BIOGEOTECHNOLOGY OF METALS

MANUAL

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BIOGEOTECHNOLOGY OF METALS

MANUAL

SCIENTIFIC EDITORS:
G.I. KARAVAIKO (USSR), G. ROSSI (ITALY),
A.D. AGATE (INDIA), S.N. GROUDEV (BULGARIA),
Z.A. AVAKYAN (USSR)

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Reviewed by:
Corresponding Member *USSR Academy of Science E.N. Kondratieva,*
Candidate of Science (Eng.) V.A. Sidorov

The MANUAL

The Manual considers theoretical and applied aspects of biogeotechnology of metals: microorganisms, active in leaching of metals from ores, methods of their isolation and cultivation, fundamentals of dump, underground and tank leaching of metals from ores, new trends in biohydrometallurgy.

The Manual is intended for experts at large who specialize in microbiology, biochemistry, hydrometallurgy, and geotechnology.

CONTENTS

PREFACE

The Manual on Biogeotechnology of Metals has been prepared within the framework of the International Project on Microbiological Leaching of Metals from Ores implemented in 1981–1985 under an official agreement between the United Nations Environment Programme (UNEP), the USSR State Committee for Science and Technology (GKNT) and the Environment Protection Committee of the People's Republic of Bulgaria, in cooperation with the Council for Mutual Economic Assistance. The Project was initiated by the UNEP/UNESCO/ICRO Panel on Microbiology.

The Institute of Microbiology of the USSR Academy of Sciences served as the basis of the Project.

The objective of the Project was to contribute to the training of specialists from developing countries in biogeotechnology of metals and to the application of low-waste technologies promoting rational use of natural resources.

The Manual deals with a set of microbiological and technological approaches to the main problems of dump, underground and tank bacterial and chemical leaching of sulfide and non-sulfide material, and with metal recovery from diluted solutions.

INTRODUCTION

The development of new effective techniques to process mineral raw material is one of the decisive factors of scientific and technological progress. The Earth's resources, however great, are limited and exhaustible.

A lower metal content and a more complicated mineral composition of ores to be processed have been observed recently, the demands for environmental protection have grown, which makes mining and recovery of values more expensive.

A growing need in metals forces to use low-grade ores, deeplying deposits and mine dumps.

It has become evident, that only an improvement and radical changes in the methods of mining and processing of minerals, i.e. a transfer to low- and non-waste technologies allowing a rational and comprehensive use of mineral resources will make it possible to provide the necessary metals for the industry at the end of the 20th and beginning of the 21st century.

Biogeotechnology is one of the approaches to the solution of many such problems.

Biogeotechnology of metals is a field in the science of metal extraction from ores, concentrates, rocks and solutions under the impact of microorganisms and/or their metabolities. Its components are:

1. Biohydrometallurgy or bacterial metal leaching.
2. Ore enrichment.
3. Biosorption of metals from solutions.

These approaches are not equally developed. The processes of dump and underground leaching of copper-zink, uranium and a number of other metals are better studied in the field of biohydrometallurgy. This technology is already used for metal extraction from low-grade and lost ores in the industrial processes of USA, Canada, USSR, Bulgaria and a number of other countries. Price of copper obtained in this way is 1.5–2.0 times lower, than that, obtained by traditional means. Metal tank-leaching processes are worked out to extract noble metals from complex in composition or poor products impossible to process by traditional means. Arsenic gold- and tin-containing concentrates, metacolloidal copper-zink concentrates, poor copper-nickel concentrates and a number of other are referred to such products. This technology is in the process of semi-industrial tests in a number of countries (South African Republic, Canada, USA, USSR). Practically all the techno-

logical schemes are closed, what to a great extent decreases or excludes the pollution of the environment at all. The new tendencies in the development of biogeotechnology of metals have already been outlined. They include the enrichment of a number of rocks and ores, for example, bauxites, with partial silicon leaching, sulfidization of oxidized ores, biosorption of metals from solutions. Utilization of biosorbent on the basis of microorganisms' biomass allows to solve both technological and nature protection problems. The real possibility for the purification of industrial waste waters from heavy metals is created.

Utilization of new bacteriochemical methods will allow to increase sources of raw materials, to provide complex metal extraction and does not require to create complicated mining systems. That gives the possiblity to automatize corresponding technological processes, to raise labour productivity and industrial efficiency, to solve many problems of the environmental protection.

The main task of the Manual is to outline the basic methodic approaches to the solution of technological problems.

Chapters 1 and 2 give a detailed description and the methods of work with bacteria, which have already found application in metal leaching. These are obligate chemolytoautotrophic and facultatively autotrophic acidophilic bacteria. A number of non-specific processes are caused by a wide spectrum of already known microorganisms, for example, destruction of silicate minerals, metal biosorption, manganese leaching, etc.

Therefore, we limited ourselves to the estimation of microorganisms' role in these processes.

Chapter 3 describes not only the investigation methods of microbiological processes of metal leaching from ores, but the methods of their securing and estimation as well. These methods deal with the study of qualitative composition of ores and concentrates, the production of active bacterial biomass, development of mathematical models of the leaching processes, creation of criteria for economic estimation of the processes.

Chapters 4 and 5 deal with the methods of studying and working out the technology of dump, underground and tank bacterio-chemical leaching. The examples of utilizing these technologies for metal production are given.

Chapter 6, taking into account the available information, describes the methods of studying bacterial methods of non-sulfide materials' processing, and Chapter 7 deals with the methods of metal biosorption studying.

The Manual shows that the combination of different microbiological, chemical and technological methods will allow to create a totally new and effective technology of metal extraction.

The present Manual presupposes, that specialists – microbiologists, chemists, process engineers will cooperate, and biogeotechnologists have already mastered the methods of using microorganisms to leach metals from ores and concentrates. Separate chapters of the Manual are written by experts in various fields of biogeotechnology who have accumulated significant experience.

The Manual will be of use to specialists and processing of mineral raw materials.

Chapter 1
MICROORGANISMS AND THEIR SIGNIFICANCE FOR BIOGEOTECHNOLOGY OF METALS

1.1. MICROORGANISMS AND AREAS OF THEIR APPLICATION IN HYDROMETALLURGY

At present we know the following microbiological processes which play an important role in hydrometallurgy:

a) oxidation of sulfide minerals, elemental sulfur and ferrous iron;

b) production by organotrophic microorganisms of organic compounds, peroxides, etc. which destruct minerals, oxidise or reduce the elements with variable valency;

c) accumulation of chemical elements or their precipitation by microorganisms.

Table 1.1 lists the microorganisms important for hydrometallurgy along with possible areas of their application.

Table 1.1

Microorganisms important for hydrometallurgy

Microorganisms	Process	Feasible area of application
Bacteria of the genera Thiobacillus and Leptospirillum **Thiobacillus ferrooxidans** **Thiobacillus thiooxidans** **Thiobacillus acidophilus** (syn. **T.organoparus)** **Leptospirillum ferrooxidans** **Leptospirillum ferrooxidans** in mixed culture with **T.thiooxidans** and **T.acidophilus**	Oxidation of sulfide minerals, S^0 and Fe^{2+} at pH 1,4–3,5 and t=5–35 °C	Dump, underground and tank leaching of metals from sulfide and mixed ores and concentrates, from wastes of pyrometallurgic industry; desulfurization of coals
Facultative thermophilic bacteria similar to thiobacilli	Same at pH 1,1–3, 5 and t=30–55 °C	The same as above
Facultative thermophilic bacteria of the genus Sulfobacillus	Same at pH 1.1–5.0 and t=20–60 °C	The same as above
Acidophilic bacteria of genera **Sulfolobus** and **Acidianus**	Same at pH 1,0–5,0 and t=45–96 °C	The same as above

Table 1.1 continued

Microorganisms	Process	Feasible area of application
Organotrophic microorganisms and their metabolites (fungi, bacteria, yeasts, algae)	Destruction of sulfide minerals and alumosilicates, silicates, reducing and oxidation manganese, solubilizing gold and biosorption of metals	Extraction of metals from carbonate and silicate ores and rocks; leaching of gold; use of bacterial biomass and metabolites in ore flotation (lipids) and selective extraction of metals from solutions

Below are the characteristics of leaching processes and microorganisms which play an important role in metal recovery from ores and concentrates and which are most efficient in metal extraction from solutions.

Some processes being studied at present (destruction of silicate minerals, leaching of manganese, gold and rare elements and metal biosorption) are performed by non-specific groups of generally well-known microorganisms. Hence the appropriate sections of the present Chapter just enumerate these microorganisms while their detailed description can be found in the existing determinative manuals for bacteriology. Preference is given to a specific number of species which are used in research but not cited in the manuals.

1.2. ROLE OF BACTERIA IN OXIDATION OF Fe^{2+}, S^0 AND SULFIDE MINERALS

Oxidation of Fe^{2+}, S^0 and sulfide minerals is mediated primarily by bacteria of the genera **Thiobacillus, Leptospirillum, Sulfolobus, Sulfobacillus** and **Acidianus.** Especially important for hydrometallurgy are acidophilic bacteria, since at low pH values the metals released as a result of oxidation of sulfide minerals are solubilized and can be eventually recovered as a marketable product.

Oxidation of Fe^{2+}. Ferrous iron is the most easily oxidized substrate for **T.ferrooxidans** and a number of other bacteria. The oxidation reaction takes place probably according to the scheme [48]:

$$4\,Fe^{2+} + O_2 + 4\,H^+ \xrightarrow{\text{bacteria}} 4\,Fe^{3+} + 2\,H_2O \qquad (1.1)$$

$$\Delta G_{30\,^oC} = -38.0\ kJ \cdot mol^{-1}$$

This reaction is rather important for leaching of metals since it enables accumulation of bacteria biomass in ores and solutions, to obtain a strong oxidizer of many of sulfide minerals – Fe^{3+}, and to create a high redox potential of the medium. Ferric iron, depending on pH and its concentration in the medium, is hydrolyzed, and as a result a part of Fe^{3+} precipitates and H-ions are formed:

$$Fe^{3+} + 3H_2O \underset{\text{chemically}}{\rightleftharpoons} \downarrow Fe(OH)_3 + 3H^+ \qquad (1.2)$$

This enables adjusting pH and ferric iron concentration in solutions.

Kinetics of Fe^{2+} oxidation has been studied in detail with **T.ferrooxidans** which accelerates Fe^{2+} oxidation by hundreds of thousands fold. Especially important is the concentration of O_2, CO_2 [220] and **T.ferrooxidans** cells (Table 1.2). Increasing the number of **T.ferrooxidans** cells by one order, namely from $2.5 \cdot 10^7$ to $2.5 \cdot 10^8$ per ml enables to accelerate Fe^{2+} oxidation rate by threefold even at the temperature of 8–9 °C [114].

Table 1.2

The dependence of Fe^{2+} oxidation rate on T.ferrooxidans biomass (t=28 °C) [114]

Bacterial concentration, g/l (wet biomass)	Oxidized Fe^{2+}, $g \cdot L^{-1} \cdot h^{-1}$
0.005	0.6
1.2	15

Oxidation of sulfide minerals. Bacteria are capable of oxidizing the following sulfide minerals: [98, 112, 207, 208] pyrite and marcasite (FeS_2), pyrrhotite (FeS), chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), covellite (CuS), chalcocite (Cu_2S), tetrahedrite ($Cu_8Sb_2S_7$), enargite ($3Cu_2S \cdot As_2S_5$), arsenopyrite (FeAsS), realgar (AsS), orpiment (As_2S_3), cobaltite (CoAsS), pentlandite ($Fe, Ni)_9S_8$, violarite (Ni_2FeS_4), bravoite $(Ni, Fe)S_2$, millerite (NiS), polydymite (Ni_3S_4), antimonite (Sb_2S_3), molybdenite (MoS_2), sphalerite (ZnS), marmatite (ZnS), galena (PbS), geocronite Pb_5 (Sb, As_2) S_8, Ga_2S_3 as well as CuSe. The oxydized compound is sulfide sulfur in such minerals as pyrite, chalcopyrite and arsenopyrite, the oxydized compounds are sulfur and iron, in Cu_2S it is copper and selenium (Se^{2-}) in CuSe.

The role of bacteria and chemical processes in oxidizing sulfide minerals and metal leaching can be illustrated by pyrite oxidation:

$$2\,FeS_2 + \frac{15}{2}\,O_2 + H_2O \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2SO_4 \quad (1.3)$$

$$FeS_2 + 2Fe^{3+} \xrightarrow{\text{chemically}} 3Fe^{2} + 2S^0 \quad (1.4)$$

$$2Fe^{2+} + \frac{1}{2}\,O_2 + 2H^+ \xrightarrow{\text{bacteria}} 2Fe^{3+} + H_2O \quad (1.5)$$

$$S^0 + H_2O + \frac{3}{2}\,O_2 \xrightarrow{\text{bacteria}} H_2SO_4 \quad (1.6)$$

$$S^0 + 6Fe^{3+} + 4H_2O \xrightarrow{\text{chemically}} 6Fe^{3+} + SO_4^{2-} + 8H^+ \quad (1.7)$$

In other words, in metal leaching one should take into account the direct bacterial oxidation of sulfide minerals related to bacterial activity and the indirect one, related to Fe^{3+} activity due to bacterial and chemical oxidation of Fe^{2+}. Therefore the leaching of metals from sulfide ores is often referred to as a bacterio-chemical process.

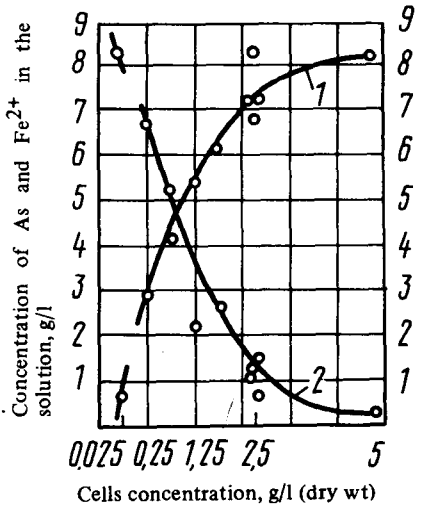


Fig. 1.1. Effect of bacteria concentration on the rate of arsenic leaching (1) and variation of Fe^{2+} concentration in the solution (2).
Time – 27 hours; S:L = 1:5; Fe^{2+}_{init} = = 10 g/l

The kinetics of bacterio-chemical oxidation of sulfide minerals depends on their thermodynamic features and electro-chemical interrelations in ores and concentrates, on the medium characteristics (pH, temperature, concentration of O_2 and CO_2 mineral particle size), on leaching techniques, as well as on bacteria concentration and activity [24, 73, 112, 122, 163, 233]. The impact of bacterial concentration on the rate of arsenopyrite oxidation is shown on Fig. 1.1 [74].

Depending on conditions, the mesophylic and thermophylic bacteria accelerate the oxidation of sulfide minerals by a factor of dozens, hundreds and thousands compared with the chemical oxidation by the molecular oxygen or ferric iron.

Ferric iron (Fe^{3+}) actively oxidizes such sulfide minerals as chalcocite, bornite, sphalerite. However, it is not very effective in oxidizing pyrite, chalcopyrite, arsenopyrite and a number of other sulfide minerals.

Oxidation and leaching of uranium [48]. T.ferrooxidans oxidizes uranium as follows:

$$2U^{4+} + O_2 + 2H_2O \rightarrow 2UO_2^{2+} + 4H^+ \qquad (1.8)$$

$$\Delta G\ 30\ ^{o}C = -130.4\ kJ \cdot mol^{-1}$$

The leading role in uranium leaching is played by ferric iron. Fe^{3+} oxidizes U^{4+} to U^{6+} which are solubilized in sulfuric acid solutions:

$$UO_2 + Fe_2(SO_4)_3 \xrightarrow{\text{chemically}} UO_2SO_4 + 2\ FeSO_4 \qquad (1.9)$$

$$UO_3 + H_2SO_4 \xrightarrow{\text{chemically}} UO_2SO_4 + H_2O \qquad (1.10)$$

In this case bacteria regenerate Fe^{3+} in the oxidation of Fe^{2+} or FeS_2.

1.3. THE MECHANISM OF BACTERIAL OXIDATION OF Fe^{2+}, S^0 AND SULFIDE MINERALS

The oxidation of Fe^{2+} and reduced sulfur compounds by bacteria is an extremely complex and multistage process which has not been deeply searched. It encompasses bacteria adhesion to minerals, their destruction, solubilization of sulfur, transport of S^0, Fe^{2+} or ions of other metals into the cell and their oxidation. At the first stage bacteria interact with the surface of sulfide minerals and modify it.

As has been demonstrated for pyrite oxidation by **T.ferrooxidans,** the mineral electrode potential (EP) is considerably lowered while the redox potential of the medium (Eh) is increased, producing oxidative environment [233]. In the absence of bacteria, when EP of pyrite and Eh of the medium have similar values, oxidation does not take place (Fig. 1.2).

This pattern has also been revealed for other sulfide minerals. In a mixture of different sulfide minerals forming galvanic couples bacteria preferentially oxidize those with a lower EP, i.e. sulfide-anode (Table 1.3):

Obviously, the direction of the microbiological oxidation of sulfide minerals coincides with that of the electrochemical oxidation. Therefore, it may be regarded as a biologically intensified electrochemical or corrosive process. Also, in attacking sulfide minerals bacteria enhance the deformation of the crystal structure, facilitating the oxidation process.

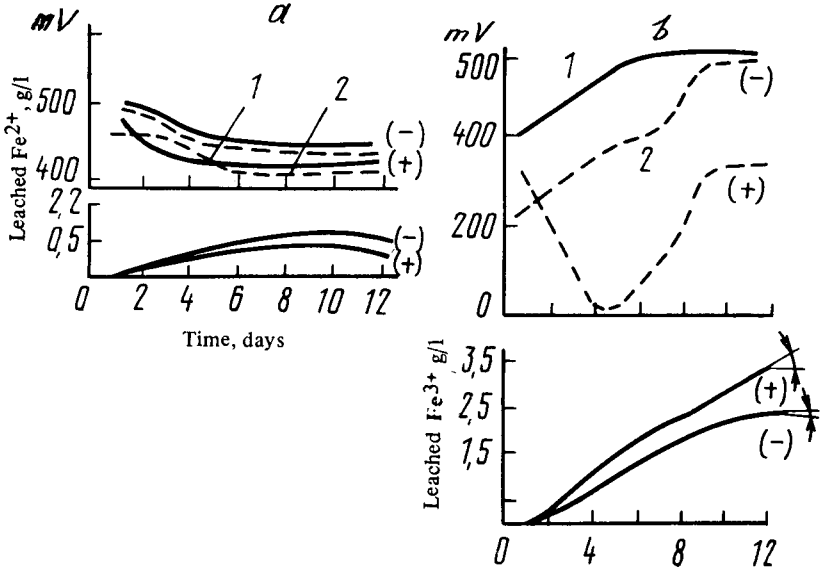


Fig. 1.2. Plots of changing Eh of the medium (1) and EP (2) of the p-type (+) and n-type (−) pyrite and leaching curves of Fe^{2+} and Fe^{3+} in experiments without (a) and with T. ferrooxidans (b)

Oxidation of mixture of sulfide minerals by T.ferrooxidans [19, 173]

Mineral mixture in concentrates	EP, V	Preferentially oxidized mineral
$CuFeS_2$ FeAsS	0.76–0.77 0.62–0.64	FeAsS
$CuFeS_2$ ZnS	0.60–0.68 0.23–0.43	ZnS
FeS_2 $CuFeS_2$	0.60 0.50	$CuFeS_2$

Fig. 1.3 presents the scheme of bacterial oxidation of sulfides for arsenopyrite as an example [173, 174].

The reactions in the diffusion layer may be illustrated as follows:

Anodic reaction: $FeAsS \rightarrow Fe^{2+} + As^{3+} + S^0 + 7\bar{e}$ (1.11)

Cathodic reaction: $3\frac{1}{2}\ O_2 + 14H^+ + 7\bar{e} \rightarrow xH_2O$ (1.12)

Bacteria accelerate the electrochemical oxidation process in two ways:

1. By decreasing the EP of sulfides and increasing the Eh of the medium.

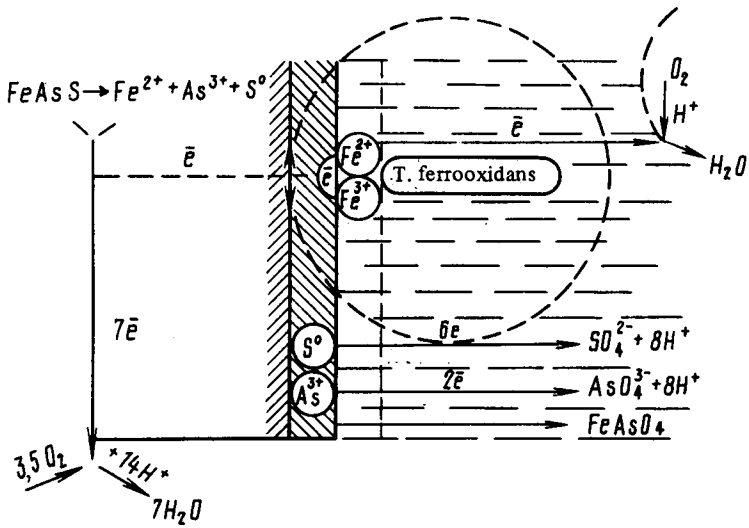


Fig. 1.3. Model of bacteriochemical oxydation of arsenopyrite

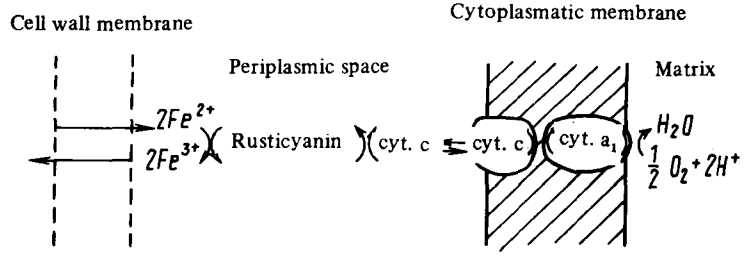


Fig. 1.4. Scheme for pathway of electron transfer in T. ferrooxidans during oxidation Fe^{2+}

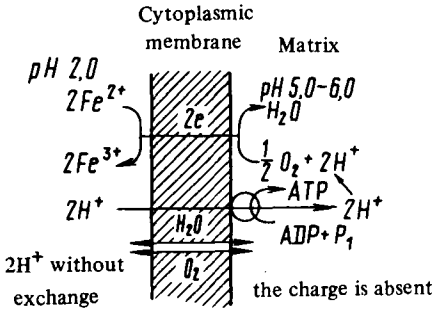
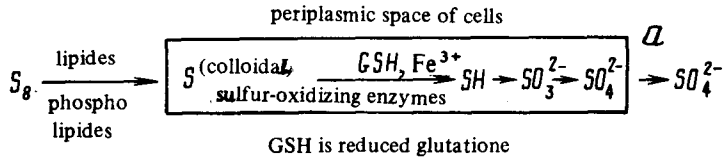


Fig. 1.5. Scheme for oxidative phosphorylation in T. ferrooxidans



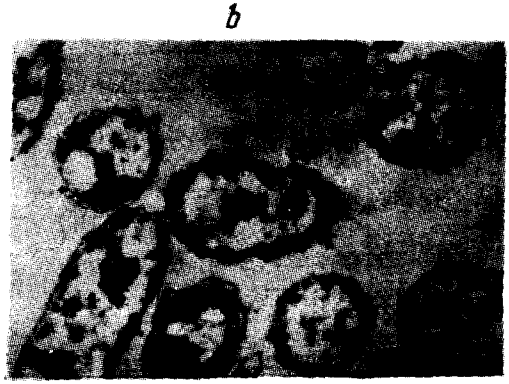


Fig. 1.6. The proposed scheme elemental sulfur oxidation T.ferrooxidans (a), P–periplasmic space in the cell (b) (black dots – product of S reaction with $AgNO_3$ – Ag_2S

2. By oxidizing S^0 and Fe^{2+}:

$$S^0 + 4H_2O \rightarrow SO_4^{2-} + 8H^+ + 6\bar{e} \quad (1.13)$$

$$Fe^{2+} \rightarrow Fe^{3+} + \bar{e} \quad (1.14)$$

Fe^{3+} in its turn reacts with sulfides and other reduced compounds of sulfur and other elements as per the following equations:

$$S^0 + 6Fe^{3+} + 4H_2O \rightarrow 6Fe^{2+} + SO_4^{2-} + 8H^+ \quad (1.15)$$

$$H_2AsO_4^- + Fe^{3+} \rightarrow FeAsO_4 + 2H^+ \quad (1.16)$$

The mechanism of bacterial oxidation of Fe^{2+} and S^0 has not been clarified yet.

