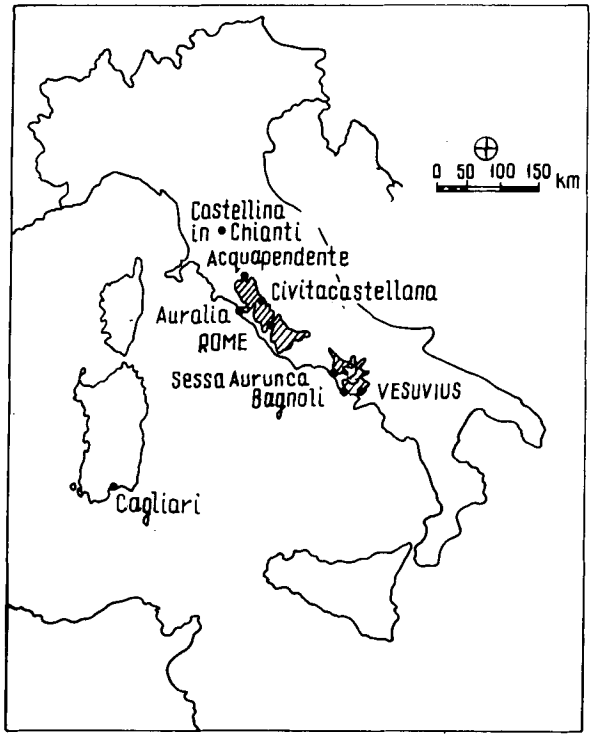

Fig. 1. Map of Italian leucite deposits (From Rossi, 1977. Copyright (1978) Pergamon Press, New York)

aluminum and potash extraction have proved unsuccessful. Several difficulties — mostly related to the elevated energy requirements of the processes currently practiced — are also encountered in the commercial utilization of other aluminosilicates.

It should be pointed out that up until the early seventies aluminosilicate biodegradation formed the object of investigation by agricultural microbiologists only. From the literature on the subject it would seem that the investigations were carried out according to two main lines of approach:

the first, which initially adopted the conventional procedures of agricultural microbiology, was mainly aimed at ascertaining the action of the known soil microorganisms, and of the products of their metabolism on aluminosilicates and carbonates;

the second, mainly followed by the Slav-speaking countries, aimed at identifying microbial strains specifically suitable for aluminosilicate biodegradation.

INVESTIGATIONS UTILIZING KNOWN MICROORGANISMS

Amongst the first — one can probably say "the first" — investigators who tackled the problem were the Italians De Grazia and Camiola [5]. The object of their investigation was to understand the

process by which plants obtain the soluble potassium supply needed for their nutrition from insoluble volcanic rock.

They worked on batches of very finely ground, museum grade leucite crystals mixed with 1000 ml of culture medium each in 2000 ml stationary Florence flasks which were inoculated with various strains of moulds. As shown by Table 4, where the results are reported, a culture yielded, in 70 days, more than 70 % dissolution of K_2O equivalent, though the sterile control grade was also quite high. De Grazia and Camiola attributed the enhanced solubilization observed in the presence of moulds to the chemical action of their metabolites, amongst which CO_2, although they did not deny the possibility of a direct action.

In 1913, Bassalik [6] reported on the enhancement of orthoclase solubilization by means of several microbial strains (Table 5). He too attributed the increase in solubilization mainly to the action of the CO_2 produced by the metabolism of the microorganism utilized — *Bacillus extorquens* n. sp. — characterized, compared to other strains examined, by a large respiratory energy.

The evidence that the metabolism products of certain soil microorganisms may furnish a marked contribution to the destruction of crystalline lattices of silicates was produced by Webley et al. [7], who worked with a strain of *Pseudomonas*. The silicates (wollastonite, apophyllite and olivine), finely ground, were mixed with agar. The plates prepared with this mixture were inoculated with the microorganism: a circular halo always formed around its colonies and inside this halo 2-ketogluconic acid and amorphous mineral residues were detected. The same authors confirmed, in two subsequent papers [8, 9], the destructive action caused by the metabolism products of several bacteria and moulds on various synthetic silicates, more pronounced however in calcium silicates and zinc silicates, as opposed to the aluminosilicates which are less amenable to this treatment. Silverman [10] gives an exhaustive list of organic biogenic acids active in silicate rocks and minerals degradation.

The evidence furnished by Holzapfel [11] that silicon can replace phosphorus and sulphur in the organism of certain microorganisms for certain functions, led Heinen to carry out investigations on the possibility that this element can be assimilated, in certain conditions, by various microorganisms, to become an integral part of their structure. The important results of Heinen's investigations [12—23] led to ascertain that several bacterial strains (*Proteus mirabilis* [17—20], *B. subtilis*, *Escherichia coli* and *Serratia marcescens*) take in silicon both from the solutions containing soluble silicon compounds, and (even though to a lesser extent) from quartz. It was also verified that this process is active — not a simple adsorption process — and depends on the environmental conditions [12] and that silicon is utilized by the microorganisms to produce organic compounds where C-Si bonds are formed [16] in the absence of phosphorus. In addition, Heinen show-

Table 4

The results of De Grazia and Camiola's tests

Name of the microorganism	Leucite, g	K_2O content, g	K_2O solubilized	
			g	%
Aspergillus niger	0.8213	0.1463	0.0758	51.82
Mould No. 1	0.8547	0.1523	0.0908	59.65
Penicillium glaucum	0.9238	0.1646	0.0895	54.37
Penicillium brevicaule	0.6750	0.1202	0.0851	70.81
Sterile Control	0.6664	0.1187	0.0365	30.74

Table 5

Orthoclase biosolubilization [6]

Material of flasks	Microbial strain	Duration of test, days	Initial mass of orthoclase, g	Final mass of orthoclase, g	Mass of orthoclase solubilized	
					g	%
Jena glass	*Bacillus extorquens*	64	10.7197	10.3282	0.3915	3.65
	Sterile control	68	16.9866	16.9704	0.0162	0.09
Quartz	*Bacillus extorquens*	62	0.8318	0.8045	0.0273	3.28
	Sterile control	60	0.7377	0.7359	0.0018	0.24

ed that silicium assimilation is enhanced by various anions [14] as well as by organic compounds [15]: the assimilation of silicon is stimulated by carbohydrates and by some aminoacids, whereas it is inhibited by phenylaniline, acetate, lactate and peptone.