Figs. 1.4 and 1.5 present the schemes of electron transfer and ATP synthesis during oxidation of Fe^{2+} by **T.ferrooxidans** [103, 104].

A transfer of electrons and protons leads to the emergence of transmembrane electrochemical protential due to which the ATP synthesis occurs.

The oxidation of S^0 also involves a close contact between the sulfur and bacteria. Phospholipids are important for elemental sulfur solubilization [117, 171]. Sulfur crystals were shown to be localized on the surface of bacterial cells while the solubilized colloidal sulfur enters the periplasmic space of cells (Fig. 1.6 b) and is oxidized to sulfuric acid probably according to the following scheme [75, 104, 115, 204], (see Figs 1.6 a).

1.4. CHARACTERISTICS OF BACTERIA OXIDIZING Fe^{2+}, S^0 AND SULFIDE MINERALS

Genus Thiobacillus [16, 45]. Small rod-shaped cells, gram-negative. Motile by means of a single polar flagellum. Non-spore-forming, strict aerobes*. Growth range of separate species at pH between 0.5–9.0, optimum temperature is 28–30 °C. Energy derived from the oxidation of reduced sulfur compounds. **T.ferrooxidans** also oxidizes ferrous compounds. The genus includes strictly chemolithoautotrophic species which derive their carbon from carbon dioxide and energy from the non-organic sulfur compounds and facultative chemolithoautotrops, capable of growing in the heterotrophic, autotrophic, and mixotrophic conditions**. The DNA G+C content ranges from 48–68 moles %. Widely occur in sulfide ore deposits, sulfur springs and soils.

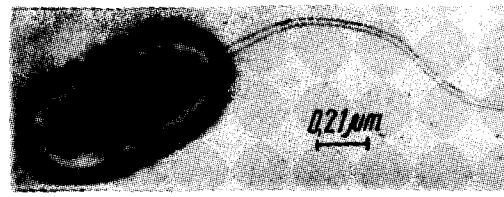


Fig. 1.7. Cell of T.ferrooxidans

* An exception is **Thiobacillus denitrificans** (facultative anaerobe) which is not considered below.

**Mixotrophic conditions are those in which microorganisms combine different nutrition patterns, e.g. capable of oxidizing both organic and inorganic compounds simultaneously.

Fig. 1.8. Cell of T.ferrooxidans on the medium with S^0

Classification of thiobacilli into species is based on different DNA nucleotide composition, fatty acids composition of the cell wall and physiological properties. Since the leading role in leaching of metals is played by acidophilic thiobacilli, only this group of bacteria is considered below (Tables 1.4, 1.5). It includes such well-studied chemolithoautotrophs as **T.ferrooxidans** and **T.thiooxidans** (Figs. 1.7, 1.8, Table 1.4) and facultative chemolithoautotrophs **T.acidophilus** (syn. **T.organoparus)** and a number of moderate thermophilic bacteria (Table 1.5).

Table 1.4

Characteristics of obligate chemolithoautotrophic acidophilic thiobacilli

Characteristics	**Thiobacillus ferrooxidans** [10, 16, 89, 95, 98, 120, 121, 130, 201, 208, 220]	**Thiobacillus thiooxidans** [16, 95, 116, 120, 121, 226]
Form and cell size (μm)	Rods, 0.3–0.5x1.0–1.7	Rods, 0.5x1.0–2.0
Cell wall	typical of Gram-negative bacteria	
Flagellation	+ (one)	+ (one)
Pili	+	no data
Intracellular granula:		
Polyphosphate granules	+	+
Sulfur	+	+
Carboxysomes	+	+
Source of energy for growth in chemolithoautotrophic conditions		
S^0	+	+
$S_2O_3^{2-}$	+	+
$S_4O_6^{2-}$	+	+
Fe^{2+}	+	–
U^{4+}	+	–
Cu^+	+	–
Se^{2-}	+	–
Sulfide minerals	+	+ (weak, certain sulfides)
pH range (optimum)	1.2–6.0 (2.5–2.8)	0.5–6.0 (2.0–3.5)
Sources of nitrogen	NH_4^+	NH_4^+

Table 1.4 continued

Characteristics	**Thiobacillus ferrooxidans** [10, 16, 89, 95, 98, 120, 121, 130, 301, 208, 220]	**Thiobacillus thiooxidans** [16, 95, 116, 120, 121, 226]
Relation to O_2	strict aerobe	strict aerobe
Temperature for growth (optimum), °C	5–40 (28–35)	5–40 (28–30)
The DNA G+C content, moles %	55.0–57.4	52.0–53.0
Ubiquinone: Q – 8	+	+
Q – 10	–	–
Hydroxylated fatty acids	3–OH 14:0	3–OH 14:0

Note: "+" – positive response
"–" – negative response

Table 1.5

Characteristics of facultative chemolithoautotrophic acidophilic thiobacilli

Characteristics	**Thiobacillus acidophilus** (syn. **T.organoparus**) [86, 89, 120, 121, 142]	Thiobacillus-like (TH–1, 2, 3) [31, 33, 132]
Forms and cell size (μm)	Rods, 0.5–0.7x1.1–1.8	Rods, 0.4–0.8x1.2–4.2
Cell wall	typical for Gram-negative bacteria	(?)
Flagellation	+ or –	+ or –
Intracellular granules:		
Polyphosphate granules	+	no data
Sulfur	no data	no data
Carboxysomes	no data	no data
Source of energy for growth in chemolithoautotrophic conditions		
S^0	+	+
Fe^{2+}	–	+
Sulfide minerals	–	+
Use of organic compounds	+	+
pH range (optimum)	1.5–6.0 (3.0)	1.1–3.5 (2.6)
Sources of nitrogen	NH_4^+, urea	NH_4^+
Relation to O_2	strict aerobe	strict aerobe
Temperature for growth, °C	25–30	50
The DNA G+C content, moles %	62.9–63.2	48.0
Ubiquinone: Q – 8	–	no data
Q – 10	+	no data
Hydroxylated fatty acids	3–OH 14:0	no data

T.ferrooxidans. Easily isolated from mine drainage water. Grows on liquid media with Fe^{2+} and sulfide minerals, as well as on solid media based on silica gel or polyacrilamid. Initially transparent the liquid medium becomes amber-hued, then changes to rusty-brown due to formation of ferric iron. Colonies on solid media are small (from 1.0 to 1.5 mm in diameter), round-shaped, smooth, with ferric iron deposits.

T.thiooxidans. Widely occurs in sulfide and sulfur ore deposits, from where it is easily isolated. The liquid medium containing sulfur becomes turbid with pH decreasing to 1.0–1.5. Small colonies (from 1.0 to 2.0 mm in diameter) are formed on solid media with $S_2O_3^{2-}$.

T.acidophilus (syn. **T.organoparus).** Isolated from ores and first described as **T.organoparus** by Markosyan [142]. Consequently the identic bacteria was isolated by Quay and Silver [86] from the museum culture of **T.ferrooxidans** and described as **T.acidophilus.** Under this name it was classified as another species thiobacilli. Utilizes the following organic substances: D-glucose, D-fructose, D-galactose, D-mannitol, D-xylose, D-ribose, L-arabinose, sucrose, citrate, malate, DL-aspartate, DL-glutamate. On 9K agar medium with glucose forms small colonies (1 to 2 mm in diameter), round-shaped, convex, slightly transparent and cream-coloured. Renders liquid media with sulfur turbid and decreases pH to 1.5.

Thiobacillus-like thermophilic bacteria (TH – 1, 2, 3). Taxonomically the thiobacillus-like bacteria are not sufficiently studied. Nevertheless they are important for hydrometallurgy and hence described in Table 1.5. These bacteria show active growth on media with Fe^{2+} and sulfide minerals in the presence of yeast extract (0.02 %). At 30 °C these bacteria exhibit poor growth on media with Fe^{2+} and none at all on medium with pyrite. Growth on pyrite does not start until temperature is as high as 40 °C. Also develop on media with yeast extract in the absence of inorganic energy sources. Yeast extract may be replaced with simple organic compounds as reduced sulfur sources (glutathione, cysteine, cystine).

Thiobacillus-like bacteria have been isolated from thermal springs with pH 4.3 and temperature 64 °C. They have been detected in the leached ores with pH 2.7 and temperature between 27 and 43.5 °C.

Other thermophilic thiobacilli capable of oxidizing iron and sulfur, were isolated from samples of sediments from hot springs, from water (pH 1.8) of Myvam Lake in the north-west Iceland, from coal (pH 2.8) and from waste water canal (pH 1.5, t=37 °C) in Great Britain [147]. Some of them oxidize sulfur at 50 °C and are described as strains close to **T.thiooxidans.** Another isolate grown on medium with Fe^{2+} at 50 °C is apparently close to **T.ferrooxidans.** For growth on medium with Fe^{2+} the pH optimum varies from 1.5 to 1.8. Wood and Kelly [232] described three thermoacidophilic iron-oxidizing bacteria capable of autotrophic and mixotrophic growth. They were isolated from coal storage. The three bacteria develop on mineral media with Fe^{2+}, although require thiosulphate or tetrathionate to be added as sources of reduced sulfur. Unable to assimilate SO_4^{2-} for biosynthetic processes. The three bacteria require Fe^{2+} for growth on organic substrates. According to

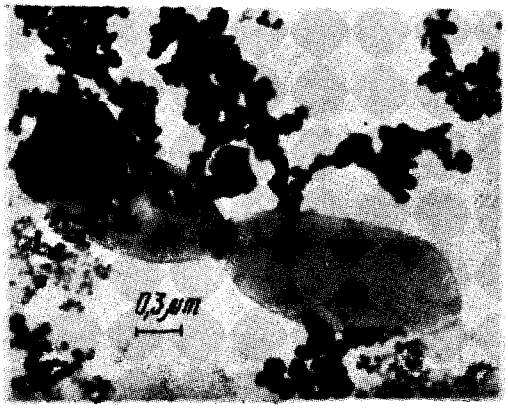


Fig. 1.9. Cells of S.thermosulfidooxidans on the medium with arsenopyrite

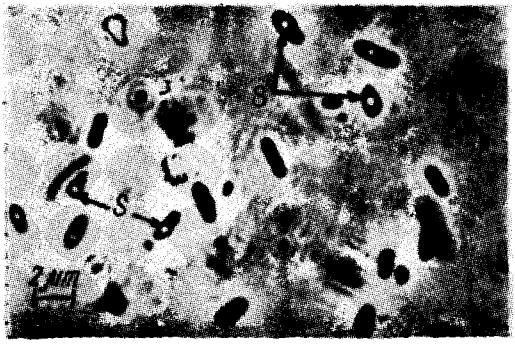


Fig. 1.10. S.thermosulfidooxidans: cells and spores

Wood and Kelly enhanced bacterial growth and oxidation of Fe^{2+} in the presence of glucose are related to accelerated biosynthetic processes.

Genus Sulfobacillus [17, 69, 70]. Rods with rounded or tapered ends. Nonmotile. Occur in pairs or short chains. Gram-positive. Endo-Spores round or slightly oval, paracentral to terminal. Sporangia swollen. Colonies on agar containing Fe^{2+} are round, shining, initially yellowish, becoming reddish-brown in colour. Strictly aerobic. Facultatively chemolithoautotrophic. Sulfur, iron and sulfide minerals like pyrite are oxidized. Can grow in heterotrophic conditions on media containing 0.1 % of glucose, sucrose or other organic substances (0.02 % YE.) Facultatively thermophilic. The mole % G + C of DNA is 45.5–49.3. Isolated from dumps of the Nikolayevsky deposit in the USSR. Widely occurs in thermal springs, sulfide ore deposits and in corrosion spots in municipal heat-supply systems. Today only one species **S.thermosulfidooxidans** and two subspecies **S.thermosulfidooxidans subsp. thermotolerans** and **S.thermosulfidooxidans subsp. asporogenes** are known (Table 1.6, Figs. 1.9 and 1.10). Type species: **Sulfobacillus thermosulfidooxidans,** strain 1A (BKM B-1269).

Table 1.6

Characteristics of species belonging to the genus Sulfobacillus

Characteristics	**Sulfobacillus thermosulfidooxidans** [69, 113]	**Sulfobacillus thermosulfidooxidans** subsp. **thermotolerans** [127]	**Sulfobacillus thermosulfidooxidans subsp. asporogenes** [135]
Forms and size of cells (μm)	Rod-shaped, aggregates of cells: palisades, Y-shaped; 0.6–0.8 by 1.0–3.0	Rods, 0.9–1.0 by 3.0–6.0	Rods, 0.5–0.9 by 2.0–4.0, with rounded ends, occur singly, in pairs or short chains. Tend to form string-like cells
Cell wall	typical of Gram-positive bacteria, with capsular layer forms glycocalyx	no data, Gram-positive staining	typical of Gram-positive bacteria
Pili	+	no data	no data
Spore formation	+	+	–
Intracellular granules (Polyphosphate granules)	+	no data	+
Energy sources for growth in chemolithoautotrophic conditions			
Sulfide minerals	+	+	+
S^0	+	+	+
Fe^{2+}	+	+	+
Energy sources for heterotrophic/ mixotrophic growth	yeast extract, glucose, sucrose glutamate	yeast extract, glucose, starch, urea	yeast extract, glucose, glutatione, cysteine, casamine acids
Source of nitrogen	NH_4^+	NH_4^+	NH_4^+
pH range (optimum)	1.1–5.0 (1.7–2.4)	1.5–5.0 (2.0–2.7)	1.5–3.9 (1.6–1.8) on Fe^{2+} 2.0–4.5 (2.3–2.5) on S^0
Temperature range (optimum), °C	20–60 (50–55)	(38–42)	(~50)
Relation to O_2	strict aerobe	strict aerobe	strict aerobe
The DNA G+C content, moles %	47.2	49.34	45.5
Size of genome (Da)	$3.7 \cdot 10^9$	no data	$3.0 \cdot 10^9$

Genus Leptospirillum [141]. Vibrions, spiral-shaped, pseudococci. Motile by means of single polar flagellum (Fig. 1.11). Non-spore-forming. Gram-negative. Colonies on silica gel impregnated with medium with Fe^{2+}, small, red-brown colour due to the formation of ferric iron compounds. Strict aerobes. Obligate chemolithoautotrophs. Use Fe^{2+} and FeS_2 as energy source. Acidophilic. Widely occur in sulfide ore deposits. One species is known – **L.ferrooxidans** (Table 1.7).

Fig. 1.11. Cell of L.ferrooxidans on the medium with Fe^{2+}

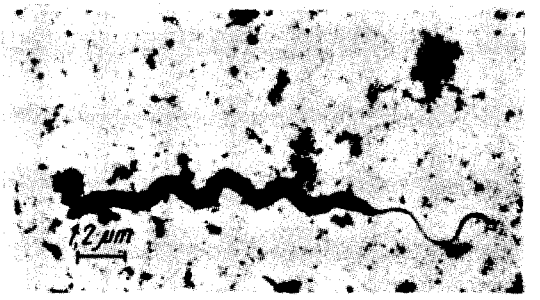


Table 1.7

Characteristics of Leptospirillum ferrooxidans strains

Characteristics	L.ferrooxidans [141, 170]	L.ferrooxidans INMI 17 [113]
Forms and size of cells (μm)	Vibrios 0.2–0.4 by 0.9–1.1, spiral-shaped, pseudococci, ball-shaped	Spiral-shaped, 0.3–0.4 in diameter, vibrio-shaped 0.3–0.4 by 1.0–1.8 form pseudococci 1.6–1.7 in diameter
Cell wall	typical of Gram-negative bacteria	typical of Gram-negative bacteria
Flagellation	+	+
pH range (optimum)	1.5–4.0 (2.5–3.0)	(1.9–2.4)
Temperature (optimum), °C	(30)	(40–45)
Relation to O_2	strict aerobe	strict aerobe
Energy source for growth in chemolithotrophic conditions		
Fe^{2+}	+	+
Pyrite	+	+
Sources of nitrogen	NH_4^+	NH_4^+
The DNA G+C content, moles %	no data	no data

Genus Sulfolobus [16, 34]. Spherical cells with lobes (Fig. 1.12), non-motile, flagella and endospores absent. Gram-negative. **Sulfolobus** and other

Fig. 1.12. Cell of Sulfolobus sp. on the medium with S^0

Table 1.8

Characteristics of thermoacidophilic archaebacteria involved in sulfur and iron cycles

Characteristics	Sulfolobus acidocaldarius [34, 150, 238]	Sulfolobus solfataricus [47, 150, 238]	Acidianus brierleyi [26, 28 193, 238]	Acidianus infernus [193]
Forms and size of cells (μm)	Cells are mainly spherical 0.8–1.0	0.8–2.0	1.0–1.5	0.8–2.0 (aerobic cond.) 0.5–1.5 (anaerobic cond.)
Cell wall structure and composition	Consists of glycoprotein subunits, S-layer murein layer is absent, Gram-negative			
Pili or branching	–	–	–	–
Sources of energy for growth in chemolithotrophic conditions:				
Sulfide minerals	–	–	+	–
S^0	+	+	+	+
Fe^{2+}	–	+	+	–
Substrates for heterotrophic/mixotrophic growth	yeast extract, triptone, pepton, casamino acids, casein hydrolysate, glutamine, alanine, asparagine, sucrose, ribose	yeast extract, glucose, xylose, sucrose, lactose, maltose, rhamnose	yeast extract, sucrose, lactose, mannitol, L-ketoglutarate, triptone, meat extract, peptone, casamino acids	–
pH range (optimum)	1.0–5.9 (2.0–3.0)	(3.0–5.0)	(1.5–2.0)	1.0–5.5 (2.0)
Temperature range (optimum), °C	55–80 (70)	50–89 (75–87)	45–75 (70)	65–96 (90)
Relation to O_2	aerobe	aerobe	aerobe	facultative anaerobe
The DNA G+C content, moles %	34.0–39.0	34.0–39.0	34.0–39.0	~31.0

archaebacteria have the following differential features: (1) unique structure of the transcription and translation apparatus, distinct from that found either in other prokaryotes (eubacteria) or in eukaryotes; (2) absence of murein in the cell wall; (3) unique structure of lipids which in archaebacteria, unlike other organisms, contain no glycerol ethers of fatty acids but consist mainly of isoprenoid and hydroisoprenoid hydrocarbons and isoprenil glycerol eithers. DNA-dependent RNA-polymerazes contain from 9 to 11 subunits. By the proteins number the ribosomes are closer to enkaryotic ones.

Colonies smooth, glistening, non-pigmented. Facultative chemolitho-autotrophs. Use elemental sulfur as energy source.

Growth and oxidation of sulfur occur or are enhanced only in the presence of yeast extract (0.01 to 0.02 %) as well as some other organic substances. May also use yeast extract, glutamate and other organic compounds as carbon and energy source.

Aerobic. May use Fe^{3+} in aerobic and Mo^{6+} in anaerobic conditions as an acceptor of electrons in oxidation of elemental sulfur.

Obligately thermophiles. Acidophilic. The DNA G+C content ranges from 60 to 68 moles %. Distributed in hot springs and ores. Type species: **Solfolobus acidocaldarius** Brock et al. [34]. Archaebacteria. Characteristics of species of the genus **Sulfolobus** are listed in Table 1.8.

Genus Acidianus [193]. Spherical cells, occasionally with lobes in the shape of tetrahedron, pyramid disk or saucer. The cell wall consists of subunits in hexagonal package, murein is absent. Cells are resistant to vancomycin, ampicillin and kanamycin. Lipids are represented by isoprenoid ethers. Facultative anaerobes. Lithotrophic growth occurs in aerobic conditions during oxidation of S^0 or Fe^{2+} and sulfide minerals, as well as in anaerobic conditions during S^0 reduction by molecular hydrogen. Grow in autotrophic or myxotrophic conditions. Thermoacydophilic. Grows in the presence of 0.1–4 % of NaCl. The DNA G+C content is about 31.0 moles %. Occurs in acid solfatares and marine hydrotherms. Type species **Acidianus infernus.** Two species in this genus are known: **A.infernus** and **A.brierleyi** (former **Sulfolobus brierleyi).** Characteristics are listed in Table 1.8.

Associates of T.ferrooxidans. Zavarzin [236] detected a heterotrophic associate in **T.ferrooxidans** culture, which were motile thin rods. The associate developed at pH 2.0 to 3.0 in the presence of Fe^{2+} in the cases when citric acid was added to the medium. Less active growth was observed on medium with sucrose, fructose, ribose, glucose, maltose, xylose, fumaric and succinic acids, mannitol and ethanol. Its taxonomical position is not clear. According to Zavarzin, it is similar to **Acetobacter acidophilum**, though it grows at lower pH values and forms less acetate from ethanol. Recently, an associate **T.acidophilus** was found by Arkesteyn [8] in stock cultures of **T.ferrooxidans** (ATCC) and Barros et al. and Harrison et al. [15, 88, 90] have shown that some stock cultures of **T.ferrooxidans** (ATCC) contain a heterotrophic associate, which has been isolated and named **Acidiphilium cryptum.** This is a Gram-negative aerobic, mesophilic, rod-shaped bacterium, forming no spores. Some strains are motile and possess one polar or two lateral flagella. Grows on poor organic media with pH from 1.9 to 5.9. Growth is severely inhibited by 0.01 % sodium acetate. Grows in medium with 0.5 % glucose at pH 3.5. The DNA G+C content is 68–70 moles %.

Four strains of **T.ferrooxidans** maintained in the National Collection of Industrial and Marine Bacteria (Great Britain) were also contaminated with acidophilic heterotrophic bacteria [108]. All heterotrophic acidophilic strains are Gram-negative motile rods, showing no growth on media with Fe^{2+} and sulfur. Their colonies on solidified tryptone-soya broth (TSB) are well

distinguished in size and colour. Sodium acetate (0.1 %) inhibits growth, however these bacteria grow in the medium containing 0.5 % glucose (pH 3.5). Tests for a possible presence of heterotrophic associates should be performed both when working with **T.ferrooxidans** pure cultures and in metal leaching.

1.5. MICROORGANISMS REDUCING SULFATES AND OTHER SULFUR COMPOUNDS

Sulfate-reducing bacteria widely occur in metal ore deposits. They grow both in anaerobic zones and in places of molecular oxygen intrusion in microzones where these bacteria are active under a layer of aerobic concomitants. Growth of sulfate-reducing bacteria is accompanied by the formation of large amounts of H_2S which facilitates metal precipitation and suppresses oxidation processes in dumps and orebodies. They also play an important role in metal precipitation in settling ponds enabling their use for waste water treatment and metal ions extraction.

These bacteria use organic substances and molecular hydrogen as donors of electrons, the electron acceptors being sulfate, elemental sulfur and some partially oxidized sulfur compounds (SO_3^{2-}, $S_2O_3^{2-}$), which are reduced to sulfide, as well as fumarate and nitrate [44, 177]. The sulfate-reducing bacteria include the genera **Desulfovibrio, Desulfotomaculum, Desulfomonas, Desulfobulbus, Thermodesulfobacterium, Desulfobacter, Desulfococcus, Desulfonema, Desulfosarcina, Desulfobacterium** [182].