The ability of several bacterial strains to solubilize various types of rocks and minerals was further confirmed by Wagner and Schwartz [24, 25] who developed some kind of biodegradability scale in which the first place is held by Rapakivi granite followed by nepheline, leucite and orthoclase. Specific examples of transformations produced in black mica (biotite) by chemical or biological agents were reported by Boyle et al. [26], who, in particular, ascertained by means of chromatographic analysis, that *A. niger* produces, from a glucose-containing medium, oxalic and citric acids; the first of which should be the most active in removing ions from the crystalline lattice of the mineral, producing a brittle matrix of amorphous material.

With the possible exception of Heinen, the researchers mentioned so far have shown a certain propensity towards considering silicate minerals degradation mediated by microorganisms not as the result of their direct action but rather of the chemical attack by the metabolic products without, however, going so far as to produce evidence in favour of either mechanism. The problem is of considerable practical

and scientific significance since if the destruction of the crystalline material is the consequence of a direct action the process kinetics would depend on the number of cells present. Investigations aimed at elucidating this problem were carried out by researchers of the Bureau de Recherches Géologiques et Minières (B.R.G.M.) in France, in the framework of an extensive research programme on rock weathering processes [27]. The rocks investigated comprised olivine, coming from basalt nodules of the Srmci region (Northern Bohemia, Czechoslovakia), dunite from New Caledonia (Dumber river tunnel) and serpentine from the region of Saint-Véran and of Cristillan, in the High Alps, whereas the microorganism was a pure strain of *A. niger*, isolated from the soil. In order to make a comparison with chemical attack, a solution was prepared composed of a 50/50 sterile mixture of 0.1 M oxalic and citric acids on the basis of the analysis of the culture medium at the 8th day. With this mixture 100 g of the same mineral (olivine) was subjected to sterile chemical attack for the same duration and *ceteris paribus*. The final results are very similar and the pattern of the curves of the solubilized metals concentrations vs. time show that the solubility of the oxalates regulates the amount of metal in solution at equilibrium. It can be concluded that the microbial alteration, as produced in the experiments described, is equivalent to chemical alteration. Similar conclusions were reached in the laboratory of the Instituto di Arte Mineraria e Preparazione dei Minerali of the Engineering Faculty of the Cagliari University, where the investigations undertaken more than half a century earlier by De Grazia and Camiola were resumed in 1975. A leucite concentrate obtained by magnetic separation from a volcanic rock ("leucitofiro") of the Civitacastellana (Central Italy) area and finely ground [28] was employed in the experiments. In 150 days, strains of *A. niger*, *Scopulariopsis brevicaule* and *Penicillium expansum* leached between 21 % and 27 % of the potassium, amounts which are much higher than those found in the sterile controls but quite close to those solubilized by a sterile solution of oxalic acid and citric acid (Fig. 2).

It must be noted, however, that it is highly likely that the microorganisms produce a medium with a more complex composition than that of a simple mixture of oxalic and citric acids.

Further research in this direction was conducted on muscovite [29] and on feldspar [30, 31] using soil bacteria and strains of *A. niger*, by Mehta et al. [32–34] on basalt, employing strains of *P. simplicissimum*, by Aristovskaya and Kutuzova [35] on nepheline, plagioclase, quartz and phytoliths, utilizing *P. notatum*, *Pseudomonas* sp. and *Sarcina ureae*, by Silverman and Munoz [36], who described the action of a strain of *P. simplicissimum*, isolated from weathering basalt rocks, on basalt, granite, granodiorite, rhyolite, andesite, peridotite, dunite and quartzite, by Karavaiko et al. [37], who worked on spodumene with several microorganisms amongst which *P. notatum* and *A. niger*, by Avakyan et al. [38], who reported on the activity of strains of

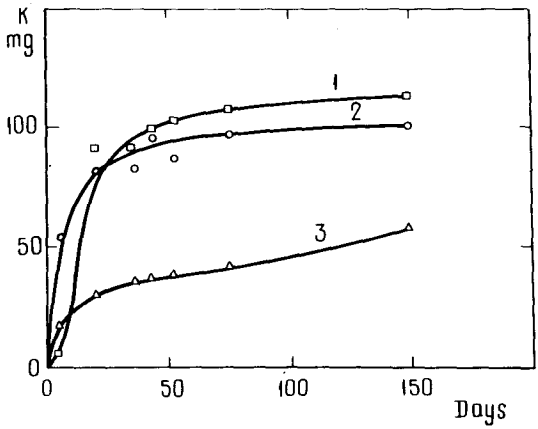

Fig. 2. Potassium solubilization of leucite vs. time

curve 1 — sterile acid mixture; curve 2 — *Penicillium expansum* culture; curve 3 — sterile control. (From Rossi, 1977. Copyright (1978) Pergamon Press, New York)

Trichoderma lignorum and *P. notatum*, isolated from the weathering zone of a pegmatitic orebody, on spodumene, pegmatite and schist, and by Groudev et al. [39, 40] who reported on the enhancement of clays and kaolin leaching by various microorganisms among which *A. niger* and *P. simplicissimum*.