By the type of organic substances oxidation included in the sulfate reduction process, sulfate reducers can be divided into two groups. The first group effects partial oxidation of organic compounds with formation of acetate by the reactions:

$$2\,CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2\,CH_3COO^- + 2HCO_3^- + H_2S, \qquad (1.17)$$

$$\Delta G_0^1 = -\,159.9\ kJ \cdot mole^{-1}$$

$$4\,CH_3COCOO^- + SO_4^{2-} + 4\,H_2O \longrightarrow 4\,CH_3COO^- + 4\,HCO_3^- + 2\,H^+ + H_2S \qquad (1.18)$$

$$\Delta G_0^1 = -\,341.2\ kJ \cdot mole^{-1}$$

As donors of electrons, they usually utilize lactate, pyruvate, malate, fumarate, some alcohols, such as ethanol, butanol, isobutanol, propanol, and molecular hydrogen [44, 177]. Most of these organic compounds are also used in biosynthetic processes. Some strains of **Desulfotomaculum nigrificans, Desulfovibrio vulgaris** and **Desulfovibrio** sp. are known to utilize sugar, e.g. glucose, fructose, galactose and ribose, in the process of sulfate reduction [44, 94, 137]. In general, however, utilizing sugars is not typical of the group of sulfate-reducing bacteria.

Growth on hydrogen is possible in the presence of acetate and carbon dioxide, which are required for synthesizing the cellular compounds [12, 202, 203].

$$4\,H_2 + SO_4^{2-} \rightarrow H_2S + 2\,H_2O + 2OH^- \qquad (1.19)$$

$$\Delta G_0^1 = -\,152.4\ \text{kJ}\cdot\text{mole}^{-1}$$

This group of bacteria includes representatives of the genera **Desulfotomaculum** (except for **D.acetoxidans**), **Desulfovibrio** (except for **D.baarsii**), **Desulfobulbus**, **Desulfomonas**, and **Thermodesulfobacterium**.

Besides sulfate-reduction, many sulfate-reducing bacteria are able to obtain energy by fermenting organic substances, most frequently choline and pyruvate.

Apart from that, the sulfate-reducing bacteria may grow on media without sulfates, performing the inter-species transport of hydrogen. Below is an illustration of the syntrophic growth of **Desulfovibrio** and methane-producing bacteria on media containing lactate and ethanol [40], what cannot be achieved by isolated microorganisms:

$$2\,CH_3CHOHCOO^- + H_2O \rightarrow 2\,CH_3COO^- + HCO_3^- + H^+ + CH_4 \qquad (1.20)$$

$$\Delta G_0^1 = -\,143.6\ \text{kJ}\cdot\text{mole}^{-1}$$

The second group include microorganisms which, apparently, possess the complete Krebs cycle and are thus able to completely oxidize organic compounds, including acetate, to carbon dioxide and water [25, 102, 205, 229–231]:

$$CH_3COO^- + SO_4^{2-} \rightarrow 2\,HCO_3^- + HS^- \qquad (1.21)$$

$$\Delta G_0^1 = -\,48\ \text{kJ}\cdot\text{mole}^{-1}$$

This group of microorganisms includes bacteria of the genera **Desulfobacter**, **Desulfococcus**, **Desulfosarcina**, **Desulfonema**, as well as **Desulfotomaculum acetoxidans** and **Desulfovibrio baarsii**.

Species of **Desulfococcus**, **Desulfonema**, **Desulfosarcina** and **Desulfovibrio baarsii** are able to utilize a wide range of organic substrates in the sulfate-reduction process: acetate, formate, propionate, butyrate, higher fatty acids (up to C_{14}–C_{18}), and are also known to ferment lactate and pyruvate. The growth of **Desulfonema limicola** and **Desulfosarcina variabilis** in autotrophic conditions has been observed in series of successive culture transfers in hydrogen and carbon dioxide atmosphere [227].

The taxonomy of sulfate-reducing bacteria, developed by Postgate [178] and Campbell [41], has been refined and several new genera and species have been included in this group of microorganisms [227]. Now, bacteria are divided into genera according to cell morphology, spore-forming ability, cytochrome composition, presence of desulfoviridine and other pigments. For species differentation, the following characteristics are taken to be of taxonomical significance: type of flagellation, relation to various organic substrates, DNA nucleotide content, resistance to chibitane, thermophily and halophily.

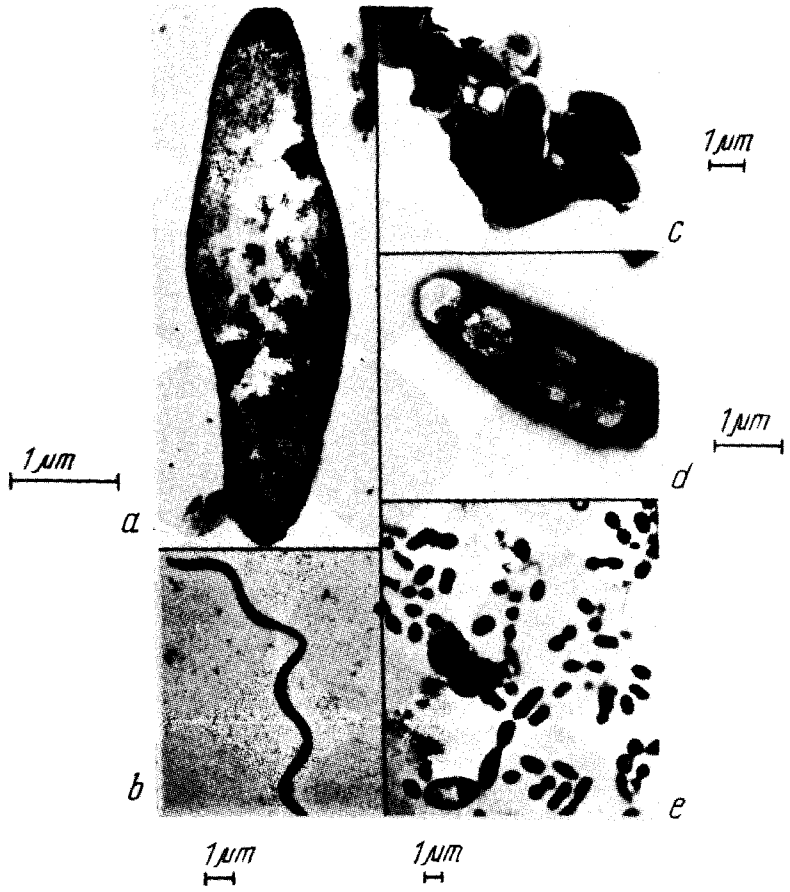


Fig. 1.13. Cells of sulfatreducing bacteria:
a – Desulfotomaculum nigrificans; b – Desulfovibrio africanus; c – Desulfovibrio baculatus; d – Desulfovibrio thermophilus; e – Desulfobulbus sp

1.5.1. Sulfate-reducing bacteria oxidizing organic substances to acetate

Genus Desulfovibrio. This genus comprises anaerobic, Gram-negative microorganisms, with no spore-forming ability, having vibrio-shaped **(D.desulfuricans, D.vulgaris, D.salexigens)**, sigma-shaped **(D.africanus)**, or spirilla-shaped **(D.gigas)** cells, and motile by means of one or more polar flagella (Table 1.9). The bacteria contain cytochromes c and b and desulfoviridin, an enzyme acting as bisulfite reductase and fluorescing in red light at 630 nm following exposure to ultra-violet radiation (365 nm) under alkaline conditions.

These microorganisms utilize few organic compounds. Universal substrates oxidized in the process of sulfate reduction are lactate and pyruvate only; malate, formate, ethanol, choline and some other organic substances can be utilized only by individual representatives of the genus **Desulfovibrio.**

Recently, new strains of non-spore-forming sulfate-reducing rod-shaped bacteria have been isolated (Fig. 1.13). They were assigned to different taxonomical groups. Rod-shaped bacteria with no desulfoviridin, **D.basulatus, D.thermophilus, D.baarsii** have remained in the genus **Desulfovibrio** [185, 186, 227]. A non-motile, rod-shaped bacterium, containing desulfoviridin and isolated by Moore et al. [153] has given rise to a new genus and species, **Desulfomonas pigra.** A thermophilic bacterium, close to **D.thermophilus,** has been assigned to a new genus and species **Thermodesulfobacterium commune** [237].

Further research into genetic relations of rod-shaped bacteria **D.baculatus, D.thermophilus, D.baarsii, D.pigra,** and **T.commune** is, apparently, needed before the existence of these genera and species can be unambiguously established [177].

D.baculatus, D.thermophilus, Thermodesulfobacterium commune and **Desulfomonas pigra** are also characterized by a narrow substrate specificity. **D.baarsii** is the only representative of the genus **Desulfovibrio** that is unable to utilize lactate or pyruvate, but grows on media with formate, acetate, propionate, butyrate, and higher fatty acids, which it completely oxidizes to CO_2 (Table 1.10).

Genus Desulfotomaculum. This genus includes spore-forming sulfate-reducing bacteria with Gram-negative staining, but with Gram-positive cell wall structure [158, 177]. The bacteria are rod- or lemon-shaped cells with peritrichous **(D.nigrificans, D.orientis, D.ruminis, D.antarcticum, D.guttoideum)** or polar **(D.acetoxidans)** flagellation, and contain cytochrome of types **b** and **c** Instead of desulfovirdine, cells of spore-forming sulfate-reducing bacteria contain bisulfite reductase with absopriton minimum at 582 nm, the pigment P_{582}. The genus includes the thermophilic species **D.nigrificans** and mesophilic **D.ruminis, D.orientis, D.antarcticum, D.acetoxidans,** and **D.guttoideum** (Tables 1.9, 1.10).

Representatives of this genus utilize, in the process of sulfate reduction, a limited range of organic substrates. In the case of **D.nigrificans,** these are lactate, pyruvate, ethanol and glucose; for **D.ruminis,** lactate, pyruvate and formate; for **D.orientis,** pyruvate and lactate; and for **D.acetoxidans,** only acetate. Besides, **D.nigrificans** and **D.ruminis** are able to grow in the absence of sulfates by fermenting pyruvate to acetate, CO_2 **and** H_2 (Table 1.9). Different species of **Desulfotomaculum** are able to oxidize organic substances to acetic acid. **D.acetoxidans** is the only representative of this genus that can oxidize acetate to CO_2 and H_2O [230].

Genus Desulfobulbus. The genus includes ellipsoid and lemon-like Gram-negative microorganisms, motile by means of a single polar flagellum, and having no spore-forming ability. The cells contain cytochromes of types **b** and **c**, and do not contain desulfoviridin. The genus currently includes two

Table 1.9

Characteristics of sulfate-reducing bacteria oxidizing organic substances to acetate

Genus and species	Cell shape	Flagellation	Cell size, μm	Desulfoviridin	Spore formation	NaCl required	G+C, mol %	Cytochromes	Substrates utilized in SO_4^{2-} reduction			Substrates utilized by fermentation	
									formate	malate	ethanol	pyruvate	choline
Desulfovibrio													
D. desulfuricans	Vibrios, spirilla	+	0.5–1 by 3–5	+	–	–	55.3±1	c_3	–	+	–	+	+
D. vulgaris	vibrios, spirilla	+	0.5–1 by 3–5	+	–	–	61.2±1	c_3	+	–	+	–	–
D. salexigens	vibrios, spirilla	+	0.5–1 by 3–5	+	–	+	46.1±1	c_3	–	+	–	–	–
D. africanus	sigma-shaped rods	+	0.5 by 5–10	+	–	–	61.2±1	c_3	–	+	–	–	–
D. gigas	spirilla	+	1.2–1.5 by 5–10	+	–	–	60.2	c_3	no data	–	no data	–	–
D. sapovorans	sigma-shaped, bent rods	+	1.5 by 3–5.5	–	–	–	52.7	**b, e**	–	–	–	+	no data
D. baculatus	rods	+	0.5–0.6 by 0.4–1.4	–	–	–	56.8±0.4	**b, c**	+ (with acetate)	+	–	–	–
D. thermophilus	rods	+	0.6 by 2	+	–	–	no data	c_3	+ (with acetate)	–	–	–	–
Desulfomonas													
D. pigra	rods	–	0.8–1 by 2.5–10	+	–	–	67.0	no data	–	no data	–	+	no data
Desulfobulbus													
D. propionicus	ovoid lemon-shaped rods	+	1–1.3 by 1.8–2	–	–	–	59.9	**b, c**	–	–	+	+	–
D. elongatus	ovoid lemon-shaped rods	+	0.5–0.7 by 1.5–2.5	–	–	–	59.0	**b, c**	–	–	+	+	no data
Thermodesulfobacterium													

T. commune	rods	–	0.3 by 0.9	–*	–	–	34.4	c_3	no data	–	–	+	no data
Desulfotomaculum													
D. nigrificans	rods	+	0.3–0.5 by 3–6	–	+	–	44.7	b	–	no data	+	+	no data
D. ruminis	rods	+	0.5 by 3–6	–	+	–	45.5	b	+	no data	–	+	no data
D. orientis	rods	+	1.5 by 5	–	+	–	41.7	b	–	no data	–	–	no data
D. antarcticum	rods	+	1.0–1.2 by 4–6	–	+	–	no data	b	–	no data	no data	+	no data
D. guttoideum	rods	+	1.0 by 2–3	–	+	–	51.6	c	–	–	–	+	no data

* – Contains desulfofuscidin.

Table 1.10

Characteristics of sulfate-reducing bacteria oxidizing organic substances to CO_2

Genus and species	Cell shape	Flagellation	Cell size, μm	G+C, mol. %	Desulfoviridin	Cytochromes	Substrates utilized in SO_4^{2-} reduction						Substrates utilized by fermentation	
							formate	alcohols C_2–C_4	lactate	benzoate	H_2+CO_2	higher fatty acids up to C_{14}	lactate	pyruvate
Desulfobacter														
D. postgatei*	Rod-shaped, ellipsoid	+	1–2 by 1.7–3.5	45.9	–	b, c	–	–	–	–	–	–	–	–
D. hydrogenophilus	oval, ellipsoid	+	1–1.3 by 2–3	44.6	–	+	–	+(C_2)	no data	no data	+	–	no data	no data
D. latus	oval, rod-shaped	+	1.6–2.4 by 4–7	43.8	–	+	–	–	no	no data	–	–	no data	no data
D. curvatus	vibrions	+	0.5–1 by 1.7–3.5	46.1	–	+	–	+(C_2)	no data	no data	+	–	no data	no data
Desulfococcus														
D. multivorans*	spherical	–	1.5–2.2	57.4	+	b, c	+	+	+	+	–	+	+	+
D. niacini**	spherical	+	1.5–3.0	45.8	–	b	+	+	–	–	+	+(C_{16})	–	–
Desulfonema														
D. limicola*	rods arranged in filaments	–	2.5–3 by 2.5–3.5	34.5	+	b, c	+	–	+	–	+	+	–	–
D. magnum	rods arranged in filaments	–	6–8 by 9–13	41.6	–	b, c	+	–	–	+	–	+(C_{10})	–	–
Desulfosarcina														
D. variabilis*	rod-shaped, ellipsoid, in packets	±	1–1.5 by 1.5–2.5	no data	–	no data	+	+	+	+	+	+	+	+
Desulfovibrio														
D. baarsii*	bent rods sigma-shaped	+	0.5–0.7 by 1.5–4	no data	–	no data	+	–	–	–	–	+	–	–

Desulfotomaculum														
D. acetoxidans*	rods	+	1–1.5 by 3.5–9	37.5	–	**b**	+	+	–	–	–	–	–	–
D. sapomandens	rods	+	1.2–2.0 by 5–7	48.0	no data	no data	+	+	–	+	no data	+	–	–
Desulfobacterium* **														
D. indolicum	rods, ovoid cells	+	0.7–1.5 by 2–2.5	47.4	–	b, c	+	+			–			
D. phenolicum	bent rods		1–1.5 by 2–3	40.6	–	no data	+	+		+	–			
D. autotrophicum	ovoid	+	1–1.5 by 1.5–2.5	42.6	–	b, c	+	+	+	–	+	+	+	+
D. catechlicum	ovoid to lemon-shaped	–	1.3–1.8 by 2.2–2.8	52.4	–	+	+	+	+	+	+	+	+	+

* [182, 227]
** [102]
*** [101]

species, **D.propionicus** and **D.elongatus** (Table 1.9). Type strains of **D.propionicus** and **D.elongatus** carry out sulfate reduction by oxidizing lactate, propionate, ethanol, propanol, and pyruvate, and form acetate as the oxidation end product. When grown in the molecular hydrogen atmosphere, they require added acetate and CO_2 for anabolic process. **D.propionicus** has a unique capacity to utilize nitrates, in addition to sulfates (SO_3^{2-}, $S_2O_3^{2-}$), as electron acceptors. Nitrates are stoichiometrically reduced to ammonium. In media lacking both sulfates and nitrates, these two organisms are able to ferment lactate and pyruvate for their energy needs [189, 227].

1.5.2. Sulfate-reducing bacteria oxidizing organic substances to CO_2

Genus Desulfobacter. The genus includes three strains of Gram-negative sulfate-reducing bacteria belonging to the same species **D.postgatei.** Bacteria are ellipsoid or rod-shaped, and either non-motile or motile by means of a single polar flagellum. The type strain of **D.postgatei,** "Dangast" 2ac9, is able to oxidize only acetate, while the two other strains can utilize lactate and ethanol as well (Table 1.10). In this process, sulfate and other oxidized sulfur compounds serve as electron acceptors. Cytochroms of **b**- and **c**-types were found in the cells, but no desulfoviridin was detected [231].

Genus Desulfococcus. The genus is represented by spherical Gram-negative bacteria, which are sometimes motile by means of a single polar flagellum. The genus includes two species, **D. multivorans** and **D.niacini,** which are able to utilize a wide range of substrates in the sulfate reduction process. Bacteria oxidize pyruvate, formate, acetate, propionate, butyrate, lower alcohols (ethanol, propanol, butanol), and higher fatty acids (up to C_{14}). In the absence of sulfates, **D.multivorans** is able to grow by fermenting lactate and pyruvate. Specific enrichment of **D.multivorans** culture can be obtained on media with benzoate, and of **D.niacini,** on media with nicotinic acid. Bacteria contain cytochromes of type **b**. **D.multivorans** was shown to contain cytochrome **c** and desulfoviridin (Table 1.10) [102, 227].

Genus Desulfonema. The genus includes rod-shaped sulfate-reducing bacteria which form filaments as long as a few millimeters. The filaments are generally attached, but can occasionally be observed moving by gliding. Though cells exhibit Gram-positive staining, they were shown to have a Gram-negative cell wall structure [229].

Complete oxidation of formate, propionate, butyrate, and higher fatty acids (with carbon chains of 10 to 14 atoms) to carbon dioxide is carried out by **D.limicola** and **D.magnum** in the process of reduction of sulfates or other oxidized sulfur compounds. Slow growth on medium with acetate can be improved by adding succinate, higher fatty acids, or extract of anaerobically degradated organic matter of sewage. None of the two organisms is capable of fermenting organic substances (Table 1.10).

D.limicola contains cytochromes **b** and **c**, as well as desulfoviridin. Cells of **D.magnum** were found to contain cytochromes **b** and **c** as well as sulfite reductase P_{582}, which is typical of spore-forming sulfate-reducing bacteria of the genus **Desulfotomaculum.**

Genus Desulfosarcina. The genus includes Gram-negative sulfate-reducing bacteria forming cell packets. Bacteria in packets have irregular shape, while non-aggregated cells are rod-shaped and ellipsoid. Motile cells with one polar flagellum can also be observed sometimes.

The genus is represented by a single species, **D.variabilis,** characterized by an extremely wide range of substrates it can utilize. In sulfate reduction process, this bacterium oxidizes alcohols (ethanol, propanol, butanol), lower acids (formate, acetate, propionate, butyrate), higher acids (up to C_{14}), lactate, pyruvate, succinate, fumarate, benzoate, etc., and can grow in autotrophic conditions in the H_2+CO_2 atmosphere. In the absence of sulfates, **D.variabilis** ferments lactate, pyruvate and fumarate (Table 1.10).

The cells contain no desulfoviridin. The cytochrome composition and the DNA G+C content of the bacterium have not been established yet.

1.6. MICROORGANISMS REDUCING AND OXIDIZING ELEMENTS OTHER THAN SULFUR AND IRON

Processes of reduction and oxidation of a number of elements with variable valency can be carried out both by autotrophic and by heterotrophic microorganisms. In the latter case these elements are either acceptors of electrons or oxidized under the impact of microorganisms metabolism.

1.6.1. Bacteria oxidizing As^{3+} [1, 2, 96]

Several strains of **Pseudomonas putida** and **Alcaligenes eutrophus** have been isolated from gold-arsenic deposits and are used to remove arsenic from industrial effluents (Chapter 7). They oxidize As^{3+} to As^{5+} in the presence of organic substances. Oxidation of As^{3+} is supposed to be carried out by atomic oxygen produced during decomposition of bacteria-synthesized peroxide by catalase.

These reactions may be represented by the following equations:

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + \frac{1}{2}\ O_2;\ AsO_2^- + \frac{1}{2} O_2 \rightarrow AsO_3^- \qquad (1.22)$$

The autotrophic bacterium **Pseudomonas arsenitoxidans** has been isolated from gold-arsenic deposit waters. It can autotrophically oxidize As^{3+} to As^{5+} utilizing atmospheric CO_2 as the carbon source. Oxidation of As^{3+} occurs by the equation:

$$NaAsO_2 + H_2O \rightleftharpoons NaAsO_3 + 2H^+ + 2e \qquad (1.23)$$

As a result of this reaction pH of the medium falls down to 6.0.

Characteristics of these bacteria are listed in Table 1.11.

Characteristics of bacteria oxidizing As^{3+}

Characteristics	**Pseudomonas putida [1]**	**Alcaligenes eutrophus [2]**	**Pseudomonas arsenit-oxidans [96]**
Forms and size of cells (μm)	Rods, 0.6 by 1.5	Rods, 0.6 by 2.0, occasionally forming chains	Rods, 0.4 by 1.2–1.5
Surface structures	no data	no data	capsula
Flagellation	+	+(peritrychous)	+
Gram staining	–	–	–
pH range (optimum)	4.0–10.5 (7.0–8.5)	4.0–10.5 (6.0–9.0)	6.0–8.5 (7.5–8.0)
Temperature range (optimum), °C	4–28	(28)	(28–35)
Relation to O_2	aerobe	aerobe	aerobe
Energy and carbon sources	arabinose, xylose, rhamnose, glucose, fructose, galactose, succrose, maltose, raffinose, ethanol, mannitol	organic substances, H_2, CO_2	As^{3+} CO_2
Sources of nitrogen	NH_4^+	NH_4^+	NH_4^+
Catalase activity	+	no data	–
Colonies on meat infusion agar	smooth, glistening, brown	no data	small, spot-like colonies on mineral medium with As^{3+}

1.6.2. Bacteria reducing Cr^{6+} [129, 131, 183]

Pseudomonas dechromaticans has been isolated from active sludge of a bioregenerator in which municipal waste water and chromate-containing industrial effluents were treated. **Pseudomonas chromatophila** has been isolated from water taken from the bottom of an open pit in the Kempirsay chrome-iron deposit and from industrial effluents. **Aeromonas dechromatica** has been isolated from industrial effluents of a machine-tool factory. These bacteria are able to utilize chromates and bichromates as acceptors of electrons when grown anaerobically on organic media. As a result Cr^{6+} is reduced to Cr^{3+} which precipitates as $Cr(OH)_3$. This led to the development of a microbiological method of industrial effluents treatment for chrome removal (see Chapter 7).

Characteristics of **P.dechromaticans, P.chromatophila** and **A.dechromatica** are listed in Table 1.12.

P.dechromaticans. Colonies on meat infusion agar are initially small and watery with pink edges. Later colonies grow in size, turn light pink, become pasty in consistency and are slightly raised at the centre.

Weak growth on potato slices, the cratch is dense and knobby, coloured from light pink to reddish in old cultures. Growth on beef-extract broth gives rise to turbidity.