All of the above-mentioned authors are of the opinion that substantially the essential mechanism of aluminosilicate degradation by the moulds appears to be one in which the organism produces either acids or alkalis which then attack the rock (respectively by acidolysis or by alkalolysis) or complexing agents which in some way subtract the elements to the crystalline lattice (complexolysis), whereas the possibility of direct attack is excluded by all of them.

Even though the moulds seem to be microorganisms capable of enhancing the aluminosilicate degradation processes, many reasons lead to consider with caution the possibility of their commercial utilization. In fact, the weathering processes of rocks by moulds are characterized by relatively slow kinetics, since in order to achieve metal recoveries higher than a few tens of percent a few weeks are required. Furthermore, the biomasses which form during the process occupy considerably larger volumes than those of the rock masses on which they act, and this fact might detrimentally affect investment costs of industrial plants designed for continuous operation. With the present state of knowledge one suitable field of application could be the in-situ leaching or leaching in large, open air basins, climate permitting, since microbial activity is negligible at temperatures close to the freezing point of water.

INVESTIGATIONS WITH SPECIFIC MICROORGANISMS

The Academician Vernadsky [41] already pointed out, half a century ago, the possibility of decomposing silicates by the soil microorganisms which liberate the potassium ion along with other

silicate-forming elements. It was on the basis of this consideration that, in the forties', Aleksandrov undertook a series of investigations aimed at isolating from agricultural soils bacteria capable of decomposing the silicates and at utilizing them for improving crops as a substitute for chemical fertilizers [42, 43].

Aleksandrov [44] provided information on the characteristics of "silicate" bacteria and, among other things, showed that:

a) soil microorganisms participate in the soil formation processes, by transforming the potassium contained in the latter in a form not susceptible to assimilation by higher plants into an assimilable form;
b) the intervention of "silicate" bacteria allows to considerably improve the crops of wheat and corn from 56 % to 105 % and from 34.0 % to 50.0 % respectively.

A more thorough knowledge of "silicate" bacteria was achieved, subsequent to further investigations, by Aleksandrov and Zak [45] who, in tests performed in Petri dishes with silicate agar, found that five days after inoculation the amount of potash solubilized in the presence of two bacterial strains was, respectively, 75.9 % and 67 % of the aluminosilicate present in the medium.

Important progress was achieved, in the definition of the characteristics of "silicate" bacteria, by Tesic and Todorovic [46, 47], who stressed the need of extending their function beyond the sole aluminosilicate potassium mobilization and oriented their investigations towards the capability of these microorganisms to utilize also the other elements of the aluminosilicates.

Tesic and Todorovic, having ascertained the existence of substantial differences in the descriptions of these heterotrophic bacteria by various authors, have come to the conclusion that they actually belong to the old species known by the name of *Bacillus circulans*, Jordan (1890).

In a later paper [48], the same authors reported on experiments which led them to ascertain that silicon (and probably aluminum) is an essential element for the normal evolution (probably for the formation of the cell capsule) of these bacteria in natural conditions; their view was confirmed by the papers subsequently published by Aleksandrov [50—55].

The experiments described so far, although they have not yet led to the achievement of the desired final aim, (i.e. the isolation of microorganisms capable of growing at the expense of the energy liberated during the biocatalytic destruction of the crystalline lattices of the aluminosilicates) have furnished encouraging results to stimulate the investigation of the possibility of transferring the utilization of aluminosilicates biodegradation processes to the ore dressing field.

It should be emphasized that one basic difference between the agricultural and the mineral-metallurgical utilizations is represented by the time factor: whereas in agricultural applications solubilization rates of the same order of magnitude of the vegetable growth are

acceptable, i.e. of the order of few milligrams per dm^3 of soil per day, and may be less, in the mineral-metallurgical applications the rates should be several thousands or tens of thousands higher, with recoveries in the region of 90 to 95 %. High rates allow, in fact, to limit the size of the plants and hence the investment costs, whereas high recoveries contribute to containing the operating costs.

The application of biohydrometallurgy to aluminosilicates may derive advantage from at least four types of technological solutions:
1) The production of a solid residue enriched in aluminum as a consequence of the selective solubilization (extraction) of silicate components.
2) The selective aluminum solubilization.
3) The selective removal of iron from the run-of-mine ore.
4) The recovery of other values contained in the mineral, as is the case of lithium from petalite and from spodumene or beryllium from beryl.

Furthermore, the possibility of utilizing as fertilizers the spent liquors, which contain substances similar to heteroauxin and giberellin [39], cannot be neglected.

The selective extraction of the silicate component has proved to be an efficient means of treating very lean bauxite run-of-mine ores, where the very fine intergrowth of the free alumina and silicate component makes the liberation of the former with ordinary mineral dressing methods impossible [55—59]. With this procedure, Pol'kin et al. [60], resorting to strains of *B. mucillaginosus*, increased the grade of a bauxite (assaying from 12 to 14 % SiO_2, from 38 % to 49 % Al_2O_3, from 15 % to 21 % iron, from 3 % to 17 % Fe_2O_3) to 58.7 % Al_2O_3, with a recovery of 87.2 % Al_2O_3. These favorable results allowed the authors to propose a flowsheet for the microbial beneficiation of this type of bauxite run-of-mine ores.

Similar results were reported by Groudev and Genchev [39, 61], who worked with strains of *B. circulans* — of which 10 were wild and 8 laboratory-bred after having been subjected to various treatments (X-rays, N-methyl-N'-nitro-N-nitrosoguanidine and ethylenimine)-on a bauxite assaying 43.4 % Al_2O_3, and 25.9 % SiO_2 [61]. The best results were obtained either with laboratory-bred mutant strains adapted to the bauxite ore and capable of growing at low pH's and high temperatures [39] or utilizing mixed populations containing several wild strains indigenous to the substrate. The leaching of alumina has been strictly selective since only the alumina bound to silica in the form of kaolinite has been solubilized. A solid residue assaying 56.8 % Al_2O_3 with a recovery of 81.1 % was obtained, whereas in the sterile controls the assay of the solid residue remained 44.3 % Al_2O_3.

These same authors [40] achieved very interesting results working on two types of clays according to the second technique with 8 strains of *B. circulans* grown on a solid medium of Bogdanovich [61]. The best results were obtained in the flasks where the biomass development was maximum, and this fact leads to believe that solubilization is re-

lated to the development of the microbial population. For certain bacterial strains the preliminary adaptation to the run-of-mine constituted a prerequisite for a faster aluminum leaching kinetics, whereas for other strains it seemed insignificant: on the basis of these observations the authors maintain that the interactions between clays and "silicate" bacteria are complex in nature and not as yet fully elucidated. With stationary cultures of a clay composed mostly of kaolinite and illite, Groudev and Genchev obtained a solution assaying 43.7 % Al, whereas from a kaolinite clay they obtained a 40.9 % Al solution; in the sterile controls the Al contents remained at about 1 %.

Finally, the results reported by Karavaiko et al. [38] and by Krutsko et al. [62] concerning spodumene biodegradation by means of mucilaginous "silicate" bacteria, are very significant. These bacteria appeared to be, in fact, among the most active microorganisms in decomposing spodumene and in solubilizing lithium, aluminum and silica. The maximum lithium solubilization is achieved in the pH range from 3.0 to 5.0, i.e. in acid or weakly acid media, whereas the conditions most favorable to silica and aluminum solubilization are in the range of neutral to weakly alkaline pH's. In 7 days 0.12 % lithium, 1.6 % SiO_2 and 1 % Al_2O_3 were solubilized.

It is worth mentioning that "silicate" bacteria can be easily recovered from a pulp in a conventional flotation machine [63].

CONCLUSION

The utilization of microorganisms for the beneficiation of aluminosilicates constitutes the most recent research field in biohydrometallurgy, and, although laboratory investigations, now numerous, have produced in many cases quite encouraging results, the processes based on them are still far from being competitive — with the present knowledge — with the conventional techniques. The latter, however, do not allow the exploitation of important aluminosilicates occurrences, amongst which the Italian leucite reserves and the bauxite orebodies located in various parts of the world and characterized by very fine intergrowths of alumina and silica. It is in view of the exploitation of these mineral resources that the development of biohydrometallurgy might play an important techno-economic role in the future.

The discovery of the specifity of "silicate" bacteria towards silica undoubtedly represents a very important step forward in the development of such processes, even though much research work remains to be done for a more accurate characterization of these microorganisms.

As a matter of fact, on the grounds of the reports of all the researchers who worked on this subject, as well as of our own experience, doubts exist that *Bacillus circulans*, Jordan, can always be identified with "silicate" bacteria: certain collection strains of this microorganism did not actually exhibit any ability to enhance aluminosilicate solubilization. In conjunction and as a result of this more accurate characterization, the selection of particularly active —, and, possibly, specific

strains for the various types of aluminosilicates, as well as the development of specific culture media should form the subject of research topics of immediate realization.

The resort to genetic improvement techniques, to which, in any case, some researchers are already turning with success [61], should make it possible, at the same time, to enhance the ability to bioattack of the microorganisms recognized as being suitable, so as to allow the achievement of solubilization rates high enough to make the investment costs for continuous operating industrial plants competitive.

The performance of laboratory reactor bioleaching tests should constitute an obligatory step of the research. In this regard, the importance of the standardization of the experimental techniques should be emphasized so as to allow a more fruitful and close cooperation of the various laboratories involved in this type of research.

The fact that the weathering of rocks can be produced by several other bacterial genera, as shown e.g. by Lyalikova et al. [64], who worked with ultrabasic rocks, may extend in the future this field of research.

Much work has also been done with moulds: and the results obtained have undoubtedly provided a lot of useful information on aluminosilicates biodegradation processes, but these microorganisms are probably less suitable than "silicate" bacteria in the development of industrial processes, since it has been demonstrated, for most of them, that there is no direct attack. One parameter suitable for the comparison of the two types of microorganisms might also be the ratio between biomass and mass of mineral involved in the leaching process: this ratio is far lower for bacteria than for moulds, with significant consequences on the dimensioning of the continuous operation plants. With the present state of knowledge, however, it cannot be excluded that, for processes to be carried out in-situ or in inexpensive open-air basins moulds may also find suitable application in aluminosilicate biodegradation.

It can be concluded that more research on pilot and semi-industrial scale and a closer cooperation between engineers, microbiologists, chemists and physicists are still required for the perfecting of suitable techniques of aluminosilicate degradation.

REFERENCES

1. Vais, J.A., *E.M.J.*, *183*, 3, 76 (1982).
2. Lyon, F.D., *E.M.J.*, *183*, 3, 129 (1982).
3. Washington, H.S., The Roman Comagmatic Region, The Carnegie Institution, Washington (1906).
4. Cavinato, A., *Giacimenti Minerari*, U.T.E.T., Torino, 27 (1964).
5. De Grazia, S., and Camiola, G., *Le Stazioni sperimentali agrarie italiane*, Modena, *39*, 829 (1906).
6. Bassalik, K., *Zeitschrift für Gärungsphysiologie*, *2*, 1 (1913).
7. Webley, D.M., Duff, R.B., and Mitchell, W.A., *Nature*, *188*, 766 (1960).
8. Duff, R.B., Webley, D.M., and Scott, R.O., *Soils Sci.*, *95*, 105 (1963).