Table 1.12

Characteristics of Pseudomonas dechromaticans, Pseudomonas chromatophila and Aeromonas dechromatica

Characteristics	P.dechromaticans [182, 183]	P.chromatophila [131]	A.dechromatica [129]
Forms and size of cells (μm)	Rods, 0.4 by 1.5	Rods, 0.4 by 6–8	Coccoid and rod-shaped shaped cells, 0.5–1.6 to 15
Flagellation	+	+	+ (1–10)
Gram staining	–	–	(+) cell wall is Gram-negative
pH (optimum) range	(7.7–8.3)	(7.0–8.5)	5.0–9.0 (6.8–7.0)
Temperature range (optimum), °C	(20–25)	17–42 (27)	4–45 (34)
Relation to O_2	facultative anaerobe	facultative anaerobe	facultative anaerobe
Denitrification ability	+	–	+
Energy and carbon sources	organic substances in anaerobic condition in the presence of Cr^{6+}	organic substances in anaerobic condition in the presence of Cr^{6+}	organic substances in anaerobic condition in the presence of Cr^{6+}
Hydrolysis of gelatine and starch	–	–	+
Sources of nitrogen	NH_4^+	NH_4^+	NH_4^+
Catalase formation	no data	+	–
The G + C content of the DNA, moles %	no data	67±0.3	59±0.2
Relation to NaCl	no data	growth at 1.0 % concentration of salt in medium	growth only in the presence of NaCl – 3 % is the optimum concentration

In the presence of Cr^{6+}, this species can grow on media with peptore glucose, ethanol, etc. Anaerobically, it can also grow on media with ethanol.

P.chromatophila. Colonies on beef extract agar and starch agar are colourless with even edges, slightly raised at the centre, pasty in consistency, 1.0 to 1.5 mm in diameter.

Grows both in aerobic and anaerobic conditions on the following organic substances: ribose and fructose; sodium salts of benzoate, fumarate, lactate, acetate, succinate; malate and tartrate. Low-molecular alcohols are not utilized.

A.dechromatica. Colonies on nutrition agar are small, transparent, smooth, glistening with uneven ends, with age (6 days) they become yellow. The growth on liquid media: dispersable with turbulance of medium, the colour of medium containing Cr^{6+} changes from yellow to slightly blue. Metabolism is respirative and fermentative. It ferments glucose, hydrolizes casein, starch and gelatin, coagulates milk, does not produce acetoin from glucose. Utilizes galactose, mannose, mellibose, sucrose, fructose, lactose, cellobiose, arabinose, mannitol, dulcit, sorbitol, glycerol.

1.6.3. Microorganisms reducing and oxidizing manganese

Microbiological leaching of manganese (Mn^{4+}) involves its reduction to the bivalent form. As can be seen from Table 1.13, this capacity is found with microorganisms of different taxonomic groups. Characteristics of these microorganisms can be found in existing manuals [16–18]. The reduction of Mn^{4+} may occur either via the enzymatic route or by the action of various metabolites, e.g., organic acids, peroxides and some other [46, 56].

Enzymatic reduction. A strain of **Bacillus** sp. 29 was studied by Trimble and Ehrlich and his coworkers extensively. They have shown that this organism reduces MnO_2 using glucose as an electron donor [210].

Table 1.13

Manganese-leaching bacteria

Microorganisms	Reference
Freshwater and sediments	
Bacillus macerans **Bacillus subtillis** **Clostridium felsineum** **Enterobacter aerogenes** **Proteus vulgaris** **Bacillus cereus** **Bacillus circulans** **Bacillus mycoides** **Bacillus polymyxa**	[212–214]
Pseudomonas fluorescens **Escherichia coli**	[209]
Oceanic and marine sediments	
Arthrobacter sp. **Arthrobacter strain B**	[3, 4, 38]
Bacillus spp.	[62, 167, 210]
Acinetobacter calcoaceticus	[234]
Pseudomonas herbicola sp. 10*, **Aeromonas sp. 125**	[187]
Rhodopseudomonas sphaeroides**	[50]

at present: *not recognized [17, 18]
**Rhodobacter sphaeroides [101]

The MnO_2 reduction was also studied with resting-cell suspensions and cell-free extracts of **Bacillus sp. 29** [46, 211]. It was postulated from the studies that an inducer in the form of Mn^{2+} or Mn^{4+} is necessary to reduce MnO_2. The reactions are summarized as follows:

$$\text{Glucose} \xrightarrow{\text{bacteria}} n\bar{e} + nH^{+} + \text{end products} \qquad (1.24)$$

$$n/2MnO_2 + n\bar{e} + nH^+ + \xrightarrow[\text{uninduced bacterial enzymes}]{\text{manganese induced bacterial enzymes or}} n/2\ Mn(OH)_2 \quad (1.25)$$

+ferri – or ferrocyanide

$$n/2\ Mn(OH)_2 + nH^+ \longrightarrow n/2\ Mn^{2+} + nH_2O \quad (1.26)$$

Several marine bacteria were shown to reduce MnO_2 by a similar mechanism [61, 62].

In these reactions, MnO_2 serves as a terminal electron acceptor and has a link with the electron transport system. It has been suggested that cytochromes b, c and o as well as metalenzyme may be involved in the reduction of MnO_2 by the probable route:

$$\text{glucose} \rightarrow \text{cyt. b} \rightarrow \text{cyt. c} \begin{cases} \rightarrow \text{cyt. o} \rightarrow O_2 \\ \rightarrow \text{ME} \rightarrow MnO_2 \end{cases}$$

Several microorganisms, e.g. **B.polymyxa** [154, 175, 176], have been shown to reduce Mn^{4+} via the nitrate reductase route is dissimilatory nitrate-reduction process. Our study has shown that **A.calcoaceticus** is able to reduce Mn^{4+} by the action of assimilatory nitrate reductase [119].

Non-enzymatic reduction of manganese. Some bacteria and probably many fungi reduce MnO_2 indirectly through due to production of various metabolites.

Formic and oxalic acids are most active manganese-reducing agents, which react with MnO_2 according to the following equations [59]:

$$MnO_2 + HCOOH + 2H^+ \rightarrow Mn^{2+} + 2H_2O + CO_2 \quad (1.27)$$

$$MnO_2 + HOOCCOOH + 2H^+ \rightarrow Mn^{2+} + 2H_2O + 2CO_2 \quad (1.28)$$

This mechanism of Mn^{4+} reduction is the most efficient among those considered above in leaching this element [234].

A peroxide mechanism of manganese reduction and oxidation has also been described [56, 118, 221]. It has been demonstrated to occur in many microorganisms. This pathway of Mn^{4+}-reduction depends on the activity of bacterial catalase and on some environmental factors, such as the organic substrate concentration. In acid and neutral media, Mn^{4+} is reduced by H_2O_2 by the reaction $Mn^{4+} \rightleftharpoons Mn^{2+}$. However, there are also other routes of manganese reduction.

For example, in the presence of Fe^{2+} and under anaerobic conditions, the following reaction has been shown to take place [56, 221]:

$$Mn^{4+} + Fe^{2+} \xrightarrow{\text{chemical}} Mn^{2+} + Fe^{3+} \quad (1.29)$$

The MnO_2 potential in acid medium is known to be 1.23 V. So, in acid media, the reduction of Mn^{4+} by ferrous iron can also proceed under aerobic or microaerophilic conditions. The reduction of Mn^{4+} was shown to be accelerated by addition of sulfide minerals, sulfur, hydrogen sulfide, and gaseous sulfur dioxide [100, 116, 155]. These processes are also facilitated by the presence of **T.thiooxidans.** It has been suggested that **T.thiooxidans,** oxidizing sulfur, can use manganese dioxide as electron acceptor [223].

Mn^{4+} is reduced to Mn^{2+} by hydrogen sulfide, and to MnS_2O_6 by gaseous sulfur dioxide. Manganese dithionate is oxidized by **T.thiooxidans** to $MnSO_4$.

When leaching manganese from ores it should be taken into account that solubilized Mn^{2+} under appropriate conditions may be oxidized by microorganisms to Mn^{4+} and precipitated.

Direct or enzymatic oxidation of manganese. In bacteria this process takes place by three different mechanisms. Two of these mechanisms involve oxidation of free Mn^{2+} ions and one involves oxidation of Mn^{2+} ions pre-bound to Mn^{4+} oxide.

Mechanism 1 – Free Mn^{2+} ions are catalyzed by constitutive or inducible manganese oxidases which transfer electrons to oxygen via cytochromes.

$$Mn^{2+} + 1/2\ O_2 + H_2O \rightleftharpoons MnO_2 + 2H^+ \qquad (1.30)$$

Mechanism 2 – Free Mn^{2+} ion oxidation is catalyzed by a catalase in a reaction with metabolically produces H_2O_2:

$$Mn^{2+} + H_2O_2 \rightleftharpoons MnO_2 + 2H^+ \qquad (1.31)$$

Mechanism 3 – The Mn^{2+} pre-bound to Mn^{4+} is oxidized with a manganese oxidase that shunts electrones to oxygen by cytochrome pathway. This enzyme is always constitutive and cannot oxidize free Mn^{2+} ions:

$$Mn{\cdot}MnO_3 + 3/2\ O_2 + 2H_2O \rightleftharpoons 2H_2 \cdot MnO_3 \qquad (1.32)$$

The enzymatic mechanism of Mn^{2+} oxidation was found in **Sphaerotilus discophorus** (now known as **Leptothrix discophora).** The free energy change at pH 7.0 for the above reaction is -16.3 kcal·mole^{-1} [58] which is enough to generate at least one ATP molecule.

Arthrobacter strain B carries out the oxidation process by reaction mechanism 1 and the enzymes are probably located in cytoplasmic membrane [60].

In **Arthrobacter** sp. No. 37, isolated from deep sea sediments and ferromanganese nodules, a constitutive enzyme was found which oxidizes pre-bound Mn^{2+} ($Mn{\cdot}MnO_3$). This organism cannot oxidize free Mn^{2+} ions, unless they are first bound to Mn^{4+} oxide in the form of MnO_2 or a synthetic Mn·Fe oxide or a substance from the nodule [57]. The reaction proceeds in two stages and the second stage is as per Mechanism 3. The enzyme system of this organism has been characterized by Ehrlich [58]. Cytochrome **c** was found to be involved in Mn^{2+} oxidation in two different strains of **Oceanospirillum** sp. [7].

The coupling to Mn^{2+} oxidation has been observed in **Arthrobacter** sp. No. 37 and six other marine bacteria by Ehrlich [58]. All these organisms, mentioned above, are heterotrophs.

The enzymatic Mn^{2+} oxidation was demonstrated in cell-free extracts of **Pseudomonas** sp. and **Citrobacter freudii** [51, 52]. Dubinina [56] has reported that **Leptothrix pseudoochracea, Arthrobacter siderocapsulatus,** and **Metallogenium** oxidize Mn^{2+} via H_2O_2 catalase (Mechanism 2).

Non-enzymatic oxidation of Mn^{2+}. Some groups of organisms produce metabolic products which cause chemical oxidation of Mn^{2+} [36, 37, 109, 222]. MnO_2 is frequently precipitated on fungal hyphae and zygospores of an alga **Chlamidomonas** [192] on cells of **Metallogenium, Caulococcus, Kuznezovia, Pedomicrobium, Hyphomicrobium, Siderocapsa** (now **Arthrobacter), Naumaniella** [60].

1.7. MICROORGANISMS SOLUBILIZING, ACCUMULATING AND PRECIPITATING NATIVE GOLD

A wide range of microorganisms and their metabolites are capable of solubilizing native gold. The representatives of genera **Bacillus, Pseudomonas** and other amino acids-producing bacteria [125, 126, 166] are the most efficient.

Amino acids and proteins synthesized by bacteria in alkaline medium (pH 9–10) produce an Au-N bond via the atom of the amino group. Complexes of anionic character are predominant. Solubilization occurs in the presence of oxidants, for example, H_2O_2:

$$Au^0 \xrightarrow[\text{amino acids}]{H_2O_2} \text{Au-N} \qquad (1.33)$$

Oxidants (peroxides) may be formed by the microorganisms. However, in practice it is preferable to introduce oxidants from the outside. In acidic medium amino acids reduce ionic species of gold to metal state producing the so-called "new" gold.

Accumulation and precipitation of gold is performed by bacteria, yeasts, fungi and microscopic algae [22, 125, 152, 191].

1.8. MICROORGANISMS INVOLVED IN DEGRADATION OF SILICATES

1.8.1. Mechanism of destruction of silicates

Microbiological destruction of silicates is studied with the aim to remove silicon from low-grade mineral raw materials (e.g. bauxites) or to extract valuable components (Al, Ti, U, Au and others) from the silicate and aluminosilicate ores and minerals.

Mechanisms underlying the impact of microorganisms upon silicates have not been studied. According to the existing data [20, 148, 197] the chemical

destruction of silicates is based on hydrolysis processes that can be generally described by the following reaction:

$$M^+ [\text{mineral}]^- + H^+OH^- \rightleftharpoons H^+ [\text{mineral}]^- + M^+OH^- \qquad (1.34)$$

where:

$M^+ [\text{mineral}]$ – is the initial mineral,

$H^+ [\text{mineral}]$ – is the weathered mineral,

M^+ – is the cation.

The above equation stands for an equilibrium reaction which makes clear that any process altering the concentration of reagents or end product will influence the hydrolysis reaction.

Experiments have proved that destruction of silicates occurs more actively in the presence of microorganisms [9, 20, 197]. Several microorganisms are known to be active producers of mineral and organic acids as well as complexing agents. A supposition can be made that the mechanism of microbial destruction of silicates is an indirect one and is related to activization of hydrolisis and formation of complexes.

1.8.2. Characteristics of microorganisms involved in destruction of silicates

Representatives of different taxonomic groups of microorganisms are involved in destruction of silicate and aluminosilicate minerals [9, 20, 197]: **Autotrophic microorganisms:** diatomaceous algae, **Thiobacillus sp., Thiobacillus thioparus, Thiobacillus thiooxidans, Nitrosospira briensis, Nitrobacter vinogradskyi; heterotrophic microorganisms: Aspergillus niger, Cephalosporium sp., Fusarium sp., Mucor sp., Penicillium glaucum, Penicillium brevicaule, Penicillium expansum, Penicillium notatum, Penicillium simplicissimum, Scopulariopsis brevicaule, Trichoderma lignorum, Spicaria sp.; Bacillus circulans, Bacillus megatherium, Bacillus mucilaginosus, Bacillus subtilis, Mycobacterium sp., Proteus mirabilis, Pseudomonas sp.; populations of microorganisms:** populations of soil microorganisms; **symbiotic cultures:** lichens. As a rule, these are well-known microorganisms described in existing manuals [16–18, 71, 180, 206]. **B.mucilaginosus** is an exception. This bacterium is close to species **B.circulans** and **B.polymyxa.** However, according to the existing data [11] it cannot be attributed to either of the above species. Since this species is widely used in laboratory studies and is not included in Bergey's Manual [16–18], its detailed description is listed below.

B.mucilaginosus. Isolated from different soils and described by Aleksandrov and Zak [5]. Consequently this species was isolated from rocks by other authors [11]. Detailed characteristics can be found in Table 1.14 and on Fig. 1.14.

Grows well on synthetic media with carbohydrates and nitrogen compounds, on potato agar. Complete cycle of development resulting in sporula-

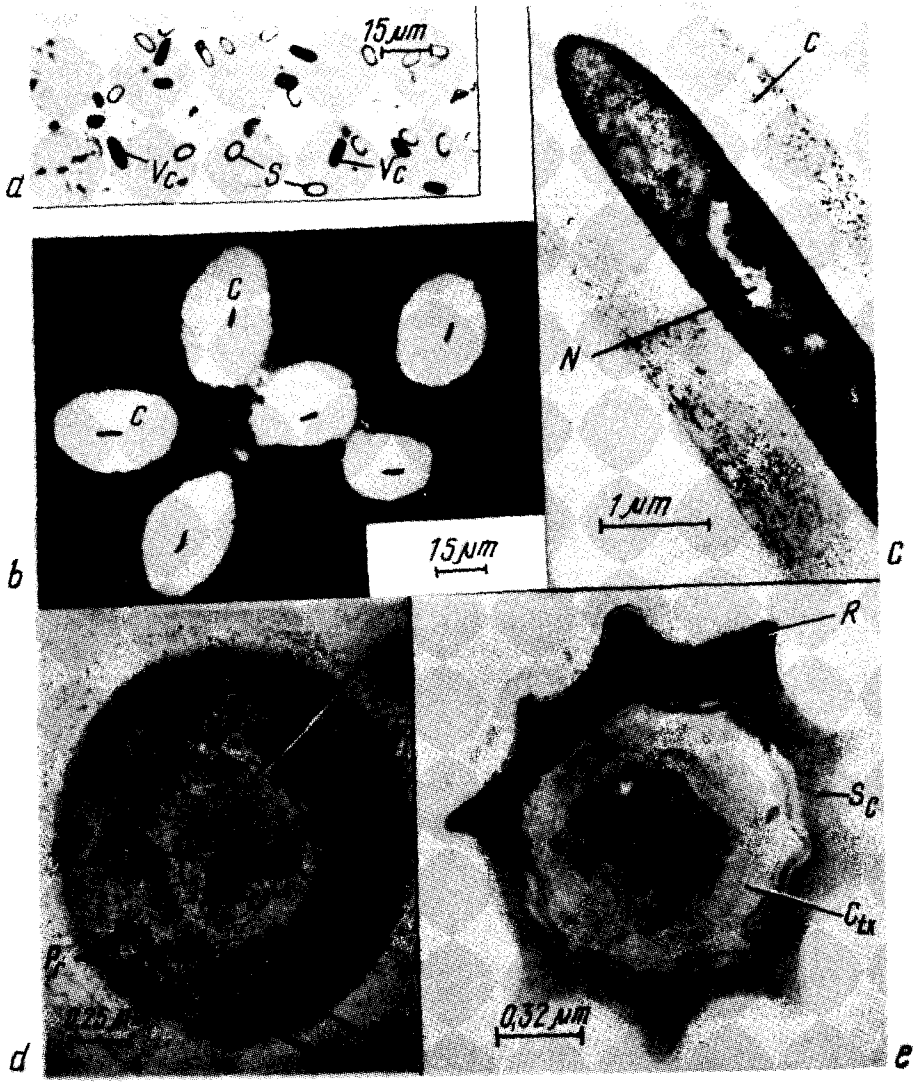


Fig. 1.14. B.mucilaginosus cells and spores:
a – vegetative cells and spores (24-hour culture on potato agar, phase-contras); S – spores, Vc –vegetative cells; b – vegetative cells and capsules (48-hour culture on Eshby medium, Indian ink colouring, phase-contrast): C – capsules; c – d – thin-sections of vegetative cells: C – capsule; Mc – microcapsule; CW – cell wall; Pr – polyribosomes; N – nucleoid; e – thin-section pf spores; Ctx – cortex; R – regular ridges, Sc – spore coats

Table 1.14

Characteristics of B.mucilaginosus BKM 1480 D [11]

Characteristics	B. mucilaginosus BKM 1480 D
Forms and size of cells (μm)	Rods, 1.0–1.2 by 4–7
Motility	–
Forms and size of spores (μm)	oval-shaped, 1.1 by 1.7–2
Comb-shaped excrescences	+
Spores location in sporangia	central, subterminal
Temperature (optimum), °C	10–42 (37–40)
pH range (optimum)	5.5–9.5 (7.5–9.0)
Relation to O_2	aerobe
Growth on media with NaCl:	
1 %	+
2 %	–
Formation of:	
crystalline dextrins	–
dehydroxyaceton	–
Denitrification	–
Hydrolysis of:	
caseine	–
gelatine	–
starch	+
Sources of nitrogen	NH_3^+, urea
Utilization of:	
citrate	+
fumarate	–
Acids formation from:	
L-arabinose	+
D-mannose	+
sorbytol	+
The G + C content of the DNA, moles %	59

tion occurs when grown on the above media. Spore formation is inhibited when grown on Ashby medium. No growth on nutrient broth, nutrient agar and nutrient gelatin.

Colonies on potato agar: light gray, smooth, with even edges, moist, glistening.

Growth on potato slice: abundant, light gray.

Colonies on Ashby medium: convex, semi-transparent, slimy, even-edged, of viscous consistency, up to 10 mm in diameter.

Does not curdle of coagulate milk through weak growth and slime formation are observed.

Does not form indole, does not form H_2S in the presence of thiosulphate.

Utilizes as a sole carbon and energy source the following compounds: starch, cellobiose, glucose, sucrose, fructose, arabinose, galactose, lactose, xylose, maltose, trehalose, raffinose, sorbose, sorbitol, inositol, mannitol, glycerol, malonate, citrate, lactate.

Does not utilize: cellulose, galactose, ethanol, butyl alcohol, arabitol, formate, acetate, propionate, butyrate, oleate, succinate, tartarate, α-ketoglutarate.

Growth in the presence of carbohydrates and polyatomic alcoholes is accompanied by formation of organic acids, gas is not produced.

Does not form nitrite from nitrate under aerobic conditions, does not utilize nitrite as electron acceptor under anaerobic conditions.

1.9. MICROORGANISMS ACCUMULATING METALS FROM SOLUTIONS

Microorganisms are able to accumulate cations of different metals extracting them from solutions. Both living and non-living cells are active in metals accumulation. This may be used both for extraction of metals from solutions and for industrial effluents treatment for metal recovery. Activity and selectivity of metal recovery processes are determined by physiological properties of microorganisms, characteristics of elements, as well as by physical and chemical factors of the medium (e.g., pH).

Two major mechanisms of accumulation can be classified as follows:

1) binding ions of metals by cell surface components (biosorption);

2) intra-cellular accumulation related to active and passive transport of metal cations.

The amount of the sorption elements may total 15–30 % of the biomass dry weight [43, 194, 195, 217, 218]. Table 1.15 lists species most active in metals accumulation. These are well-known microorganisms the characteristics of which can be found in existing manuals [16–18 et al.]. The given list illustrates that microorganisms of different taxonomic groups are able to accumulate metals. However, most listed species are known to accumulate single elements. There are grounds to suppose that further research will not only enlarge the list of microorganisms active in metals sorption but will also prove that cations extracted by concrete species are not limited to those listed in Table 1.15.

Table 1.15

Microorganisms, active in sorption and accumulation of metals

Microorganisms	Metal cations	References
Autotrophic bacteria		
Thiobacillus ferrooxidans	Cd^{2+}, Co^{2+}, Cu^{2+}, Cr^{2+}, Fe^{3+}, Ni^{2+}, UO_2^{2+}, Ag^{+}, Au^{3+}	[49]
Cyanobacteria	Cd^{2+}, Au^{3+}	[76, 165]
Algae		
Chlorella pyrenoidosa, **Chlorella regularis**	Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Sr^{2+}, UO_2^{2+}, Mo	[91, 156]
Non-identified algae	UO_2^{2+}, Mo cations of other heavy metals	[28, 30, 64, 65, 188]
Heterotrophic bacteria		
Acinetobacter sp.	UO_2^{2+}	[239–241]
Alcaligenes eutrophus	Cd^{2+}	[199]
Bacillus subtilis, **Bacillus cereus**	Cd^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, Au^{3+}, UO_2^{2+}	[23, 42, 53, 54, 136, 157]

Table 1.15, continued

Microorganisms	Metal cations	References
Escherichia coli	Cd^{2+}	[123]
Gluconobacter oxydans	Cd^{2+}	[136]
Klebsiella aerogenes	Co^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}	[39]
Leptothrix discophora	Mn, Fe^{3+}	[68]
Pseudomonas sp.		
Pseudomonas aeruginosa		
Pseudomonas fluorescens	Cd^{2+}, Co^{2+}, Ni^{2+}, UO_2^{2+}, Th	[136, 143, 144,
Pseudomonas putida		157, 194]
Pseudomonas saccharophila		
Pseudomonas stutzeri		
Sphaerotilus natans	Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}	[92]
Zoogloea ramigera	Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, UO_2^{2+}, Zn^{2+}, Ag^{+}, UO_2^{2+}, Ni^{2+}	[55, 159–161]
Mixed cultures of bacteria	Ag^{+}, UO_2^{2+}, Ni^{2+}	[43, 195]
Actinomyces		
Actinomyces flavovirides		
Streptomyces niveus		
Streptomyces albus		
Streptomyces cineroruber	Th, UO_2^{2+}	[63, 93, 156, 157, 217]
Streptomyces echinatus		
Streptomyces scabies		
Streptomyces viridochromogenes		
Yeasts		
Candida lipolytica		
Candida utilis		
Rhodotorula mucilapinosa	Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}	[63, 157, 161–163, 194]
Saccharomyces cerevisiae		
Rhodotorula glutinis		
Fungi		
Aspergillus niger		
Aspergillus ustus	Co^{2+}, Ra^{2+}, Th, UO_2^{2+}	[53, 63, 93, 157 215]
Aspergillus oryzae		
Aspergillus terreus		
Fusarium sp.	Co^{2+}, Cr^{3+}, Mn^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}	[157]
Mucor rouxii		
Fusarium oxysporum		
Penicillium chryzogenum		
Penicillium levitum		
Penicillium chrochloron	Th, UO_2^{2+}	[63, 93, 157, 215–218, 225]
Rhizopus arrhizus		
Rhizopus javanicus		
Rhizopus oligosporus		
Rhizopus oryzae		

Chapter 2
METHODS OF ISOLATION, EVALUATION AND STUDYING OF MICROORGANISMS

The present Chapter deals with methods of isolation, quantitative evaluation, determination of activity and studying of microorganisms which are already used for bacterial leaching and industrial effluents purification. Generally speaking, these are bacteria oxidizing S^0, Fe^{2+} and sulfide minerals, as well as sulfate-reducing bacteria.

Such processes as destruction of silicate minerals, leaching of Mn, Au and other elements, biosorption of metals are caused by a wide spectrum of well-known microorganisms. However, these processes are not widely used in practice yet. Thus methods of isolation of those species which are widely used in laboratory research but not listed in existing manuals are described in this chapter.

2.1. ISOLATION OF MICROORGANISMS

Isolation of microorganisms important for hydrometallurgy is performed by inoculating the nutrient media with appropriate samples of ores or solutions. By this method the enrichment culture is obtained. Even when elective media are used the enrichment cultures of isolated bacteria contain concomitant microorganisms. Hence isolation of pure cultures often presents considerable difficulties. Below the methods for obtaining both enrichment and pure cultures of bacteria that are most perspective for hydrometallurgy are listed.

2.1.1. Acidophilic bacteria of the genus Thiobacillus [108, 116, 139, 151]

Enrichment. 100 ml Erlenmeyer flasks with 30 ml of sterile Silverman and Lundgren nutrient medium 9K (No. 2) are inoculated with samples of drainage water or ore from sulfide ore deposit and incubated at 30 °C until growth is observed. Bacterial growth is assessed from the appearance of brown colour in the medium due to formation of ferric salts. In order to isolate bacterial strains active in oxidation of sulfide minerals and resistant to metal ions it is reasonable to use sulfide minerals instead of Fe^{2+} as an energy source and to add heavy metal ions to the medium.

Isolation of pure culture. Pure culture can be isolated by using solid agar medium 9K [139, 151]. However it is better to use silica gel plates impregnated with Leathen medium (No. 1) or medium 9K (No. 2) [99, 116] or solid

media prepared on polyacrylamide gel. Small rusty brown colonies appear on solid media.

From the colonies grown on solid medium, inoculations are made in tubes containing small amounts (4–6 ml) of liquid nutrient medium with Fe^{2+} (No. 2) or in flasks with sulfide minerals. Thereafter purity of the culture is verified.

To isolate a pure culture of **T.ferrooxidans**, the method of end-point dilutions may be used. From an enrichment culture which, according to the analytical data, contains, for example, 10^7-10^8 cells of **T.ferrooxidans**, 1 ml of solution is taken and is diluted 10, 100 . . . 1 million and 10 million times. Consequently, it may be expected that the terminal dilutions will contain single cells of **T.ferrooxidans.** From the terminal dilutions transfers are preferably made into medium with the appropriate energy sources (Fe^{2+} and sulfide minerals).

A pure culture of **T.ferrooxidans** may be obtained on Leathen medium (No. 1) containing 400 mg/l of Fe^{2+} or on 9K medium (No.2) containing 9 g/l of Fe^{2+} with the addition of 1–5 g/l of Cu^{2+} ($CuSO_4 \cdot 5H_2O$). Heterotrophic concomitants usually die in the presence of copper ions [108].

Purity check. As mentioned above, strains of **T.ferrooxidans** may be contaminated with a facultative autotroph **T.acidophilus (T.organoparus),** a chemolithoautotroph **T.thiooxidans,** heterotroph. **A cryptum** and other bacteria. Purity check of **T.ferrooxidans** is carried out by inoculation into the media intended for the above mentioned microorganisms as well as onto beef-extract agar, beef-extract broth, Ashby medium (No. 27), potato agar, starch-ammonium agar, wort agar and medium with glucose and Fe^{2+}.

Maintenance and storage of culture. T.ferrooxidans is maintained on the 9K liquid medium (No. 2) with Fe^{2+} or on the same medium with sulfide minerals in a refrigerator at 4 °C. Transfers should be made at least once a month. Different methods of long-term storage of **T.ferrooxidans** are known. Some of them are listed below:

1) **Mixing with inert or slowly degrading substrates.**

a) The culture is grown in Erlenmeyer flasks with 250 ml of liquid 9K medium for 48 hours on shakers that transfer it to aseptic test-tubes or ampoules containing a mixture of sand and pyrite (1:3). The ratio of sand and pyrite may differ. Prior to sealing sand and pyrite should be washed and dryed. Ampoules are sealed, the test-tubes are covered with rubber stoppers and sealed with paraffin. The sand-and-pyrite mixture is applied at 2–3 g to 1–2 ml of culture containing 10^9 cells/ml at pH 2–2.5, Eh 350-370 mV, and Fe^{2+} content at 4,5 – 4,7 g/l. Under such conditions the culture may be stored at room temperature for 2.5–3 years [97].

b) A culture of **T.ferrooxidans** with a known number of cells is prepared, ampoules with 1 g of chalcopyrite ore are sterilized by autoclaving at 121 °C for 20 min for three successive days; hypodermic syringes and needles are sterilized.

A known amount of cell suspension is introduced by a sterile hypodermic syringe into sterile ampoules with an inert carrier – lygnite or chalcopyrite

(slowly oxidied substrates). The ampoules are sealed and may be stored at a room temperature. The active cell number is checked periodically.

Cells stored in this way can remain in active state for one year.

Gupta and Agate [87] mixed a suspension of **T.ferrooxidans** cells containing 10^{12} calls/ml with 1 g of lygnite or 1 g of chalcopyrite-bearing ore. Upon 8 months of storage with chalcopyrite ore the bacterial oxidation activity decreased by 5 %, while that of bacteria stored on lygnite dropped by 30 %.

2) **Lyophilization.**

Machee proposed a method of a long-term storage of **T.ferrooxidans** [138] which reveals a quick freezing of **T.ferrooxidans** cells grown at 30 °C on a medium with Fe^{2+} at pH 1.6. Precipitation of Fe^{3+} compounds at this pH is absent. Thereafter, 48–72-hour old culture is transferred to a glass container with liquid nitrogen using a pipette. The container is placed in copper wire net and immersed in polystyrene foam block. The frozen droplets of the culture are transferred into a cooled container (2 ml capacity) which is closed then and is immersed into a vacuum container with liquid nitrogen from −120 to −180 °C.

It has been demonstrated that cultures of **T. ferrooxidans** stored in this way retained iron-oxidizing activity even after 36 months while bacteria maintained at +4 °C completely lost this activity after 12 months of storage.

Cells are also dried in vacuum on a medium containing defatted milk powder, 100 g; sodium glutamate, 10 g; distilled H_2O, 1 l [72]. The medium is prepared with a small quantity of distilled water and is stirred to avoid lumps. Then the rest of water is added and agitated with a mixer. Sterilization is conducted in autoclave for 15 min at 121 °C and 1 atm.

0.2 ml of a dried medium and one leep of bacteria culture from the thiosulphate-agar medium*, or, for instance, 0.3 ml of the centrifugate (5 min at 6,000 x g) obtained from 50 ml from the culture grown on the medium with sulfur** are added to each tube. With the certain amount of sulfur being adhered to **T.ferrooxidans** is transferred into the dry medium. The medium used in **T.ferrooxidans** lyophilization, inhibited the culture's growth, when further transferred. Thus the following steps are taken then: lyophilized cells are transferred into 0.3 ml of the iron-containing medium***, the suspension

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***Agar medium with thiosulphate:** KH_2PO_4, 2.0 g; NaCl, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2SO_4$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.03 g; $FeCl_3 \cdot 6H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 0.02 g; $Na_2S_2O_3 \cdot 5H_2O$, 10.0 g; Ion-Agar No. 2 (Oxoid), 10.0 g; H_2O dist. – 1 l, pH 4.4–4.6.

****Medium with sulfur.** Corresponds to the medium with $S_2O_3^{2-}$* but instead of thiosulphate sterile sublimated sulfur is added.

*****Medium with Fe^{2+}. Solution A:** $(NH_4)_2SO_4$, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; KH_2PO_4, 0.1 g; $K_2HPO_4 \cdot 3H_2O$, 0.1 g; H_2O dist., 700 ml. **Solution B:** trace elements solution SL4 according to Pfennig: EDTA (disodium salt), 500 ml; $FeSO_4 \cdot 7H_2O$, 200 mg; $ZnSO_4 \cdot 7H_2O$, 10 mg; $MnCl_2 \cdot 4H_2O$, 3 mg; H_3PO_3, 30 mg; $CoCl_2 \cdot 6H_2O$, 20 mg; $CuCl_2 \cdot 2H_2O$, 1 mg; $NiCl_2 \cdot 6H_2O$, 2 mg; $Na_2MoO_4 \cdot 2H_2O$, 3 mg. H_2O dist. – 1 l; pH 3–4, 10 mg/l of the medium is added. **Solution C:** $FeSO_4 \cdot 7H_2O$, 45 g, H_2O dist. – 300 ml. Solutions A, B, and C are mixed and sterilized by filtration.

is shaken from time to time for 30 min and then put on a membrane filter sterilized in autoclave for 15 min at 121 °C and 1 atm. Then the residue is washed three times with 20 ml of the Fe^{2+} containing medium.

The membrane filter with cells is introduced into a 300 ml Erlenmeyer flask containing 50 ml of the medium. After 21 days of incubation at 28 °C an intensive growth is observed.

Lyophilized cultures should be kept in dark place at temperatures from 4 to 8 °C.

3) **Storage in liquid nitrogen** [87]. 1 ml of the cell suspension containing 1.8–2.0 x 10^{12} cells/ml is mixed with 1 ml of sterile glycerol (10 %). Capillaries (2.5 cm long, 1 mm of internal diameter) are placed into the suspension, sealed on tops and then transferred into liquid nitrogen. Upon 8 months of storage the bacterial activity decreased not more than by 15 %.

T.acidophilus (T.organoparus) [86, 142]

Enrichment. Nutrient medium 9K (No. 2) containing 0.01–1.0 % of glucose and 2 mg – 2 g/l of Fe^{2+} is used for inoculation. Leathen medium containing glucose (5.0–10.0 g/l) in lieu of Fe^{2+} with pH 3.0 (No. 1) can be used as well.

Isolation of pure culture. Colonies of **T.acidophilus** are isolated on 9K agar medium with glucose in lieu of Fe^{2+} at 25 °C and pH 4.5. A pure culture is isolated by consecutive transfers into liquid medium.

Markosyan isolated pure culture of these bacteria on silicagel plates impregnated with medium No. 1 with glucose.

Glucose is added in lieu of Fe^{2+}. Colonies are transferred in to liquid Waksman medium with elemental sulfur (No. 9).

Maintenance of the culture. T.acidophilus is maintained on 9K medium with glucose containing 1 mg/l of Fe^{2+} at 25 °C. Transfers are made once a week. On medium with sulfur (1 %) containing 1 mg/l Fe^{2+} transfers are made once a fortnight. The culture is also maintained on agar media at 5 °C for 1 month.

Thiobacillus thiooxidans [116, 226].

Enrichment. Liquid Waksman nutrient medium with sulfur (No. 9) is inoculated with samples of mine drainage water or ore and is incubated at 28–30 °C. In 3–4 days turbidity appears and pH of the medium falls down. The enrichment culture often contains **T.ferrooxidans** and other acidophilic bacteria oxidizing sulfur and its compounds, as well as heterotrophs, especially fungi.

Isolation of pure culture. Solid Waksman medium with thiosulfate (No. 10) is used. In preparing the medium, thoroughly washed agar or Difco agar is used. In 3–10 days very small colonies of **T.thiooxidans** appear on the surface of agar plates. Fungi grow very poorly on this medium. To obtain a pure culture, bulk inoculation of bacteria from colonies grown on solid medium is carried out into test-tubes or flasks containing medium No. 9. Growth of **T. thiooxidans** is assessed from the drop in the medium pH to 2.0 and lower, and from increased turbidity of the medium.

Maintenance of the culture. T. thiooxidans is maintained on liquid Waksman medium with elemental sulfur (No. 9) in a refrigerator at 5 °C. Transfers should be made at least once a month.

Thiobacillus-like bacteria [31, 133]

Media No. 9 and No. 10 are inoculated with water samples and incubated at 50 °C.

Media supplemented with 0.02 % (w/v) of yeast extract, 0.2 % (v/v) metal trace solution and medium 9K without Fe^{2+} but with sulfide minerals or sulfur are used as well. Cultures are incubated in stationary conditions or on shakers at 200 rpm at 50 °C.

Medium No. 1 containing Fe^{2+} may be also used.

Thermophilic iron- and sulfur-oxidizing bacteria close to thiobacilli [147].

Enrichment. Samples of water, sediments from hot springs, samples of coal and drain water are inoculated into medium No. 11 containing Fe^{2+} ($FeSO_4 \cdot 5H_2O$) or pyrite (FeS_2) and are incubated at 50 and 65 °C. Yeast extract or thiosulfate (0.2 g/l) is added as reduced sulfur source.

Isolation of pure culture. A pure culture is obtained on Manning medium (No. 3) using end-point serial dilutions and agar media with Fe^{2+} ($FeSO_4 \cdot 5H_2O$) at 50 °C. K_2HPO_4 and $Na_2S_2O_3 \cdot 5H_2O$ are added to the medium to the final concentrations of 0.01 and 0.2 g/l respectively. Cultures on agar media are incubated at 50 °C in the atmosphere of 5 % (V) CO_2. Colonies are transferred into liquid medium containing $S_2O_3^{2-}$ and Fe^{2+}, in CO_2 – enriched atmosphere.

Maintenance of culture. The bacterial culture is maintained by frequent transfers on medium No. 11 without yeast extract. Wood and Kelly [232] reported three similar iron-oxidizing bacteria capable of oxidizing Fe^{2+} and FeS_2. These strains are maintained on medium No. 11.

2.1.2. Genus Sulfobacillus [69]

S.thermosulfidooxidans and **S.thermosulfidooxidans subsp. asporogenes** [135].

Enrichment. Active cultures are obtained by inoculation of ore samples into medium containing S^0, Fe^{2+}, sulfide concentrates, pyrite and 0.02 % Difco yeast extract (No. 12).

Growth of **S. thermosulfidooxidans** is manifested in the appearance of turbidity and acidification, while on the medium with Fe^{2+} in its colouring as well (rusty-brown). Colonies are obtained on the same agar media.

Isolation of pure culture. A pure culture is isolated by end-point serial dilutions. To remove non-spore-forming concomitants **S.thermosulfidooxidans** culture is heated at 100 °C for 30 min. Purity check is made by transfers onto meat-peptone and potato-peptone media, thiosulfate agar, and on agar mineral medium No. 12 with Fe^{2+}, Fe^{2+} plus 0.1 % glucose or glucose alone (0.1 %).

Maintenance and storage of culture. S. thermosulfidooxidans is maintained in laboratory conditions on medium No. 12 with sulfide concentrates or

pyrite as an energy source. Yeast extract is also added (0.2 g/l). Transfers are made once a month.

S.thermosulfidooxidans subsp. thermotolerans [127]

Enrichment. Nutrient Manning medium (No. 3) with Fe^{2+} is inoculated with ore or mine drainage water. Growth is assessed from oxidation of Fe^{2+} and appearance of rusty-brown colour of the medium.

Isolation of pure culture. A pure culture is obtained on solid Manning medium with organic substances added at a concentration 0.01–0.1 % (glucose, starch, urea, yeast extract). Subsequently, single colonies are transferred into liquid medium.

Maintenance of culture. The pure culture is maintained on Manning medium (No. 3) with sulfide minerals.

2.1.3. Genus Sulfolobus [34]

S.acidocaldarius. Enrichment. The simplest way to obtain an enrichment culture of **S. acidocaldarius** is to add 0.1 % of yeast extract acidic solution to water samples from springs and to incubate them at 70 °C. In 3 or 7 days heavy turbidity is observed, frequently a film appears on the surface of the medium. Microscopic examinations reveal a large number of cells typical of **Sulfolobus.** Later, the culture is transferred into medium No. 13 containing 0.1 % of yeast extract. To isolate a culture from soil, 0.1–0.5 g of soil is transferred into the medium containing 0.1 % of yeast extract. For a heterotrophically growing culture pH is adjusted to 2.0. However, when an enrichment culture is obtained at 55 °C, pH is adjusted to 1.0 to inhibit growth of **Bacillus acidocaldarius.**

When **S.acidocaldarius** enrichment culture is obtained water or soil samples are added to the medium containing about 1 % of elemental sulfur and pH is adjusted to 3.0.

Isolation of pure culture. Colonies of **Sulfolobus** are obtained on agar medium No. 13 with 0.1 % yeast extract, although growth is not reproducible on this medium [34]. To obtain a pure culture Zillig et al. [238] used silica gel plates. Growth of colonies was observed upon 2–3 days of incubation at 85 °C. In one week colonies reach their maximum size (about 2 mm). Bacteria from single colonies are transferred into liquid medium No. 13.

Maintenance of culture. A pure culture is maintained in liquid medium No. 13, or on the same medium with pH 6.0 at 4 °C at one month interval between transfers.

2.1.4. Genus Acidianus [193]

A.infernus and **A.brierleyi.**

Enrichment. To isolate **A.infernus** nutrient medium No. 14 is inoculated with water samples (5 %) and incubated in the presence of S^0 in the atmosphere with H_2–CO_2 (80:20, v/v) at 85 °C in anaerobic conditions. A pure culture is obtained from enrichment culture by serial dilutions. Colonies are grown on solid medium containing 9 % of starch, about 12 g of

colloidal sulfur and 0.2 g yeast extract per 1 l, at 80 °C in H_2–CO_2 atmosphere.

To isolate **A.brierleyi** medium No. 14 is used containing Fe^{2+} ($FeSO_4 \cdot 7H_2O$) or sulfur as an energy source, as well as 0.02 % of yeast extract. The bacteria are incubated at 60 °C.

A criterion of the culture purity is the genetic identity of aerobically and anaerobically grown cells DNA homology and a very short lag-phase of growth upon transfer from aerobic to anaerobic conditions.

2.1.5. Genus Leptospirillum

L.ferrooxidans [141] and **L.ferrooxidans INMI 17** [113].

Enrichment. Leathen nutrient medium (No. 1) or 9K medium (No. 2) is inoculated with samples of mine drainage water or ore from sulfide ore deposit. Growth is assessed from appearance of rusty-brown colour due to formation of ferric iron compounds. Norris [162] used pyrite (FeS_2) as an energy source.

Isolation of pure culture. To isolate a pure culture silica gel plates impregnated with media No. 1 or No. 2 are used. After inoculation with samples from the enrichment culture microscopic colonies appear. These colonies are coloured rusty-brown due to precipitated ferric salts. A pure culture can be obtained by transferring the colonies into liquid medium. Pure culture of **Leptospirillum**-like bacteria can be also obtained from the enrichment culture by serial end-point dilutions and transfers on Manning solid medium (No. 3) containing 0.5 % (w/v) of agar [139]. K_2HPO_4 (final concentration of 0.01 g/l) and $FeSO_4 \cdot 7H_2O$ (final concentration of 2.78 g/l) are added to the Manning medium No. 3 and the dishes are kept at 30 °C. Purity check is done by inoculation into organic media for heterotrophs and mineral media for thiobacilli and by microscopic studies. Special attention should be paid to the presence of **T. ferrooxidans** capable of growing on the same media.

Maintenance of cultures. Pure cultures of **L. ferrooxidans** are maintained in mineral media with Fe^{2+} at 5 °C, transfers are made once a month.

2.1.6. Genus Acidiphilium

Acidiphilium cryptum [88].

Enrichment. Nutrient medium containing trypton, soybean and glucose (No. 15) is inoculated with soil or samples from the enrichment culture of **T. ferrooxidans,** pH is adjusted to 2.0. The culture is incubated at 28 °C on a shaker. After several days it is transferred onto agar trypton-soybean medium with glucose.

Isolation of pure culture. A pure culture of **A. cryptum** is obtained from single colonies.

Maintenance of culture. A. cryptum is maintained on agar trypton-soybean medium containing glucose, in a refrigerator. Transfers are made once a month.

2.1.7. Other heterotrophic contaminants of T. ferrooxidans

To isolate heterotrophic contaminants close to **Acetobacter acidophilium** Leathen medium (No. 1) with addition of 0.01–0.02 % of yeast extract is used, pH is adjusted to 2–3 [236].

A pure culture of a contaminant can be obtained by dilutions in Leathen medium with addition of yeast extract and glucose Johnson and Kelso [108] and Manning [139] used media Nos. 16 and 17, respectively, to isolate non-identified heterotrophic contaminants of **T.ferrooxidans.**

2.1.8. Syntrophic association of iron- and sulfur-oxidizing bacteria

Several syntrophic associations of bacteria are known to oxidize sulfide minerals more intensively under autotrophic conditions than the respective members of these associations when used separately. They include **L. ferrooxidans** and **T.thiooxidans; L. ferrooxidans** and **T. acidophilus (T. organoparus); S. thermosulfidooxidans** and **L. ferrooxidans** (the thermotolerant strain) [113, 164]. They can be isolated on appropriate media with sulfide minerals or sulfide concentrates as an energy source. The same media should be used for maintenance at 5–^{0}C with transfers at least once a month.

2.1.9. Sulfate-reducing bacteria

When preparing media for sulfate-reducing bacteria, the following factors should be taken into account.

Nutrient media should contain major mineral components: phosphates, ammonium salts, potassium, magnesium, sulfates used as acceptors of electrons and ferrous iron.

Since all sulfate-reducing bacteria are obligate anaerobes, it is necessary to add reductants to the medium, usually sodium sulfide, though sometimes sodium thioglycolate, cysteine sulfide, sodium ascorbate or dithionite are used.

The majority of representatives of the genera **Desulfovibrio** and **Desulfotomaculum** grow on media prepared with tap water and do not require addition of trace elements. Other sulfate-reducing bacteria require addition of certain trace elements or natural water originating from the same location as the strain under investigation. When grown on individual substrates **D. multivorans** and **D. variabilis** display specific requirements for selenite and molybdate [227].

Sulfate-reducing bacteria isolated from saline habitats require addition of sodium chloride to the medium, Na^{+} and Cl^{-} being essential components for **D. desulfuricans** subsp. **aestuarii** and **D. salexigens,** respectively. From brackish and marine habitats Widdel [227] isolated sulfate-reducing bacteria obligately requiring high concentrations of NaCl, $MgCl_2$ and $CaCl_2$ for their growth. Halotolerant and halophilic bacteria include **D. postgatei** with optimal salinity of 0.7 % NaCl as well as **D. variabilis, D. magnum** and **D. limicola** with optimal salinity of 1.5–2 % NaCl.

In addition, media for sulfate-reducing bacteria should include substances required for the constructive metabolism of bacteria, such as yeast extract, a set of vitamins, higher fatty acids, acetate. Bacteria of the genera **Desulfovibrio** and **Desulfotomaculum** as a rule can develop on synthetic media, but for better growth yeast extract is added. Many sulfate-reducing bacteria oxidizing organic compounds to CO_2 have specific growth requirements. For example, **D. postgatei** requires para-amino benzoic acid and biotin; **D. acetoxidans,** para-amino benzoic acid; **D. magnum,** 4-amino benzoic acid, biotin and B_{12}; growth of **D. multivorans** is enhanced by a small amount of higher fatty acids.