9. Webley, D.M., Henderson, M.E.K., and Taylor, I.F., *J. Soil Sci., 14*, 1, 102 (1963).
10. Silverman, M.P., In: Biogeochemical Cycling of Mineral-Forming Elements, P.A. Trudinger and D.J. Swaine (Eds.), Elsevier Scientific Publishing Company, Amsterdam, 446 (1979).
11. Holzapfel, L., *Z. Elektrochem., 55*, 577 (1951) (mentioned in Ref. 12).
12. Heinen, W., *Arch. Mikrobiol., 37*, 199 (1960).
13. Heinen, W., *Arch. Mikrobiol., 41*, 229 (1962).
14. Heinen, W., *Arch. Mikrobiol., 45*, 145 (1963).
15. Heinen, W., *Arch. Mikrobiol., 45*, 162 (1963).
16. Heinen, W., *Arch. Mikrobiol., 45*, 172 (1963).
17. Heinen, W., *Arch. Mikrobiol., 52*, 49 (1965).
18. Heinen, W., *Arch. Biochem. Biophys., 110*, 137 (1965).
19. Heinen, W., *Arch. Mikrobiol., 52*, 69 (1965).
20. Heinen, W., *Arch. Biochem. Biophys., 120*, 101 (1967).
21. Heinen, W., *Acta Bot. Neerl., 17*, 105 (1968). Mentioned by Ehrlich, H.L.: Geomicrobiology, Marcel Dekker Inc., New York (1981).
22. Lauwers, A.M., and Heinen, W., *Arch. Mikrobiol., 95*, 67 (1974).
23. Heinen, W., In: Biochemistry of Silicon and Related Problems, G. Bendz and J. Lindquist (Eds.), Nobel Symposium, Stockholm, Plenum, New York and London, 129 (1978).
24. Wagner, M., and Schwartz, W., *Z. Allg. Mikrobiol., 7*, 1, 33 (1967).
25. Wagner, M., and Schwartz, W., *Z. Allg. Mikrobiol., 7*, 2, 129 (1967).
26. Boyle, J.R., Voigt, G.R., and Sawhney, B.L., *Science, 155*, 3759, 193 (1967).
27. Department Laboratoires du BRGM, *Bulletin du BRGM* (deuxième série), Section II, No. 5, 35 (1971).
28. Rossi, G., In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, L.E., Murr, A.E. Torma and J.A. Brierley (Eds.), Academic Press, New York — San Francisco — London, 297 (1978).
29. Goni, J., Gugalski, T., and Sima, J., *Bulletin du BRGM* (deuxième série), Section IV, No. 1, 31 (1973).
30. Leleu, M., Sarcia, C., and Goni, J., Congrès International de Géochemie organique, Rueil, Sept. 1973 (mentioned in Ref. 31).
31. Goni, J., Greffard, J., Gugalski, T., and Leleu, M., *Bulletin de la Société française de Minéralogie et de Cristallographie, 96*, 252 (1973).
32. Mehta, A., Torma, A.E., Murr, L., and Berry, V.K., *Scanning Electron Microscopy, I*, 171 (1978).
33. Mehta, A., Torma, A.E., and Murr, L.E., In: IRGS Medical Science: Biogeochemistry; Environmental Biology and Medicine, Parasitology and Infectious Diseases, 416 (1978).
34. Mehta, A.P., Torma, A.E., and Murr, L., *Biotechnol. Bioeng., 21*, 875 (1979).
35. Aristovskaya, T.V., and Kutuzova, R.S., *Soviet Soil Science, 12*, 59 (1968).
36. Silverman, M.P., and Munoz, E., *Science, 169*, 985 (1970).
37. Avakyan, Z.A., Karavaiko, G.I., Mel'nikova, E.O., Krutsko, V.S., and Ostroushko, Yu.I., *Mikrobiologiya, 50*, 156 (1981).
38. Karavaiko, G.I., Krutsko, V.S., Mel'nikova, E.O., Avakyan, Z.A., and Ostroushko, Yu.I., *Mikrobiologiya, 49*, 547 (1980).
39. Groudev, S.N., Genchev, F.N., and Groudeva, V.I., Paper presented at the Symposium "Alumina production until 2000", Tihany, Hungary, October 6—9 (1981).
40. Groudev, S.N., and Genchev, F.N., In: 13th International Mineral Processing Congress, Warszawa, Proceedings of Round Table Seminar "Beneficiation of Clay Raw Materials", Polish Scientific Publishers, 87 (1979).