Isolation and purification of bacteria of the genus **Desulfonema** are considerably facilitated under conditions providing for their gliding motility, i.e. when 0.2 % agar or aluminium phosphate are added to the medium [228].

For mesophilic species the optimum growth temperature is usually from 30 to 37 °C; for the thermophilic bacteria **D. nigrificans, D. thermophilus** and **T. commune** it is 55, 65 and 70 °C, respectively.

The extreme pH values at which sulfate-reducing bacteria and sulfate reduction occur in nature are 2.8 and 9.9 [105, 190]. For the majority of pure cultures, the optimum medium pH is 6.8–7.3.

The redox potential of the medium is from –100 to – 200 mV. Isolation of **D. gigas** capable of growing at Eh +80 mV is carried out on media containing a weak reducer, e.g. sodium ascorbate.

For details of medium compositions, purification procedures and substrates preferable for isolation of different species of sulfate-reducing bacteria, see original publications [168, 179 227, 230].

Below is the composition of most frequently used media for isolating many sulfate-reducing microorganisms.

Sulfate-reducing bacteria oxidizing organic compounds to acetate. Postgates media B, C, D and N (No. 18) are the most favourable for isolation, cultivation and studying the properties of sulfate-reducing bacteria which incompletely oxidize organic compounds to acetate [179].

Medium B is used for detection, count and isolation of the bacteria. By replacing sodium lactate in medium B with other organic compounds, such as salts of lower organic acids, lower alcohols, sugar, volatile and higher fatty acids, it is possible to find the substrates utilized by these bacteria. Media C and N have no precipitate and are used for biomass accumulation. Medium D practically contains no sulfates (0.01 mg/l $FeSO_4 \cdot 7H_2O$) and may be used to test fermentation ability of bacteria. By adding sulfate, sulfite or thiosulfate it is possible to test the ability of the microorganism being studied to utilize different electron acceptors

Inoculation procedure. To remove dissolved oxygen from medium No. 18 (solution 1), the medium should be heated till boiling and cooled down quickly in cold running water, and then the following ingredients should be added in the required amounts in the sequence given below: (1) yeast autolysate; (2) ferrous sulfate; (3) trace elements and vitamins (whenever necessary); (4) HCl and $NaHCO_3$ solutions to adjust the medium pH to 7.0–7.4 according to bromothymol blue which turns blue-green at this pH

value; (5) sodium sulfide (~100 mg/l) due to which the medium turns dark grey or black. The culture is transferred onto the medium prepared for inoculation and is poured into airtight sealed vessels.

Sulfate reducing bacteria are cultivated in test-tubes which are filled to the top with medium and closed by sterile rubber stoppers so that there were no air bubbles under the stopper, using a steel wire heated in a flame.

To use anaerobic equipment proposed by Hungate is recommended if unavailable Hungate test-tubes may be replaced with glass vials closed by rubber stoppers which are fixed by means of metal or plastic screw caps. The medium thus prepared for inoculation may be stored in this fashion for a long time. The vials are convenient for transportation and are also used for quantitative estimation of sulfate-reducing bacteria from natural materials. In an appropriate gaseous phase these microorganisms may be cultivated under chemolithoautotrophic conditions. Inoculum is transferred into the vials with a sterile syringe. Before inoculation the surface of the rubber stopper should be sterilized by wiping it with acidified ethanol and heating the rubber surface slightly in a burner's flame.

Sulfate-reducing bacteria oxidizing organic compounds to CO_2. These bacteria are cultivated in Widdel and Pfennig medium (No. 19) [231]. The basic principles for work with this group of bacteria are similar to those described above.

Enrichment. Enrichment cultures of sulfate-reducing bacteria are obtained by inoculating liquid medium B with lactate or Widdel and Pfennig medium with acetate (or other substrates) with samples from natural materials. The appearance of black colour of the medium and smell of hydrogen sulfide during incubation are indicative of the bacterial growth. To obtain an active enrichment culture, it is necessary to carry out a number of consecutive transfers into liquid nutrient media by using 2–4-day old inoculum, preferably from precipitate where sulfate-reducing bacteria are usually accumulated. The amount of inoculum is 2–4 % (v/v).

It is necessary to establish the optimum medium for an enrichment culture by changing salinity, by adding different vitamins, trace elements, etc. The aim is to obtain a maximum formation of hydrogen sulfide.

Investigation of the enrichment culture begins with detecting the presence or absence of spore-forming sulfate-reducing bacteria. To this aim, 1 ml of 8–10-day old culture is transferred into a 1–2 ml ampoule to which a rubber hose with a clamp is fitted. By means of a syringe needle, inert gas is blown into the ampoule during 3–5 min to remove oxygen. The the clamp is closed, the ampoule is sealed quickly and submerged into boiling water bath for 10 min. It is possible to prepare more than one ampoule. In such a case, the other ampoules are submerged into water bath for 20 and/or 30 min. The boiled material is inoculated into one of Postgate media of into Widdel and Pfennig medium. Growth of spore-forming bacteria of the genus **Desulfotomaculum** is manifested by the blackening of medium and presence of motile cells after 3–4-day incubation. An experiment on spore germination permits simultaneously to eliminate non-spore-forming concomitant strains.

An original method of isolation of multicellular gliding sulfate-reducing bacteria has been proposed by Widdel [228, 229]. The concomitant microorganisms are washed off from filamentous bacteria placed on electron microscope metal gauze mounted into glass tubes.

Isolation of pure culture. Pure cultures are isolated by obtaining single colonies on agar media (1.5 % agar). It should be noted that it is necessary to neutralize the medium to pH 6–7, otherwise it will not solidify after sterilization. The procedure of preparing solid medium for inoculation is similar to that of preparing liquid medium. As inoculum, 2–3-day old enrichment cultures are used. Inoculum is obtained by serial dilutions in liquid medium of the same composition, and is transferred into solid medium. The solid medium is poured into test-tubes as a high layer or sucked into Vinyal's glass tubes the ends of which are sealed in a burner's flame after solidification of medium. After 2–5 days of incubation in solid medium black colonies of sulfate-reducing bacteria may be detected. Colonies are removed from test-tubes using Pasteur pipettes, from Vinyal's tubes by cutting them near the colonies and squeezing agar out into Petri dishes.

It is possible to obtain single colonies of these bacteria on the surface of solid medium using a rotating test-tube method of Hungate or by inoculation into Petri dishes incubated in an anaerostat.

Purity check of sulfate-reducing bacteria is made using techniques suggested by Postgate [179]. The culture under investigation is transferred into beef-extract agar medium to detect facultative anaerobic heterotrophic contaminants; into media with sugars and peptone to detect bacteria causing fermentation; and into Beijerinck medium [184] to detect thiobacilli. The data obtained in this way should be compared to the control (sterile medium).

Maintenance of cultures. Pure cultures are maintained on optimized liquid medium, transfers are made at least every two months.

2.1.10. Manganese leaching bacteria

Enrichment. Enrichment culture of bacteria able to leach manganese are obtained on Bromfield medium (No. 20) containing 5–10 g/l of glucose or sucrose. The nutrient medium is inoculated with samples of manganese ores, dressing tailings, etc.

For obtaining anaerobic enrichment culture the nutrient medium (50–100 ml) is inoculated and incubated at 28–30 °C under aerobic conditions, preferably under constant agitation to ensure the necessary mass exchange in the system.

For isolation of anaerobic or facultatively aerobic cultures, nutrient medium is poured into test-tubes as a high layer. To ensure a uniform distribution of manganese-containing material in the medium, the ore is rubbed into a loose cotton wool wad which is placed into a test-tube filled with the medium. The medium is covered with a layer of petroleum jelly. Before inoculation the sterile test-tubes are boiled and cooled down in a water batch. Incubation is carried out in a thermostat at 28–30 °C.

The growth of manganese-leaching bacteria and the intensity of Mn^{4+} reduction processes are assessed by the rate of Mn^{2+} solubilization.

Isolation of pure cultures. To obtain pure cultures of manganese-reducing bacteria solid Bromfield medium (No. 20) is used. Inoculation is made by using samples from the 10^{-4} and 10^{-5} dilutions of 0.1–0.2 ml of enrichment culture per dish. The dishes are incubated aerobically or anaerobically depending on the conditions of enrichment at 28–30 °C.

When Mn^{4+}-reducing bacteria develop on solid medium, light zones appear around their colonies, by the size of which the Mn^{4+}-reducing activity may be assessed.

Purity of the culture is checked by inoculations on different media for autotrophic and heterotrophic microorganisms. Bacteria are identified with the help of the existing manuals.

Maintenance of culture. A pure culture should be maintained on liquid nutrient medium with ore. Transfers should be made every 10 days adding 10 % inoculate. Mn^{4+}-reducing efficiency of the stored cultures is tested once a month.

2.1.11. Bacteria oxidizing arsenic (As^{3+})

P.arsenitoxidans [96].

Enrichment. An enrichment culture is obtained by inoculating mine drainage water of gold-arsenic deposit into medium No. 22 containing 1.3 g/l of As^{3+}.

A pure culture is obtained by drop inoculations [235] and further transferred on silica gel plates impregnated with mineral medium No. 22 containing 1.0 g/l of As^{3+}. Purity check is made by transfers on beef-extract agar, potato agar, and beef-extract broth.

2.1.12. Bacteria reducing Cr^{6+}

P.dechromaticans [183, 184].

Enrichment. An enrichment culture is obtained by inoculation of medium No. 23 with samples of activated sludge from biore-generator to which household sewage and industrial effluents containing Cr^{6+} are coming. A pure culture is obtained by multiple transfers from terminal dilutions, as well as from single colonies transferred onto beef-extract agar and grown aerobically.

P.chromatophila [131].

Enrichment. An enrichment culture is obtained by inoculating medium No. 24 with specimens of water from bottom of a pit in a chromium deposit as well as from industrial effluents. A pure culture is obtained from single colonies grown in columns of agar medium No. 24.

A.dechromatica [129].

Enrichment and pure cultures are isolated from commercial water treatment installation at a machine-building factory using traditional methods on medium No. 25.

2.1.13. Bacillus mucilaginosus [11]

Enrichment. Enrichment culture of **B.mucilaginosus** is obtained by inoculation of liquid Ashby medium No. 27 with water suspensions of soil and ore

samples. To facilitate isolation of a pure culture the sample suspension in sterile tap water is heated at 80 °C for 10 min in water bath, then quickly cooled and used for inoculation.

Pure culture is obtained from single colonies grown on Ashby agar medium No. 26 or potato agar upon inoculation of enrichment culture on these media. Purity is tested according to characteristic growth features on the above media, as well as by absence of growth on beef-extract agar and beef-extract broth, and morphological features of vegetative cells and spores. Identification is based on features listed in Table 1.16 (Chapter 1), and by a method of DNA-DNA hybridization with other species of the genus **Bacillus.**

Maintenance and storage. B. mucilaginosus is maintained on potato agar with periodic transfers (every 2–3 months). Slants with fresh-grown cultures upon spore-formation are stored in a refrigerator. Long-term storage of **B.mucilaginosus** spores is maintained in liquid nitrogen or in lyophilized state as well as in air-dry state in a mixture with sterile soil or quartz sand.

2.2. COMPOSITION OF NUTRIENT MEDIA

Successful isolation of new and already known microorganisms active in biogeochemical processes and attaining high rate of metal leaching largely depends on the quality of nutrient media. Below is the composition of the tested nutrient media for isolation and cultivation of microorganisms most important for biogeotechnology of metals.

No. 1. Leathen medium for **T. ferrooxidans** and other iron-oxidizing bacteria [130]:

$(NH_4)_2SO_4$, 0.15 g; KCl, 0.05 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.10 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.01 g; distilled H_2O, 1 l.

10 ml of 10 % solution of $FeSO_4 \cdot 7H_2O$ acidified to pH 3.5 and sterilized separately by filtration through an asbestos filter, is added to the medium. Sterilization of the $FeSO_4 \cdot 7H_2O$ sofution can be carried out in an autoclave at 0.5 atm following adjustment of pH to 2.0–2.5 with H_2SO_4. The final pH of the medium is adjusted to 3.5.

No. 2. Silverman and Lundgren medium 9K for **T. ferrooxidans** [198]:

Solution I: the following materials are dissolved in 700 ml of distilled water: $(NH_4)_2SO_4$, 3.0 g; KCl, 0.1 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $Ca(NO_3)_2$, 0.01 g.

Solution II: the following materials are dissolved in 300 ml of distilled water; $FeSO_4 \cdot 7H_2O$, 44.2 g; 1 ml of 10 N solution of sulfuric acid is added. In place of $FeSO_4 \cdot 7H_2O$, Mohr's salt $[FeSO_4(NH_4)_2SO_4 \cdot 6H_2O]$ may be used. In this case $(NH_4)_2SO_4$ is not added, and solution II is prepared as follows: 63 g of $[FeSO_4(NH_4)_2SO_4 \cdot 6H_2O]$ is dissolved in 300 ml of distilled water and 1 ml of 10 N H_2SO_4 is added. Such quantity of Mohr's salt corresponds to 9 g/l of iron and 21.4 g/l of $(NH_4)_2SO_4$.

The solutions are sterilized separately: solution I at 1 atm and solution II at 0.5 atm. Before use both solutions are mixed. This medium is most favourable for **T.ferrooxidans** growth and therefore is used more frequently.

No. 3. Manning medium for iron-oxidizing bacteria [139]:

Solution A. $FeSO_4 \cdot 7H_2O$ (33.4 g/l) is dissolved in 300 ml of water adjusted to pH 2.5 with 6 M H_2SO_4 and sterilized by filtration.

Solution B. $(NH_4)_2SO_4$, 6.0 g; KCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; $Ca(NO_3)_2$, 0.02 g; distilled H_2O, 550 ml, pH 3.0.

The solution is sterilized in an autoclave at 121 °C for 15 min.

Solution C. 7.0 g of purified agar L28, is added to 150 ml of distilled water, exposed for 15 min and sterilized at 121 °C for 15 min.

Solutions B and C are cooled for 5 min at ambient temperature and decanted with agitation. Then solution A is added with agitation and the medium is dispensed to Petri dishes.

No. 4. Medium for **T. ferrooxidans** and **Beijerinckia lacticogenes** [219]:

K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Na_2SO_4, 0.15 g; KCl, 0.1 g; $CaSO_4 \cdot 2H_2O$, 0.01 g; $Na_2MoO_4 \cdot 2H_2O$, 0.0005 g; H_2O, 1 l; pH 2.5.

No. 5. Medium for **B. lacticogenes** [219]:

KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.1 g; $FeCl_3$, 0.005 g; $Na_2MoO_4 \cdot 2H_2O$, 0.0005 g; yeast extract (Difco), 0.5 g; sucrose, 2.5 g; agar, 15.0 g; H_2O, 1 l; pH 3.0.

No. 6. Medium for **L. ferrooxidans** [141]:

Media Nos. 1 and 2 are used. Fe^{2+} or FeS_2 are added as an energy source.

Norris and Kelly [162] used nutrient medium of the following composition: $(NH_4)_2SO_4$, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; K_2HPO_4, 0.1 g; KCl, 0.1 g; $FeSO_4 \cdot 7H_2O$, 50 mM; yeast extract and sulfide minerals (pyrite, chalcopyrite concentrate), H_2O – 1 l.

No. 7. Waksman medium for **T. thiooxidans** [116, 226]:

$(NH_4)_2SO_4$, 0.2 g; KH_2PO_4, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 6H_2O$, 0.25 g; $FeSO_4 \cdot 7H_2O$, traces; sulfur flower (S^0), 10 g; distilled H_2O, 1 l; pH 4.0. Sulfur is pre-sterilized with flowing steam for 3 days or with alcohol for 2 hours. Then alcohol is evaporated, sterile sulfur powder is recovered and transferred aseptically into the sterile medium.

No. 8. Solid Waksman medium for **T. thiooxidans** [226]:

$Na_2S_2O_3 \cdot 5H_2O$, 5 g; NH_4Cl, 0.1 g; $CaCl_2 \cdot 6H_2O$, 0.25 g; $MgCl_2 \cdot 6H_2O$, 0.1 g; KH_2PO_4, 3.0 g; leached agar-agar or Difco agar, 20 g; distilled H_2O, 1 l; pH 5.0. pH is adjusted with sulfuric acid added to the medium after sterilization.

No. 9. Postgate medium 'S' for bacteria close to **Thiobacilli** [179]:

Sulfur, 10 g (or $Na_2S_2O_3 \cdot 5H_2O$, 5 g); $(NH_4)_2SO_4$, 2–4 g; KH_2PO_4, 2–4 g; $CaCl_2$, 0.25 g; $MgSO_4$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 10 mg; distilled H_2O, 1 l. The initial pH is 5.0 for bacteria close to **T. thiooxidans** and 7.5 for those close to **T. thioparus.** Media with elemental sulfur are sterilized with flowing steam. Media with thiosulfate are sterilized at 0.5 atm for 20 min. In the latter case thiosulfate is sterilized separately and added to the basic medium.

No. 10 Medium for bacteria close to **Thiobacilli** [31]:

$(NH_4)_2SO_4$, 0.5 g; NaCl, 0.3 g; KH_2PO_4, 0.1 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.01 g; trace elements solution, 0.5 ml. Difco yeast extract, 2 g; either Fe^{2+} or pyrite, or sulfur is also added, distilled H_2O – 1 l.

25 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of distilled water with 0.75 ml of concentrated H_2SO_4 is sterilized by autoclaving at 15 psi (or 1 atm) for 15 min. 4 ml of this solution is added to 96 ml of salt solution and pH is adjusted to 2.2 or 1 g of pyrite is added to 100 ml of the medium.

Sulfur flower (S^0) is sterilized with flowing steam for 30 min during 3 days and added in the quantity of 0.5 g to 100 ml of sterile medium with pH from 2.5 to 3.0. The trace elements solution contains (g/l): Na_2EDTA, 50.0; $ZnSO_4 \cdot 7H_2O$, 11.0; $CaCl_2 \cdot 2H_2O$, 7.34; $MnCl_2 \cdot 4H_2O$, 2.5; $CoCl_2 \cdot 6H_2O$, 0.5; $(NH_4)_2MO_2O_{27}$, 0.5; $FeSO_4 \cdot 7H_2O$, 5.0; $CuSO_4 \cdot 5H_2O$, 0.2; NaOH about 11 g; pH 6.0; distilled H_2O – 1 l.

No. 11. Medium for other iron- and sulfur-oxidizing bacteria [147, 232]:

$MgSO_4 \cdot 7H_2O$, 0.4 g; $(NH_4)_2SO_4$, 0.2 g; KCl, 0.1 g; K_2HPO_4, 0.1 g; $FeSO_4 \cdot 7H_2O$, 13.9 g. FeS_2 (2 g) or sulfur flower (5 g) are also used as energy source; distilled H_2O, 1 l; pH 2.0–3.0.

Bacteria are cultivated at 50 and 60 °C after addition of yeast extract (0.2 g/l) or in closed vials after enrichment of the medium with 5 % (v/v) CO_2. In the absence of yeast extract, $Na_2S_2O_3 \cdot 5H_2O$ (0.2 g/l) is added to the medium as source of reduced sulfur.

For bacteria grown in myxotrophic conditions glucose, yeast extract (0.2 g/l), and cysteine as solutions with pH 1.7 are added to the medium.

Instead of CO_2 $NaHCO_3$ as a solution (5 mM) sterilized on a filtre with 0.45 μm pores may be added. Yeast extract (2 %, w/v) solution is sterilized at 15 psi (or 1.05 atm) for 15 min, the other components of the medium are sterilized at 10 psi for 10 min.

For preparing solid medium, agar (0.5 %, w/v) is added to Manning medium (No. 3). The final concentration of Fe^{2+} is adjusted to 2.78 g/l. To this medium K_2HPO_4, 0.01 g/l and $Na_2S_2O_3 \cdot 5H_2O$, 0.2 g/l (the final concentration) are added.

No. 12 Medium for **S. thermosulfidooxidans** [69]:

$(NH_4)_2SO_4$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; NaCl, 0.2 g; KH_2PO_4, 0.2 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.07 g; yeast extract, 0.2; distilled H_2O, 1 l; pH 2.5.

Pyrite powder (FeS_2), sulfur flower (S^0) or powdered copper-zinc ore, 2 %, is used as energy source.

Yeast extract is sterilized separately. Pyrite, S^0 and ore slightly humidified with water are also sterilized separately with flowing steam for 3 days.

No. 13. Allen medium modified by T. Brock for **S. acidocaldarius** [34, 47]:

$(NH_4)_2SO_4$, 1.3 g; KH_2PO_4, 0.28 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2 \cdot 2H_2O$, 0.07 g; $FeCl_3 \cdot 6H_2O$, 0.02 g; $MnCl_2 \cdot 4H_2O$, 1.8 mg; $Na_2B_4O_7 \cdot 10H_2O$, 4.5 mg; $ZnSO_4 \cdot 7H_2O$, 0.22 mg; $CuCl_2 \cdot 2H_2O$, 0.05 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.03 mg; $VOSO_4 \cdot 2H_2O$, 0.03 mg; $CoSO_4$, 0.01 mg; distilled H_2O, 1 l; pH is adjusted to 2.0 with 10N H_2SO_4. Organic components are added to the basic medium either dry after autoclaving or from a 100-fold concentrated sterile solution (sugars).

Sulfur is sterilized with flowing steam for 3 hours during 3 days and added at about 1 g/100 ml of sterile medium.

Solid agar medium is prepared as follows.

Double-strength autoclaved salt solution is cooled to 45 °C and mixed with

autoclaved double-strength agar gel. Then yeast extract is added aseptically to the final concentration of 0.1 %. The mixture is dispensed into Petri dishes or test tubes. A better growth of colonies is observed when Ionagar 2 (Oxoid) is used in place of Difco agar. Ionagar is used in the final concentration of 1.2 %.

Polysilicate nutrient medium is prepared by mixing 94 ml of H_2O, 6 ml of sodium silicate solution and 5 g of sodium bentonite. pH is rapidly adjusted to 6.0 with H_2SO_4 and the medium is transferred to Petri dishes. For a better visibility of colonies an indicator, such as bromocresol purple or chlorophenol red, 0.01 %, is added. The inoculated dishes are incubated at 80 °C in humid chambers.

No. 14. Medium for **A. infernus** and **A.brierleyi.**

Allen medium is used for isolating **A.infernus.** The medium contains 2 g/l of S^0 (pH 2.5). Anaerobic conditions are created according to Balch [14] and 0.75 g/l of Na_2S is added. Reasazurine (1 mg/l) is used as an indicator. Medium is dispensed by 20 ml into test tubes pressurized by H_2–CO_2 (80–20, v/v, kPa) which are sterilized by tindalization. Brierleyi medium is used for isolating **A.brierleyi** [238]:

$(NH_4)_2SO_4$, 3.0 g; $K_2HPO_4 \cdot 3H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.1 g; $Ca(NO_3)_2$, 0.01 g; yeast extract, 0.2 g; sulfur flower, 10 g; distilled H_2O, 1 l; pH is adjusted to 2.0 with H_2SO_4. Preparation of the medium is similar to No. 13.

No. 15. Medium for **A.cryptum** [88]:

$(NH_4)_2SO_4$, 2.0 g; KCl, 0.1 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; dehydrated trypticase-soybean broth, 0.1 g; glucose, 1 g; H_2O, 1 l. pH is adjusted to 3.0 with 1 N H_2SO_4. Glucose is sterilized by autoclaving separately (10 % solution) and after cooling added in the required quantity to the sterile medium (pH 3.0). Solid medium is prepared by adding agar, 12 g/l. Agar and glucose (double strength) and other components are sterilized separately at 15 psi (or 1.05 atm) for 15 min and are mixed after cooling.