41. Vernadsky, V.I., A. Treatise on Geochemistry, Gosizdat (1934) (mentioned in Ref. 42) (in Russian).
42. Aleksandrov, V.G., *Dokl. vsesojuzn. Akad. sel'skokhoz. Nauk im. V.I. Lenina*, No. 3, 34 (1949).
43. Aleksandrov, V.G., Sbornik Kuibyshevskogo Sel'skokh. Inst. "Tesisy dokladov V nauchnoi konferenzii Instituta 27—30 Janvar' 1947 g., Kuibyshev (1947).
44. Aleksandrov, V.G., *Dokl. vsesojuzn. Akad. Sel'skokhoz. Nauk im. V.I. Lenina*, No. 12, 12 (1949).
45. Aleksandrov, V.G., and Zak, G.A., *Mikrobiologiya, 19*, 2, 97 (1950).
46. Tesic, Z., and Todorovic, M., *Zemljiste i biljka, I*, 3 (1952).
47. Tesic, Z., and Todorovic, M., In: Atti del VI Congr. Internaz. di Microbiologia (Roma, 1953), *6*, 356 (1955).
48. Tesic, Z., and Todorovic, M., *Zemljiste i biljka, 8*, Nos. 1—3, 233 (1958).
49. Aleksandrov, V.G., Trudi sovesch. Vopr. bacter. Udobr., 1956, Kiev, Izd. Akad. Nauk SSSR, 62 (1958).
50. Aleksandrov, V.G., Burangulova, M.N., and Solov'eva, E.P., *Trudy Odess. Sel'skokhos. Inst., 13*, 91 (1958).
51. Aleksandrov, V.G., and Ternovskaya, M.I., *Trudi Odess. Sel'skokhos. Inst., 14* (1961).
52. Aleksandrov, V.G., Silicate bacteria, Selhozgiz, Moscow (1953) (in Russian).
53. Aleksandrov, V.G., Ternovskaya, M.I., and Blagodir, R.N., *Vest. Sel'skokhos. Nauki, 11*, 95 (1963).
54. Aleksandrov, V G. and Kodra, V.A., *Dokl. Akad. Sel'skokhos. Nauk SSSR, 1*, 10 (1965).
55. Andreev, P.I., Pol'kin, S.I., Shavlo, R.A., Karavaiko, G.I., and Morozova, R.D., *Tsvetnaya Metallurgiya*, No. 3, 8 (1975).
56. Andreev, P.I., Shavlo, R.A., and Morozova, R.D., In: Mineral Processing, Tekhnika, Kiev, *18*, 42 (1976) (in Russian).
57. Andreev, P.I., Shavlo, R.A., Karavaiko, G.I., Pol'kin, S.I., Anishchenko, N.M., and Morozova, R.D., In: Ecology and Geochemical Activity of Microorganisms, M.V. Ivanov (Ed.), USSR Academy of Sciences, Pushchino, 126 (1976) (in Russian).
58. Andreev, P.I., Shavlo, R.A., Anishchenko, N.M., Semenchuk, K.L., In: Combined Methods in Complex Mineral Processing, S.I. Pol'kin and V.K. Zadorozhnyi (Eds.), Nauka, Moscow, 59 (1977) (in Russian).
59. Andreev, P.I., Licheva, L.V., and Segodina, V.Ya., *Tsvetnaya metallurgiya*, No. 5, 7 (1979).
60. Pol'kin, S.I., Adamov, E.V., Panin, V.V., Karavaiko, G.I., Andreev, P.I., and Kazintseva, S.I., *Rudy i metale nieżelazne* (Poland), No. 5, 15 (1979).
61. Groudev, S.N., and Genchev, F.N., In: Proceedings of the 4th International Congress for the Study of Bauxites, Alumina and Aluminum, Athens, Greece, October 9—12, *1*, 271 (1978).
62. Krutsko, V.S., Mel'nikova, E.O., Ostroushko, Yu.I., Karavaiko, G.I., and Avakyan, Z.A., In: Proceedings of the International Conference on Use of Microorganisms in Hydrometallurgy, B. Czegledi et al. (Eds.), Hungarian Academy of Sciences, Pécs, Hungary, 143 (1980).
63. Marjanovic, D., *Rudarski glasnik, 10*, 2, 63 (1970).
64. Lebedeva, E.V., Lyalikova, N.N., and Bugel'sky, Yu.Yu., *Mikrobiologiya, 47*, 6, 1101 (1978).

SULFATE-REDUCING BACTERIA AND SOME MICROSCOPIC FUNGI IN ORE PREPARATION AND HYDROMETALLURGY

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INTRODUCTION

Microbiological processes are widely used in various branches of industry and science.

Bacteria *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are successfully used in recovery of non-ferrous and radioactive metals from ores [1, 2]. Little attention, however, is devoted to the prospects of using other species of bacteria as flotation reagents and as agents for leaching of mineral raw materials.

The present report, therefore, represents an attempt to draw attention to a wider range of applications in non-ferrous metallurgy of a number of valuable properties of microorganisms, sulfate-reducing bacteria in particular.

SULFATE-REDUCING BACTERIA AS MODIFIERS OF THE FLOTATION PROCESS

Sulfate-reducing bacteria are a specific group of anaerobic bacteria capable of utilizing sulfate oxygen for oxidation of organic matter, i.e. of performing anaerobic respiration. The sulfates are thereby reduced to hydrogen sulfide. Sulfate-reducing bacteria are the main producers of hydrogen sulfide in nature [3].

The present study employed an enriched culture of bacteria belonging to the genus *Desulfovibrio*. The bacteria were grown on the Postgate medium containing sodium lactate.

The study resulted in the development of a simpler nutrient medium containing cheap components of cotton seed processing and bitumens of coal and oil as a carbon source.

Optimal conditions for development of bacterial culture were determined by the amount of hydrogen sulfide produced. Kinetic studies revealed that maximum amount of hydrogen sulfide was produced on the fifth day when the concentration reached 300—400 mg hydrogen sulfide per liter ($220 \cdot 10^6$ cells/ml), i.e. 10 times as low as the solubility limit (0.1 M).

A treatment of oxidized lead and antimony minerals with a culture of sulfate-reducing bacteria drastically improved the flotation and ensured a 85—92 % extraction. Floatability of cerussite pretreated with the microorganisms was increased by 20—25 % [4, 5].

When minerals are kept in contact with the bacteria for a short time flotation is activated, however, prolonged treatment results in a deep depression of flotation [6]. Floatability of minerals can be restored by washing them to reduce the residual hydrogen sulfide concentration.

The efficiency of bacterial treatment has been tested in the flotation of oxidized lead ores of Tajik deposits, under laboratory conditions and on a pilot flotation plant in a continuous cycle [7]. The ore minerals consisted mainly of galena, cerussite and plumbojarosite.

Sulfate-reducing bacteria were used to sulfidize lead ores of varying degree of oxidation and various elemental composition. Extraction of lead was improved by 5—8 % as compared to flotation using sodium sulfide under same conditions.

In pilot plants, the lead concentrate produced with the aid of sulfate-reducing bacteria contained 43.3 % lead and the extraction amounted to 85.4 %. For sodium sulfide flotation, the concentration and extraction were 43.1 % and 83.56 %, respectively.

A treatment of tailings from flotation of antimony sulfide with sulfate-reducing bacteria increased antimony extraction by 8 % owing to utilization of antimony oxides.

The bacteria exert no depressing action on molybdenite and galena, which is used in selective separation of molybdenite from chalcopyrite and of sphalerite from galena.