No. 16. Medium for isolating other heterotrophic concomitants of **T.ferrooxidans** [108]:

Acidified mannitol-triptone-soybean medium is used containing; $MgSO_4 \cdot 7H_2O$, 0.5 g; $(NH_4)_2SO_4$, 0.1 g; KH_2PO_4, 0.05 g; KCl, 0.05 g; $Ca(NO_3)_2$, 0.01 g; mannitol, 1.0 g; triptone-soybean broth (TSB, Oxoid), 0.1 g; agar (bacto, Oxoid) or agarose (BDH), 12.0 g; H_2O, 1 l. pH of the medium (excluding agar and agarose) is adjusted to 3.5 with H_2SO_4 prior to autoclaving (120 °C, 15 min). The gelatinous solution (4.5 %, w/v) is sterilized in an autoclave separately to prevent hydrolysis and the two solutions are poured together after cooling.

No. 17. Manning medium for heterotrophic acidophilic bacteria [139]:

The medium containing glucose-salt solution, yeast extract and agar (GSYE) is prepared by mixing the following solutions:

1. 25 ml of 20 % glucose solution;

2. $(NH_4)_2SO_4$, 1 g/l; KCl, 0.1 g/l; K_2HPO_4, 1.0 g/l; $MgSO_4 \cdot 7H_2O$, 0.2 g/l; yeast extract, 0.5 g/l; distilled H_2O, 750 ml. The solution is adjusted to pH 2.8 with 6 M H_2SO_4.

3. 225 ml of distilled water containing 15.0 g of agar (Difco).

Each part of the medium is sterilized by autoclaving, solutions 2 and 3 are mixed, and then solution 1 is added. Yeast extract is not an obligatory component of the medium.

Liquid medium (GSYE) is prepared by mixing solutions 1 and 2.

No. 18. Postgate media B, C, D, N [179] for sulfate-reducing bacteria oxidizing organic compounds to acetate.

Component	Medium, g/l			
	B	C	D	N
KH_2PO_4	0.5	0.5	0.5	0.5
NH_4Cl	1	1	1	1
$CaCl_2 \cdot 2H_2O$	–	–	0.1	–
$CaSO_4 \cdot 2H_2O$	1	–	–	–
$MgSO_4 \cdot 7H_2O$	2	0.06	–	0.06
Na_2SO_4	–	4.5	–	–
$(NH_4)_2SO_4$	–	–	–	5
Sodium lactate	3.5	6	–	6
Sodium pyruvate	–	–	3.5	–
Yeast extract	1	1	1	1
Sodium citrate	–	0.3	–	0.3
Sodium ascorbate	–	–	1	–
$Na_2S \cdot 9H_2O$	0.1	0.1	–	0.1
$FeSO_4 \cdot 7H_2O$	0.5	0.1	0.01	0.1
Tap water	1	–	–	–
Distilled water	–	1	1	1

Medium with sodium lactate is sterilized in an autoclave at 1 atm, the other components are sterilized at 0.5 atm in sealed ampoules or air-tight screw-capped vials and then transferred into sterilized medium (solution 1).

Distilled water is used for preparing the following solutions:

1) 5 % solution of yeast autolysate or extract;

2) 5 % solution of $FeSO_4 \cdot 7H_2O$ in 1 % hydrochloric or sulfuric acid;

3) 5 % solutions of HCl and $NaHCO_3$ used for adjusting pH to desirable value;

4) 10 % solution of sodium pyruvate and 5 % solutions of ascorbic and thioglycolic acids in water from which oxygen had been removed by boiling. The latter acids are neutralized with NaOH, sterilized in sealed ampoules filled to capacity and stored in a refrigirator. Sodium sulfide ($Na_2S \cdot 9H_2O$) is prepared as a 5 % solution in 1 % solution of $NaHCO_3$. Other reducers, e.g. sodium thioglycolate, is prepared and stored in the same manner as sulfide; dithionite ($Na_2S_2O_4$) is sterilized in 1 % solution of $Na_2S \cdot 9H_2O$, and while working with an enrichment culture $Na_2S_2O_4$ is transferred into the medium as dry crystals with a sterile spatula (10–30 mg/l).

No. 19. Widdel and Pfennig medium [231] with sodium acetate for sulfate-reducing bacteria oxidizing organic compounds to CO_2.

The basic medium has the following composition: Na_2SO_4, 3.0 g; KH_2PO_4, 0.2 g; NH_4Cl, 0.3 g; KCl, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.15 g; H_2O, 1 l.

For isolating freshwater strains 1.0 g of NaCl and 0.4 g of $MgCl_2 \cdot 6H_2O$ per 1 l of the medium are added; for isolating brackish water strains, 7.0 g of NaCl and 1.2 g of $MgCl_2 \cdot 6H_2O$; and for isolating marine strains, 20.0 g of NaCl and 3.0 g of $MgCl_2 \cdot 6H_2O$ are added.

The medium is sterilized by autoclaving at 1 atm, boiled, cooled in cold running water, then the following components are added from sterile solutions: $NaHCO_3$, 2.5 g; $Na_2S \cdot 9H_2O$, 0.36 g; solution of vitamins according to Pfennig and Lippert (ng/ml of medium): biotin, 10; calcium pantothenate; 25; thiamine, 50; p-amino-benzoic acid, 50; nicotinic acid, 100; pyridoxamine, 250; trace elements see below, 1 ml/l; sodium acetate, 2.8 g; H_2O, 1 l; pH is adjusted to 7.1–7.3 with HCl or $NaHCO_3$ solutions. The medium is inoculated with the sample, dithionite (10–30 mg/l) is added and the vials are immediately hermetically closed. All additional components are sterilized as indicated above at 0.5 atm (medium No. 18).

Trace element solution after Widdel and Pfennig [231]: for sulfate-redusing bacteria.

HCl, 25 %, 10 ml; $FeCl_2 \cdot 4H_2O$, 1.5 g; $CoCl_2 \cdot 6H_2O$, 190 mg; $MnCl_2 \cdot 4H_2O$, 100 mg; $ZnCl_2$, 70 mg; H_3BO_3, 62 mg; $Na_2MoO_4 \cdot 2H_2O$, 36 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; $CuCl_2 \cdot 2H_2O$, 17 mg. As the first step, iron chloride is dissolved in hydrochloric acid, then the volume is adjusted to 1 l with distilled water, and other components are added.

No. 20. Bromfield medium for manganese-reducing bacteria [35]:

Glucose or sucrose, 5–10 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $(NH_4)_2SO_4$, 1.0 g; yeast autolysate, 5 ml; distilled water, 1 l; finely ground manganese ore or other product. Addition of $CaCO_3$ to the medium is not obligatory because the acids released by microorganisms will be neutralized with MnO_2 and consumed for leaching manganese. For preparing solid medium agar, 20 g/l, is added, pH of the medium is adjusted to 7.0. The ore is ground, sterilized separately and added to the medium at 50–100 g/l. Since other elements are also present in ores and are solubilized after leaching, it is possible in some cases to add only sources of carbon to the medium. In scale-up experiments solutions of molasses containing glucose concentrations similar to those of Bromfield medium can be used as nutrient medium.

Before using media with molasses or other carbohydrate containing products it is necessary to examine them with respect to different forms of nitrogen (ammonium and organic) in order to decide whether nitrogen is to be added or not.

While dispensing the agar medium with manganese ore into Petri dishes it is necessary to agitate it continuously by even rotations to prevent precipitation of manganese ore at the bottom of the flask. Air bubbles formed in the dishes are easily removed by rotating them carefully in the table plane while the medium is still not solidified.

No. 21. Medium for **R.sphaeroides** reducing Mn^{4+} (modified Bromfield medium) [50]:

KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $(NH_4)_2SO_4$, 1.0 g; glucose, 20.0 g; $CaCO_3$, 5.0 g; Fe_2O_3/MnO_2, 1.0 g; Difco yeast extract, 150.0 mg; distilled H_2O, 1 l; pH 7.2.

No. 22. Medium for **P. arsenitoxidans** [96]:

$(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 0.5 g; KCl, 0.05 g; $Ca(NO_3)_2$, 0.1 g; $NaHCO_3$, 0.5 g; $NaAsO_2$, 2.0 g; distilled H_2O, 1 l; pH 7.5–8.0.

$NaHCO_3$ and $NaAsO_2$ solutions are sterilized separately.

No. 23. Medium for **P. dechromaticans** [183, 184]

NH_4Cl, 0.3 g; K_2HPO_4, 0.3 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; NaCl, 0.1 g; $CaCO_3$, 0.05 g; $FeCl_3 \cdot 6H_2O$, 0.05 g; K_2CrO_4, 0.1 g; sodium acetate, 0.2 g; trace elements according to Hogland, 0.5 ml (No. 34), B_{12} vitamin, 10 Mg; 25–100 ml; H_2O tap water, 1 l; pH 7.0

The medium is sterilized at 0.5 atm. Solutions of acetate, microelements, B_{12} vitamin and beef-extract broth are sterilized separately and added to the medium before inoculation.

No. 24. Medium for **P.chromatophila** [131]:

NH_4Cl, 0.3 g; KH_2PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; NaCl, 0.1 g; $(NH_4)_2CrO_4$, 0.2 g; sodium lactate, 2 g; agar, 15 g; distilled H_2O, 1 l. After sterilization pH of the medium is adjusted to 7.0 with 5 % $NaHCO_3$ solution.

No. 25. Medium for **A.dechromatica** [129]:

NH_4Cl, 0.3 g; K_2HPO_4, 0.3 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; NaCl, 0.1 g; $CaCO_3$, 0.05 g; $FeCl_3 \cdot 7H_2O$, 0.05 g; K_2CrO_4, 0.2 g; Na acetate, 0.2 g; tap water, 100 ml.

The medium is sterilized at 0,5 atm for 30 min. Acetate solution is sterilized separately and introduced into the medium before inoculation.

Components	Medium No.					
	28	29	30	31	32	33
	G medium components/l tap water					
Carbohydrates	100–150	100–150	100–150	100–150	100–150	50–100
NH_4NO_3	3	–	–	–	–	–
$NH_4H_2PO_4$	–	0.05	–	–	–	–
NH_4Cl	–	3.5	–	–	3.5	–
NH_4NO_3	–	–	–	2.3	–	–
$NaNo_3$	–	–	–	–	–	5.5
K_2HPO_4	–	–	–	–	–	1
KH_2PO_4	1	–	0.5	1	0.5	–
$MgSO_4 \cdot 7H_2O$	0.5	–	0.5	0.25	0.5	0.5
$K_4[Fe(CN)_6]$	–	0.2	–	–	–	–
$ZnSO_4 \cdot 7H_2O$	–	0.01	0.05	–	0.05	0.05
$FeSO_4 \cdot 7H_2O$	–	–	0.025	–	0.025	0.025

Medium pH 6.5 – 7.8

No. 26. Potato agar [184]:

Potato, 200 g; tap water, 1 l.

Potato tubers are washed, peeled, cut finely, put into cold water and boiled for 30 min. The liquid is filtered on cotton wool with cheesecloth, agar (15 g/l) is added and heated till it is dissolved, the medium pH is adjusted to 7.0 by the indicator bromphenol blue and the mixture is sterilized for 1 hour at 120 °C.

The medium is used for cultivation of **B. mucilaginosus.**

No. 27. Ashby medium for **B.mucilaginosus** [184]:

Mannitol, 20 g; K_2HPO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0, 2 g; NaCl, 0,2 g; K_2SO_4, 0.1 g; $CaCO_3$, 5,0 g; Na_2MoO_4, 0,005 g; distilled water, 1 l; pH 7,5 – 8,0. The medium is sterilized at 0,5 atm for 30 min. To obtain a solid medium, 15 g/l of agar is added.

Nos. 28–33. Media for the cultivation of fungi applied to aluminium and iron leaching.

No. 34. Solution after Hogland:

pH 7,0; H_2Odist. – 3.61; $AlCl_3$, 1g; KJ, 0.5 g; KBr, 0.5 g; LiCl, 0.5 g; $MnCl_2 \cdot 4H_2O$, 7 g; H_3PO_3, 11g; $ZnCl_2$, 1g; $CuCl_2$, 1g $NiCL_2$, 1g; $CoCl_2$, 5g; $SnCl_2 \cdot 2H_2O$, 0.5g; $BaCl_2$, 0.5g; Na_2MoO_4, 0.5g; $NaVO_3 \cdot H_2O$, 0.1g; salt of cesium, 0.5 g.

2.3. METHODS OF ENUMERATION OF MICROORGANISMS

2.3.1. Serial end-point dilution method

The serial end-point dilution method is the oldest and most labour-consuming. Yet it is widely used for enumerating viable cells of both autotrophic and heterotrophic microorganisms. These methods are well-known and can be found in microbiological manuals [124, 149]. In order to obtain end-point tenfold dilutions while investigating chemolithoautotrophic bacteria use should be made of respective nutrient media (Chapter 2) instead of water, as is frequently done. In practice to enumerate bacteria in 1 ml solution or 1 g ore use is made of tables based on the variation statistical methods [124]. The accuracy of calculating the most probable number (MPN) of cells is higher with greater number of inoculated test-tubes (e.g. from 5 to 10) of each dilution. However in mass tests of samples especially in field conditions not more than two test-tubes for each dilution are inoculated. It enables enumerating bacterial cells at an accuracy of one factor, e.g. 10^5, 10^6, etc. The incubation is carried out at the optimal temperature. Incubation time for each group of bacteria is established experimentally. Difficulties may arise in enumerating certain bacterial species since same nutrient media may be used for growth of different bacteria species with close physiological properties. For example, same media are used to cultivate **T.ferrooxidans** and **L.ferrooxidans,** or **T.ferrooxidans, T.thiooxidans** and **T.acidophilus,** etc. Hence when enumerating bacterial cells it is necessary to identify them. For this purpose a researcher should study by microscope media samples of end-point dilutions and inoculate the elective media with them, as described in Chapter 2.

Enumeration of sulfate-reducing bacteria by inoculation to selective media leads to underestimation, one of the reasons being the ability of some sulfate-reducing bacteria to form aggregates that do not dissociate into single cells (**Desulfomena** filaments, **Desulfosarcina** packets, etc.).

A more reliable method of revealing sulfate-reducing bacteria in natural sources is estimating the intensity of sulfate-reducing process using the labelled $^{35}SO_4^{2-}$ [184].

2.3.2. Enumeration of colonies

Colonies are usually obtained by inoculating the solid medium or by placing on it membrane filters after filtration.

According to the existing rule the optimum number of the estimated colonies per plate is within 30–300. The lower limit is fixed for the sake of statistical accuracy, the upper – to avoid the intermingling of separate colonies.

To cultivate chemolithoautotrophic acidophilic bacteria use is made of the following solid media: washed agar, agarose, gel plates or polyacrilamid gel plates.

Since the number of bacterial cells in water or ore samples is usually unknown before the analysis, several dilutions are prepared, i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, etc.

Then 0.1 ml of diluted culture is applied to the solid medium surface in a Petri dish. For that purpose 2–3 drops are applied to the surface, and the remainder is disposed from the pipette. The culture is eavenly distributed on the solid medium surface with a spatule. The Petri dish is turned upside down then and placed in a thermostat at the temperature optimum for bacterial growth. After incubation and appearance of colonies they are enumerated. Data interpretation depends on how the number of grown colonies corresponds to the initial cell number in the inoculum. To estimate this dependance for the studied bacteria, one should use, at least at the start, a number of methods of quantitative determination of bacterial in the inoculum.

To enumerate **T.ferrooxidans** the solid medium is prepared by mixing of 0.06 N H_2SO_4 containing (g/l): K_2HPO_4, 0.8; $MgSO_4 \cdot 7H_2O$, 0.8; $(NH_4)_2SO_4$, 0.8, and $FeSO_4 \cdot 7H_2O$, 66.6 with an equal volume of water containing agar or agarose. The two parts of the medium are autoclaved separately at 5 psi (0.35 kg/cm^2) for 10 min. The volume is poured into sterile Petri dishes, 40 ml per each [220].

The initial pH of the solid medium is 1.5. Serial dilutions of a **T.ferrooxidans** culture are made in 0.01 N H_2SO_4. From 5 to 100 ml of a bacterial suspension is filtrated through membrane filters 47 or 50 mm in diameter (pore size 0.45 μm).

Before filtration, the filters are boiled in sterile distilled water for 10 min and then left in the water for 24 hours.

After filtration, the filters with bacteria are placed onto the solid medium, and the plates are incubated at 30 °C for at least 15 days.

Optimal conditions for colony growth exist when agar concentration is 0.3 % (w/v) or less. Colonies are formed when there is at least 10^4 cells in the inoculum per plate.

When incubated the membrance filters are placed on a piece of filter paper (Whatman No. 17) and moistened with a 1 % (v/v) solution of acid fuchsin for staining the colonies. Then the filters are dried at room temperature, and the colonies are enumerated under a stereomicroscope (x 20).

Replica method on paper with thallium sulfide was proposed by Gallisi and Ferrari to identify a number of thiobacilli [66]. The method is based on the ability of **T.thioparus** and **T.thiooxidans** to oxidize thiosulphate to sulfate with the formation of elemental sulfur which is deposited in the bacterial colonies. This fact is used to determine the presence of sulfur-forming bacteria. Plates with bacterial colonies are replicated on paper with thallium sulfide which is consequently placed in diluted (0.12 N) nitrous acid. Prior to that the paper is wetted with pyridine to quickly dissolve crystal sulfur. The black paper with thallium sulfide is decolourized but not in the places where free sulfur is present. Decolourization takes place due to the formation of polysulfides of thallium which is insoluble in diluted mineral acids. If sulfur is present, the brown sports appear onto the paper on the side touching the bacterial colonies. Brown patches let us make a quantitative assay of the present thiobacilli in the initial sample. This method is more sensitive and more accurate for enumerating **T.thioparus** in soil than the end-point tenfold dilution method.

2.3.3. Enumeration using a microscope

Direct enumeration of bacterial cells is performed on microscope slides or by using different types of bacterial counting chambers, e.g. Thoma, Petrov, Hauser chambers, etc. [124, 149]. These methods are well-known to microbiologists although not widely practiced to enumerate chemolithoautotrophic bacteria. Below are listed various modern methods for **T.ferrooxidans** enumeration. These methods enable one not only to enumerate bacteria but also to differentiate various species of thiobacilli. This is extremely important since same media can be used to cultivate bacteria of different taxons making their identification difficult.

Method of indirect staining with fluorescent antibodies [67]. This method enables identification of the number of **T.ferrooxidans** cells in a mixed medium within several hours. For this purpose one will need: a) polycarbonate type of filters; b) commercially available FITC-conjugated goat-rabbit serum; c) unpurified anti-**T.ferrooxidans** rabbit serum. Below is the description of this method.

T.ferrooxidans cells to be used for obtaining specific antiserum are grown at 25 °C for 7 days on a shaker on medium 9K (No. 2). Suspension of cells is obtained by centrifugation at 40.000 x g and 4 °C for 30 min. Cells are washed twice in 0.1 N H_2SO_4 to remove iron, suspended in 0.01 N H_2SO_4 and stored at 4 °C.

Prior to injection the cells are washed 3 times in phosphate-salts buffer (FSB) (0.85 % NaCl in 0.1 M Na-K-phosphate buffer, pH 7.4) and resuspended in basal salts solution up to the density of 10^9 cells/ml. The basal salts solution contains: NaCl, 8.0 g; KCl, 0.4 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaCl_2$, 0.1 g;

Na_2HPO_4, 0.04 g; KH_2PO_4, 0.06 g; dextrose, 0.8 g; galactose, 0.8 g, and 2.5 ml of 0.4 % solution of phenol red per 1 l of distilled water. The solution is sterilized by filtration and stored at 4 °C. Aliquots of the suspension corresponding to 1.0, 1.5 or $2.0 \cdot 10^9$ cells are injected into the otic vein of young rabbits at 4-day intervals. A week after the last injection blood is taken from the rabbits by a cardiac puncture. The serum is prepared by centrifugation of the obtained blood at 1000 x g for 30 min at 4 °C in order to remove bacterial cells. Agglutination titer of immunoglobulins obtained by using **T.ferrooxidans** culture by Bailer and Scott method is more than 1:2500 [13]. It is maintained in rabbits by regular hypodermic injections of 2×10^9 cells.

Immunoglobulins not specific to **T.ferrooxidans** are removed from the serum by adsorbing on cells mixture of **Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli** and the unknown species of **Thiobacillus** isolated from the same mine drainage water. The serum is mixed with these cells and left overnight at 4 °C. Then the mixture is centrifuged at 20,000 x g for 15 min at 4 °C. Purified serum is diluted 10-fold in 0.1 M Na-K-phosphate buffer (pH 7.4), sterilized by filtration, divided into 0.5 ml aliquots and stored at −10 °C. Before and after adsorption the serum is tested for specificity to **T.ferrooxidans** by staining different bacteria described above on glass slides using the fluorescent antibodies method.

The method of enumerating **T.ferrooxidans** is as follows. 50 ml samples of acid mine drainage water are filtered through polycarbonate filters (Nucleopor, D=25 mm, 0.4 μm pore size), which had been soaked in India ink for several minutes. Then the filters are neutralized with 25 ml of PBS. For inhibiting the nonspecific fluorescence 0.2 ml of bovine serum albumin (BSA) (20 mg/ml in 0.01 M Na-K-phosphate buffer at pH 7.4) are added to these filters. After 30 min the unreacted bovine serum albumin is drawn off and the filters are covered with 0.2 ml of rabbit serum obtained by using **T.ferrooxidans** culture. After 30 min the unreacted serum is removed by washing with at least 50 ml of PBS. After that 0.2 ml of fluorescent isothiocionate (FITC) – conjugated goat antirabbit serum (Cappel, Labor) (antibody concentration – 2.8 mg/l, total protein – 2.3 mg/l) are applied to the filters and left to react for 30 min. Excess FITC-conjugated serum is drawn off and the filters are washed with at least 100 ml of PBS in order to remove all the unreacted FITC-conjugated serum.

The stained filters are mounted on glass slides in several drops of PBS-glycerol mixture (1:9 v) and a coverslip is applied.

Filters of the slides are observed with a luminescent microscope. The number of fluorescing cells per 0.01 mm^2 of filter area is determined by counting 50 such areas for each sample with the aid of an ocular grid. The number of cells in 1 ml of sample is calculated as the number of cells per 0.01 mm^2 of area multiplied by 10^3.

Other modern methods of enumerating and differentiating **T.ferrooxidans** are known, namely the method of immunological and electroforetic identification of different **T.ferrooxidans** strains in a culture containing other iron-oxidizing bacteria or heterotrophic bacteria [107].

Jates and Holmes [106] proposed for this purpose a method using molecular samples consisting of DNA cloning sequences. With appropriate equipment available these methods can be used in laboratory practice.

Large bacterial biomass is involved in tank leaching of metals. It should be noted that the biomass is evaluated in the pulp, i.e. in liquid and solid phase during short period of time. The above methods are hardly applicable for these purposes. Below are listed several methods to evaluate **T.ferrooxidans** biomass during technological processes.

2.3.4. Rapid assy of T.ferrooxidans biomass

Wet biomass concentration in a culture can be determined according to Kovrov et al. [128] by a centrifugation method. A volume of culture, e.g. 5 ml, is poured into a special plexiglas tube (Fig. 2.1) and subjected to centrifugation at 6000 rpm for an hour. After that the volume of precipitated cells determined by using a narrow graduated tube's cylinder. The tube volume should be about 5 ml, but when working with diluted cultures containing about 0.1 g/l of biomass the volume can be increased to 25–50 ml. External dimensions of the tube are predetermined by a centrifuge being used which should have a rotor with turning cups. The measuring part of the tube is a cylindrical capillary of 1.5–3 mm in diameter (the thiner is the capillary and the longer is the measuring cylinder, the more accurate is measurement). The volume of the whole measuring channel of the tube should be about twice as

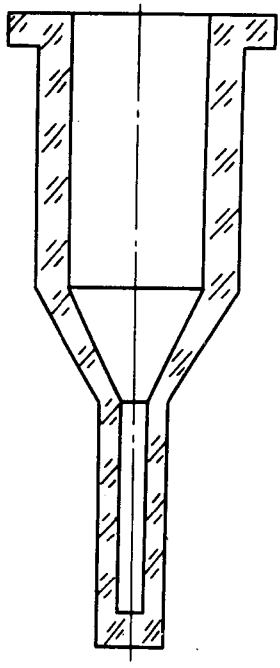

Fig. 2.1. Centrifuge tube to determine of T.ferrooxidans wet biomass concentration

large as the average expected amount of cells biomass in the volume of culture placed in the tube to measure concentration. The tube is graduated in the following way. Water is poured into the tube in amount which according to calculations is sufficient to fill the measuring channel (the amount of water is determined by weighing the tube). By short-time centrifugation water is forced in the measuring channel of the tube and the upper level of water is marked. After that all intermediate marks are calculated and traced. For example in a tube for 5 ml of culture, it is convenient to trace marks for each 5 mg of water in the channel. In such a case each division of the measuring channel of the tube corresponds to the wet biomass concentration in g/l. It is assumed that the specific weight of wet biomass differs slightly from 1. The accuracy of determining the measuring channel is 1/4 of the division value, i.e. 0.25 g/l, which corresponds to an error of ±2.5 % of the measured value when wet biomass concentration is 10 g/l.