Experiments were also performed on the use of microorganisms to desorb xanthogenate from the mineral surface [8—10]. Minerals were first flotated, the foamy product of flotation was dehydrated and then exposed to different concentrations of bacteria.

It has been found that a large portion of xanthogenate is desorbed under these conditions, and the minerals lose their floatability.

The findings were checked in separation of an artificial mineral mixture composed of galena and sphalerite, with sulfate-reducing bacteria as desorption agent.

The minerals were first subjected to bulk flotation under optimum reagent conditions corresponding to maximum extraction. Selective flotation of the concentrate was performed following its treatment with sulfate-reducing bacteria and supply of appropriate reagents.

Extraction of galena into lead concentrate after treatment with 10^6 bacteria per ml of culture fluid was 83.63 %, and the galena content of the concentrate was 94.65 %. Sphalerite extraction under these conditions was 4.48 %. No selective separation of the lead-zinc concentrate was observed in the control experiments.

It is worth mentioning that hydrogen sulfide is oxidized by oxygen at a lower rate than sodium sulfide, and hence the concentration of H_2S and the S^{-2} and SH^- ions in the flotation pulp will drop slower than in the case of Na_2S. For this reason, desorption of xanthogenate from the surface of sulfide minerals can be more effectively performed with sulfate-reducing bacteria than with sodium sulfide solution.

It is evident, therefore, that sulfate-reducing bacteria is a promising means for desorption of xanthogenate from the surface of sulfide minerals in bulk-selective flotation of lead-zinc and copper-molybdenum concentrates.

Calculations have shown that sulfidization expenses in the case of using sulfate-reducing bacteria are reduced 30-fold as compared to the sodium sulfide method, without taking into account the higher concentrate quality and the greater extraction of valuable components.

LEACHING OF ANTIMONY- AND TIN-BEARING ORES BY SULFATE-REDUCING BACTERIA

Sulfate-reducing bacteria can be effectively used as reagents for leaching of antimony-containing materials, such as antimonite and antimony flotation concentrate [11—13].

In antimony hydrometallurgy only sulfide-alkaline solvents are used on a commercial scale.

For bacterial leaching of an antimony-containing material, the solvent used was a solution of sodium hydrate (120 g/l) supplemented with various amounts of enriched culture of sulfate-reducing bacteria.

The pulp (solid:liquid = 1:16) was heated to 90 °C.

The leaching of antimony was performed in two stages. The duration of the first stage was one hour, after which the solution was removed, and a new solution containing all the required reagents was added to continue the leaching for another 0.5 hour.

Extraction of antimony under these conditions was between 96.5 and 98.04 %.

As compared to the use of sodium sulfide, this method of leaching has a number of advantages for the given material.

The presence of free alkali in the solution considerably increases its conductivity, thereby improving characteristics of the subsequent electrolysis process.

Products of oxidation of sulfide sulfur are known to adversely affect electrolysis of sulfide-alkaline solutions of antimony [14].

Polysulfide ions, for example, diffuse to the cathode and dissolve metallic antimony as well as oxidize trivalent to pentavalent antimony.

The conventional method of antimony leaching requires higher concentrations of Na_2S as compared to leaching with sulfate-reducing bacteria, resulting in a lower current efficiency.

The ability of sulfate-reducing bacteria to produce a large amount of hydrogen sulfide has also been used for leaching tin from difficult-to-enrich hydrostannite-warlamovite ore. The ore was subjected to roasting-magnetic concentration which resulted in a concentrate containing 9.5 % tin. This concentrate was then used for bacterial leaching.

Following incubation for 4 days, the culture fluid was used as a working solution for leaching of tin from the concentrate. The con-

centrate with particle size —125+71 μ (total weight 10 g) was placed into a porcelain beaker and treated with an enriched culture of sulfate-reducing bacteria (solid:liquid = 1:20) at continuous stirring for 2 hours.

Characteristics of tin leaching with sulfate-reducing bacteria and with sodium sulfide are compared in Table 1.

Table 1

Effect of sulfate-reducing bacteria and sodium sulfide on tin leaching (2 hours, S:L = 1:20)

Material	Yield, %	Tin content, %	Tin extraction, %	Experimental conditions
Cake	84.1	1.31	11.6	30 % sodium sulfide, 60 °C
Solution	—	50.3	88.4	
Concentrate	100.0	9.5	100.0	
Cake	86.2	0.99	9.2	Sulfate-reducing bacteria, H_2S, 330 mg/l
Solution	—	62.5	90.8	
Concentrate	100.0	9.5	100.0	

Bacterial leaching has improved extraction of tin into the solution by 2.4 % and obviated the need for heating the pulp.

TRANSFORMATION OF SULFATES INTO CARBONATES BY SULFATE-REDUCING BACTERIA

It has been previously found that gypsum could be used for obtaining calcium carbonate [15].

The treatment of poorly soluble sulfates, such as $CaSO_4 \cdot 2H_2O$, with sulfate-reducing bacteria results in conversion of sulfur to hydrogen sulfide and in stimulation of calcium carbonate formation:

$$2CH_3CHOHCOONa + CaSO_4 \cdot 2H_2O \xrightarrow{S-R\ bacteria} H_2S +$$

$$+ 2CH_3COONa + CaCO_3 + 3H_2O + CO_2$$

This ability of sulfate-reducing bacteria was also successfully used by the authors for processing of some alkaline earth metal sulfates. Enriched culture of sulfate-reducing bacteria was first adapted to a number of alkaline earth metals by means of gradually substituting their sulfates for the water-soluble sulfates of Postgate medium. After five successive passages, while increasing each time the amount of alkaline earth sulfates and decreasing water-soluble sulfates (down to 20 %), the enriched culture of sulfate-reducing bacteria thus obtained was able to promote conversion of alkaline earth sulfate to carbonate.

The process of converting sulfate to carbonate was carried out in a 0.5 liter fermenter with two gas-supply tubes (to remove hydrogen sulfide and to supply nitrogen) and a valve (to feed nutrient medium

and inoculate), at continuous stirring with a magnetic stirrer, constant temperature 28—30 °C and pH 7—8.

The fermenter was filled with a nutrient medium containing 0.9 g/l sulfates, and inoculate (10 % of the fermenter volume) consisting of the adapted strain was added. Hydrogen sulfide was removed from the fermenter once every 5 days by blowing nitrogen at a rate of 26 ml/hour.

These optimal conditions for the growth of sulfate-reducing bacteria offered high efficiency in converting sulfates of alkaline earth metals to carbonates which were suitable for an immediate use by the consumer.

USE OF MICROSCOPIC FUNGI FOR THE RECOVERY OF ARSENIC FROM SOLUTION

Ecological problems call for development of novel processes for recovery of arsenic from solutions. One such method is based on reduction of arsenic to a gaseous state (trimethyl arsin) using fungi, *Fungi imperfecti* [16]. Subsequent burning of the gas at 550—650 °C allows a recovery of arsenic in the metallic form.

The fungi grow more rapidly on a neutral or alkaline medium, which is essential for using them in extraction of arsenic from carbonate-rich ores. A maximum extraction of arsenic into the gaseous phase has been found to occur following cultivation of the fungus on the Chapek medium. In arsenic-containing solutions the growth of the fungus is somewhat inhibited, the development starting only after 2 days. The process is affected by arsenic concentration in the solution. Intensive growth of the biomass, for example, is observed at arsenic concentrations between 5 and 10 g/l.

Table 2 shows the kinetics of arsenic extraction from solution into gas as a function of arsenic concentration in solution.

It is clear from the Table that arsenic is extracted into the gaseous phase almost completely. The method affords extraction of arsenic from concentrated solutions and can be used for the processing of arsenic-containing solutions at hydrometallurgy plants.

Table 2

Kinetics of arsenic extraction from solutions of different concentration into a gaseous phase by means of microscopic fungi

Time, days	Sodium arsenite solution		Industrial arsenite solution	
	Arsenic content, g/l	Arsenic extraction, %	Arsenic content, g/l	Arsenic extraction, %
0	0.5	0	5.12	0
3	0.39	22	1.98	61.4
6	0.16	68	0.34	93.4
9	0.06	88	0.18	96.5
12	0.009	99.79	0.14	99.8

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REFERENCES

1. Karavaiko, G.I., Kuznetsov, S.I., and Golomzik, A.I., Role of Microorganisms in Leaching Metals, Nauka, Moscow (1972) (in Russian).
2. Adamov, E.V., Panin, V.V., and Pol'kin, S.I., Bacterial and Chemical Leaching of Metals from Ores, Progress in Science and Technology of Concentration of Useful Minerals, T-8, 5 (1974) (in Russian).
3. Sokolova, G.A., and Karavaiko, G.I., Physiology and Geochemical Activity of Thionic Bacteria, Nauka, Moscow (1964) (in Russian).
4. Solozhenkin, P.M., Lyubavina, L.L., Lyalikova, N.N., Bogdanov, N.I., Petrova, O.G., and Lebedeva, E.V., *Doklady AN Tajik SSR, 19*, 6, 32 (1976).
5. Solozhenkin, P.M., Lyubavina, L.L., and Samokhvalova, L.F., *Izvestiya vuzov tsvetnaya metallurgiya*, No. 3, 13 (1979).
6. Lyalikova, N.N., Lyubavina, L.L., and Solozhenkin, P.M., In: Conference Bacterial Leaching, W. Schwartz (Ed.), Verlag Chemie, Weinheim, 93 (1977).
7. Solozhenkin, P.M., Lyubavina, L.L., Yemelyanov, A.F., Pulatov, G.Yu., and Aknazarova, T.N., In: Material Composition and Enrichability of Mineral Raw Materials, Nauka, Moscow, 29 (1978) (in Russian).
8. Solozhenkin, P.M., Lyubavina, L.L., and Ilyina, S.I., *Information leaflet of TajINTI*, ser. Ore Mining Industry, No. 220, Dyushambe (1977) (in Russian).
9. Kupeeva, R.D., Solozhenkin, P.M., and Khan, G.A., In: Ore Concentration, Irkutsk, 5, 158 (1977) (in Russian).
10. Kupeeva, R.D., Solozhenkin, P.M., and Khan, G.A., In: Theoretical Basis and Control of Flotation Processes, Moscow, 134 (1980) (in Russian).
11. Solozhenkin, P.M., In: International Conference on "Advances in Chemical Metallurgy", Bombay, 2, paper 37, 1 (1979).
12. Solozhenkin, P.M., and Lyubavina, L.L., In: Biogeochemistry of Ancient and Modern Environment, P.A. Trudinger, M.R. Walter, and B.J. Ralph (Eds.), Australian Academy of Sciences and Springer Verlag, Canberra — New York, 82 (1980).
13. Solozhenkin, P.M., Lyubavina, L.L., and Levchenko, O.G., In: Proceedings of the International Conference on the Use of Microorganisms in Hydrometallurgy, Pécs, 135 (1980).
14. Mel'nikov, S.M. (Ed.), Antimony, Metallurgiya, Moscow, 277 (1977) (in Russian).
15. Corrick, J.D., Rose, M.J., Sutton, J.A., Microbial conversion of hydrous calcium sulphate to hydrogen sulphide. U.S. Dept. of the Interior, Bureau of Mines, report 7355 (1970).
16. Solozhenkin, P.M., Lyubavina, L.L., and Sherepova, S.A., In: Physico-chemical Bases of Wasteless Technology of Processing Useful Minerals, Alma-Ata, 99 (1981) (in Russian).

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