When measuring biomass concentration in thicker samples, a smaller amount of culture, e.g. 2.5 ml instead of 5 ml, can be poured in the tube. In this case the graduation value will double, correspondingly. When measuring biomass concentration in liquid cultures to increase measuring accuracy it is possible to accumulate cells precipitates of several portions of culture centrifuged consequently in the same tube.

To determine concentration of dry cells biomass the scaling factor of 0.26 is used, this factor is the relationship between the cell's dry and wet weight, which in the course of the experiments is checked periodically by the weight method.

When determining bacteria cells concentration in pulp, the solid part is separated by centrifugation at 300xg for 10 min [200]. To determine biomass concentration 50 cm^3 centrifuge tubes of polished plexiglas are used with capillaries 0.8, 1.13 and 3.0 mm in diameter (Fig. 2.1). An aliquote of solution (1–5 ml and more) is placed in the centrifuge tube and the bacteria are pelleted in the tube capillary by centrifugation at 6500xg for 45 min. Fine suspended particles of jarosite, iron hydrates, etc. are dissolved by adding HCl to a pH 0.8–1.1 to the sample before centrifugation. Some authors calibrate the tube capillary in milligrams of biomass per 1 mm of capillary height (assuming wet biomass density of 1.01). The height of biomass column formed after the centrifugation indicates the amount of biomass in the aliquote (mg), and, knowing the solution volume, bacteria concentration in the pulp liquid phase can be calculated – m in g/l. As a control, some other methods of biomass assay, e.g. the end/point dilution method by using the McCready tables [149] or the modified Peterson method of protein determination should be used in some cases [169].

An equation has been proposed for calculating biomass quantity (g/l) into cell concentration (cells/ml):

$$X = m \cdot 3.8 \cdot 10^9, \qquad (2.1)$$

where X is bacteria concentration in the liquid phase of the pulp, cells/ml,
m is bacterial mass, g/l.

2.3.5. Determining T.ferrooxidans biomass by measuring its protein content

Pulp. The Peterson method is used [169] which is slightly modified depending on the chemical composition of leached minerals. The analysis comprises the following stages:

1. Obtaining cells hydrolysate from the pulp.
2. Protein precipitation from hydrolysate with trichloroacetic acid (TCA) in the presence of sodium deoxycholate.
3. Quantitative determination of protein in the precipitate.

After thorough stirring, 5 ml of pulp are taken and subjected to hydrolysis for 15 min in 25 ml of 0.5 M of NaOH solution in a water bath with periodical stirring. The cells both in the solution and attached on concentrate particles are subjected to hydrolysis. The extent of cells hydrolysis is checked by a similar procedure with a further protein measurement.

The hydrolysate is separated from the solid phase by centrifugation at 5000 rpm for 5 min. After centrifugation the hydrolysate should be transparent and colourless. Otherwise, protein may be overvalued. Colourization of the hydrolysate may be caused by metal ions and sulfide sulfur which pass into solution as a result of the sulfide minerals oxidation.

In cases when the hydrolysates are coloured, they are treated with hydrogen peroxide (1 drop of H_2O_2 per 1 ml of hydrolysate) and kept for 3 min under room temperature.

As a result of such treatment sulfide is oxidized to sulfate. Excess hydrogen peroxide is removed by heating in a boiling water bath. The extent of hydrogen peroxide removal is checked using ammonium rhodanide or potassium iodide.

The next stage is precipitation of protein from the hydrolysate. This is done by adding 0.3 ml of 0.15 % sodium deoxycholate to 1 ml of hydrolysate. Then it is kept for 10 min under room temperature and 0.3 ml of 72 % TCA is added and centrifuged. The precipitate is dried by turning over centrifuge tubes on filter paper. If the protein content in the sample is low, protein can be precipitated from 2 or 3 ml of hydrolysate. However, in such a case the amount of deoxycholate and TCA added to the hydrolisate must be increased proportionally.

The obtained protein precipitate is subjected to a colorimetric reaction which is a modification of the Lowry method [134]. For this purpose 1 ml of water and 1 ml of solution A* is added to the precipitate. The reaction mixture is thoroughly stirred and kept for 10 min at room temperature. The 10-fold diluted Folin reagent** (0.5 ml) is added to the mixture, and after

*Solution A composition: solution A is composed of a mixture of equal volumes of 0.8 M NaOH solution, 10 % sodium dodecilsulfate, water and a mixture of solutions of 10 % sodium carbonate, 0.2 % potassium tartarite and 0.1 % copper sulfate.

**Folin reagent is prepared in the following way. 100 g of sodium tungstate, 25 g of sodium molybdate, 750 ml of H_2O. 8 ml of 85 % phosphoric acid (ortho) and 100 ml of H_2O are heated for 10 h to moderate boiling (the flask is equipped with a return cooler). After this 150 g of lithium sulfate, 50 ml of water and a few drops of bromine are added. Excess bromine is removed by boiling (without a cooler), then is cooled, filtered, and brought to 1 l.

stirring it is kept for 30 min at room temperature and then the optical density is measured at the wavelength of 750 nm. The instrument is set in zero position by measuring control solution composed of 1 ml of alkaline hydrolysate obtained from the initial concentrate with addition of all reaction components.

The reference curve is plotted according to a standard solution of bovine albumin. 5 ml of 0.5 N NaOH are added to 1 ml of the sample with certain concentrations of protein then 1 ml of the mixture is taken, protein is precipitated and determined as described above. Plotting the curve by using this method allows to take into account all dilutions and some errors of the method.

The accuracy of the analysis is also influenced by ions of some metals, copper in particular. To find an error in the analysis 1 ml of the pulp liquid phase, from which bacteria have been previously removed by centrifugation, should be added to samples with standard albumin solutions of 1 ml volume. 4 ml of 0.5 N NaOH are added to each sample and then 1 ml is taken for analysis. Protein precipitation and its colorimetric assay are done using the above-described method. The obtained data are compared with indices of standard protein solutions. Direct proportional relationship between the protein concentration and optical density of the final solution is observed within protein range in the pulp from 0.3 to 2.0 mg/ml.

The above method is checked and used in leaching sulfide concentrates containing metals other than copper.

Liquid phase of the pulp. To determine protein of biomass in the pulp liquid phase the solid part is separated by centrifugation at 1000 rpm for 1 min, then the cells are concentrated at 6000 rpm for 20 min. The precipitate is dried by turning over centrifuge tubes on filter paper. Due to this procedure, the heavy metals ions in leach solutions have no effect on the quantitative determination of protein. The cells are hydrolyzed in the same way as when measuring biomass protein in pulp. Ferric iron precipitate is removed by centrifugation at 6000 rpm. Protein in supernatant is determined by the Lowry method. For this purpose 5 ml of solution C* are added to 1 ml of hydrolyzate. The mixture is kept for 10 min at room temperature, after that 0.5 ml of 2-fold diluted Folin reagent is added. Optical density is measured 30 min later at the wavelength of 750 nm.

According to the difference between protein content in pulp and supernatant the protein content of cells attached on concentrate particles is estimated. To determine the amount of dry biomass, protein content is determined in a sample of lyophilized cells.

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*Solution C (prepared before use): 25 ml of reagent A + 0.5 ml reagent B

Reagent A: 1.45 g Na OH + 500 ml H_2O + 10 g Na_2CO_3;

Reagent B: 0.5 g $CuSO_4 \cdot 5H_2O$ + 100 ml H_2O + 1 g of sodium citrate.

2.3.6. Electron microscopy methods of studies

Morphologic and cytologic studies of bacterial populations taking part in metal leaching make it possible to assess their status in the technological process and to identify a variety of bacteria in ores and concentrates during their leaching.

For example, studying the fine structures of bacteria in dynamics allows to examine the effect of physical and chemical conditions on the ultrastructural cells organization and in the long run to determine the relationship between the structure and function of the organism as a whole. Major techniques of the electron microscopy methods of studies are well developed. When working with solutions the cells biomass is usually obtained by centrifugation or, in field conditions, by filtration through membrane filters (0.2–0.3 μm pore size). However, working with ore pulp is specific, as the bulk of cells is attached to the solid phase which prevents obtaining the high-quality preparations. In this case the task is to separate cells from solid particles and to obtain a cell suspension containing cells free from the mineral surface. To obtain such suspension the pulp is centrifuged at 1000 rpm for 1 min in order to separate coarser particles of minerals. Then the pulp is centrifuged at 6000 rpm for 20 min. This makes the cells to separate from solid particles and accumulate as a film on the surface of the solid precipitate. The cells film is carefully washed out from the mineral precipitate surface with the 9K iron-free medium, after that the precipitate is washed 3 times by using the same medium with further centrifugation, each time the cells being collected from precipitate surface. Mineral particles are removed from the combined cell suspension by centrifugation at 1000 rpm for 3 min, the cells being concentrated by centrifugation at 6000 rpm for 20 min.

Preparations are obtained in the following way. Bacterial suspension drops equal in volume are applied on the grid with a backed film and 1 % solution of phosphorotungstic acid (pH 2) is added. Exposure time is 30 sec. If iron hydrate precipitates, the grids must be washed in acidic water (pH 2).

To obtain ultrathin sections the cell suspension must be thoroughly washed from ferric iron by multiple centrifugation with 9K iron-free medium (pH 2) at 1000 rpm for 3 min. A fixation which permits obtaining well-preserved bacterial structures can be achieved by using the Riter-Kallenberger method. It is based on fixation of cells with 2 % osmic acid diluted 1 : 1 with a buffer of the following composition: the Michaelis buffer (medinal, 1.47 g; sodium acetate, 0.97 g; NaCl, 1.70 g per 50 ml a distilled water) – 5 ml, 0.1 N HCl – 7 ml; H_2O – 13 ml; 1 M $CaCl_2$ – 1 ml. Fixation is carried out for 18–24 hours at 5–6 °C with additions of 1 % (v/v) of meat peptone broth. Then the cells are collected by centrifugation at 5000 rpm for 15 min, enclosed in 2 % agar, dehydrated with ethanol and absolute acetone and enclosed in Epon 812. Prepolymerization is carried out at 37 °C for 2 days, polymerization at 63 °C for 2 days as well. Sections are obtained by using a suitable ultratome, then contrasted with 3 % solution of uranilacetate (pH 4.5) for 12 min at 50 °C and stained with solution of lead citrate for 10 min at the same temperature. The sections are observed with an electron microscope.

2.4. DETERMINATION OF BACTERIAL ACTIVITY

2.4.1. Determination of $^{14}CO_2$ fixation activity [116, 184]

The activity of chemolithoautotrophic bacterial growth in dump, tank and underground metal leaching can be determined by $^{14}CO_2$ fixation intensity. Below are listed several examples of applying this method. Freshly collected acid water containing bacteria and Fe^{2+} (sample volume 10 ml) is poured into 12–15 ml flasks and sealed with rubber stoppers so as to leave some air. Then exactly 0.1 ml of a $Na_2{}^{14}CO_3$ solution (specific activity $1 \cdot 10^7$ cpm/min in 1 ml) is added by puncturing the rubber stoppers with a syringe. The flasks should be sealed since the solutions being tested have low pH and the radioactive carbonate is in the form of $^{14}CO_2$. Each variant of the experiment is run in triplicate. The solution of radioactive $Na_2{}^{14}CO_3$ is filtered through a membrane filter with pore size 0.25–0.3 μm, aliquoted into ampoules which are sealed and boiled in water bath 3 times. In this form the solution of radioactive $Na_2{}^{14}CO_3$ can be stored for a long time. Some of the flasks are placed into drainage mine water under natural temperature, others are kept at room temperature. The exposure time depends on a number of factors (temperature, quantity of bacteria, Fe^{2+} concentration, etc.) and may range from several hours to a few days.

Upon the experiment is over, 0.5 ml of 40 % formaline is added to each flask and the contents are mixed. Subsequent operations are performed in the laboratory. The solution is filtered through membrane filters with pore size 0.25–0.3 μm, then the filters are treated with 2 % hydrochloric acid to remove precipitate (iron hydrates) and radioactivity of the solution is measured by using a Spectral Liquid Scientillation Counter.

Bacteria are enumerated before and after the experiment either by the end-point tenfold dilution method, or according to protein concentration.

When working with acid waters the activity of $^{14}CO_2$ fixation is usually determined either by the biomass radioactivity or by calculating the fixed carbon content. In the latter case the total carbon content in the medium is determined either analitically or through calculations. The daily CO_2 fixation value is calculated according to the formula:

$$C_X = \frac{C_{carb.} \cdot r}{R \cdot t} \qquad (2.2)$$

where:

C_X is the value of daily CO_2 fixation in mg carbon per 1 l;

$C_{carb.}$ is the carbon content in water;

R is the total radioactivity of water in the flasks upon adding the $Na_2^{14}CO_3$ solution, in cpm/min per 1 l;

r is the ratioactivity of bacteria on the filter, in cpm/min per 1 l of the filtered water;

t is the incubation period of flasks in days.

In the pulp 50–80 % bacteria are adsorbed on solid particles. Consequently, the real radioactivity of intact cells adsorbed on minerals cannot be found since

solid particles distort the results. Hence the pulp is initially hydrolyzed and then radioactivity of organic compounds in pulp hydrolysate is determined upon removal of mineral components by centrifugation and precipitation of organic components by trichloracetic acid in the presence of sodium deoxycholate. The main high-molecular mass compounds (proteins and nucleic acids) precipitate and can be collected on the membrane filter, and their radioactivity can be determined.

It can be concluded that the above-described methods allows enumeration of cells adsorbed on solid particles as well as of cells in the liquid phase.

The scheme of the experiment is described below. Well mixed pulp is added to 10-ml vials (5 ml to each). The vials are sealed and 1 ml solution of $Na_2{}^{14}CO_3$ with radioactivity of 1 Ku/ml is added with a syringe. The vials are placed on a shaker for 4–6 hours at a temperature of 20 °C, thereupon 0.5 ml formaline is added to each vial to stop the process. Then 2 ml pulp is taken from each vial and hydrolyzed in 10 ml of 0.5 M NaOH in a water bath for 20 min. The solid mineral part is removed by centrifugation. To measure protein using the above-described Peterson method 1 ml of hydrolysate is taken from the supernatant. To determine radioactivity 2–3 ml hydrolysate are taken into centrifuge tubes, 1.5 % solution of sodium deoxycholate is added (0.3 ml per 1 ml hydrolysate). The mixture is stirred and 15 min later the same amount of 73 % TCA is added. The formed precipitate is gathered on a membrane filter (0.2–0.3 μm pore size) and washed with a 2 % HCl solution. The filters are tested with a liquid scintillation counter. Each sample is studied for radioactivity in duplicate. The obtained data is expressed in cpm/min and calculated per 1 mg protein for a time unit. For example, if radioactivity of organic components of 5 ml equals 1476 cpm/min and protein content is 0.212 mg/ml, the value of relative CO_2 fixation by bacteria is

$$\frac{1476}{5 \cdot 0.256} = 1153 \text{ cpm/min/mg of protein.}$$

2.4.2. Other methods of determining bacterial activity

In dump and underground metal leaching it is possible to determine the rate of Fe^{2+} bacterial oxidation in solution alone. The activity of bacteria oxidizing Fe^{2+} in solutions has been shown not to be always favourable, and the number of bacterial cells is often low varying from $2.5 \cdot 10^4$ to $2.5 \cdot 10^6$ cells/ml. Water samples also differ in bacterial activity. Therefore, efficiency of bacterial oxidation of Fe^{2+} should be determined in different sites of the leaching operation. 30 ml of mine drainage water samples are added into 100 ml Erlenmeyer flasks, covered with aluminium foil and placed into water streams at the natural temperature. If Fe^{2+} is absent in the solutions, $FeSO_4 \cdot 7H_2O$ is added at varied quantities and potential bacterial efficiency in Fe^{2+} oxidation is determined in actual conditions. Similar tests are also run at room temperature to estimate the impact of temperature on bacterial activity in oxidizing Fe^{2+}, since drainage mine water usually has low temperature. In each case two control flasks with bacteria killed by formaline are used to assess the chemical Fe^{2+} oxidation under the concrete conditions. Assay of Fe^{2+}, Fe^{3+} and bacterial biomass is done periodically.

To evaluate the rate of gas (oxygen) uptake during bacterial leaching processes the Warburg vessel is used in most cases.

1.5–2.5 ml pulp is introduced into the Warburg vessels and 0.5 ml of 20 % KOH solution is introduced into the internal cylinder for carbon dioxide uptake. Then the vessels are connected with manometers, put into a thermostatic bath and when the vessels contact the atmosphere the agitation mechanism is switched on.

During experiments with the pulp the frequency of vessels agitation should be no less than 250–270 rounds per minute. When the process of temperature equalization is finished (10–20 min) the vessels are disconnected from the atmosphere. The volume of gases uptaken for the period of time $\Delta\tau$ is determined by the difference between liquid levels in bends of a U-shaped manometer. For kinetic calculations one can use values of oxygen consumption rate expressed in $\mu l\ O_2 \cdot min^{-1}$, $\mu l\ O_2 \cdot min^{-1} \cdot g^{-1}$ biomass, $g\ O_2 \cdot l^{-1} \cdot h^{-1}$.

It should be noted that values of oxygen uptake rates obtained during experiments are apparent. To determine the real values one should find the dependence of oxygen uptake on the effecting factors. The apparent activation energy of the bacterial leaching process can be found using the equations knowing the V_{O_2} dependence on temperature and amount of substrate.

2.4.3. Determination of temperature coefficient

The dependence of the chemical reaction rate on temperature is characterized by a temperature coefficient Q_{10} which shows by how many times the reaction rate is changed with a temperature increase by each 10 °C.

The Q_{10} value is found by the equation:

$$Q_{10} = \frac{V_2}{V_1}^{\frac{10}{T_2 - T_1}}$$

where:

T is temperature, °K;

V is the maximum oxidation rate.

2.5. METHODS OF OBTAINING ACTIVE BACTERIAL STRAINS

2.5.1. General

Obtaining and using active strains of bacteria oxidizing Fe^{2+}, S^0 and sulfide minerals or performing other functions is an important factor of intensifying metal leaching from ores and concentrates, biosorption, etc.

For instance, to obtain the active **T.ferrooxidans** strains the following parameters should be taken into account:

1. Rate of bacterial biomass accumulation (growth rate).
2. Rate of CO_2 fixation.

3. Rate of sulfide oxidation (measured by accumulation of metal ions in the solution and by O_2 consumption).

4. Resistance to heavy metal ions.

5. Rate of bacterial adhesion on solid particles and their distribution into solid and liquid phase in Pachuka tanks.

6. Storage stability.

Table 2.1 shows the differences between a wild strain of **T.ferrooxidans** and the active strain originating from it using the example of zink leaching from a copper-zink concentrate.

The ability to form amino-acids is important for bacteria solubilizing the native gold. Below is the description of several approaches to obtain the active bacterial strains.

2.5.2. Selection and induced mutagenesis of T.ferrooxidans

"Wild" strains of **T.ferrooxidans** have different efficiency in oxidizing sulfide minerals and resistance to the extremal conditions. Apparently, in natural conditions numerous populations of genetically stable mutants are adapted to the concrete environmental factors (type of sulfide mineralization, pH, metal concentration). The variability level determines the rate of oxidation processes occuring in ore deposits.

More active strains of **T.ferrooxidans** can be isolated during dump, tank and underground metal leaching where the oxidation processes are active. A new ecological niche characterized by a higher metal concentration and lower pH provides conditions for more active and metal resistant strains of **T.ferrooxidans** and other bacteria (see Table 2.1).

Table 2.1

Characteristics of T.ferrooxidans strains [173]

Parameters	Initial strain BKMB-458	Active strain
Number of cells in 1 ml solution or pulp	10^6	10^9
Efficiency of Fe^{2+} oxidation, %	100	300
Coefficient of bacterial efficiency in oxidizing sulfide concentrates, μl O_2 per 1 min	0.1	2.0–3.5
Copper concentration suppressing cells activity by 30–40 %, g/l	1.5	15.0
Zink concentration suppressing cells activity by 30–40 %, g/l	8.0	100.0
Time for establishing equilibrium between cells in solution and adsorbed on minerals, h	120–300	1–5

The classical genetic methods of obtaining active strains of **T.ferrooxidans** and other acidophilic bacteria are not adequately elaborated, although a great interest has been displayed [77–85]. The strains with the following main characteristics are used as initial ones:

a) high iron- and/or sulfur-oxidizing activity;

b) reproducible growth on solid nutrient media or membrane filters;

c) considerable natural variability.

Natural variability is determined by flating 0.1 ml of diluted cell suspension on solid media containing Fe^{2+} or S^0 as an energy source. Oxidative efficiency of a single variant, i.e. of each single colony grown on solid media is determined upon inoculation on liquid medium with the similar energy substrate and after comparing its efficiency with that of the initial strain taken as 100 %. The variants are distributed into separate classes at the intervals equal to 20 %. Only the strains with high variation coefficients are selected to undergo the induced mutagenesis.

The procedure starts with the preparation of a washed cell suspension of bacteria being at the end of their logarithmic growth phase. Until now the following mutagens were used to obtain **T.ferrooxidans** mutants: UV rays, ethylenimine, and N-methil-N'-nitro-N-nitrosoguadine. The following dosages of mutagens were found to be the most effective: 100 to 160 J/m^2 for UV rays, 0.04 to 0.06 % at 1 to 3 hours of exposition for ethylenimine, and 100 μg/ml at 10 to 30 min of exposition for nitrosoguanidine.

Following the exposure to mutagens 0.1 ml of cell suspension is flated on solid media containing Fe^{2+} or S^0 in order to determine the survival rate of cells as well as to identify the mutants. The survival rate is determined on the basis of comparison with that of the untreated cells. Mutants with high iron- and/or sulfur oxidation efficiency are identified by the total collection method or using a method based on different rates of colony formation of single variants grown on solid media. In the latter case, only those colonies are selected that were the first to grow on media, as the activity of cells forming these colonies was found to be the highest. Upon selecting the most active variants they are subcultured not less than 3–4 times on the liquid media 9K with Fe^{2+} or S^0. Prior to each successive subculturing their activity is tested. Only stable mutants are used as selection material at further stages of this step-wise selection. Selection based on the combined use of the above-mentioned mutagens, as well as on the natural variability of bacteria enables obtaining the mutant strains with oxidation efficiency exceeding that of the relevant wild strains.

2.5.3. Genetic engineering methods

Obtaining highly resistant and active strains of thiobacilli, particularly **T.ferrooxidans** using the genetic engineering methods is being studied in a number of laboratories. Scientists have discovered the plasmids – carriers of the genes making **T.ferrooxidans** resistant to Ag, Hg and U. They study plasmid biology and work out methods for obtaining the genetically modyfied thiobacilli [106, 140, 181, 224].

In future these bacterial strains will be possibly used in hydrometallurgy.

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Chapter 3

METHODS FOR EVALUATING BACTERIAL LEACHING OF METALS

The present Chapter considers the methods for solving technological problems to be met in designing and practical application of bacterial leaching of metals.

The bacterial leaching technology implies as the first step the amenability study of ore for bioleaching.

An important technological aspect is to intensify bacterial leaching processes which is attained by using concentrated or immobilized **T.ferrooxidans** biomass and by studying the minerals properties.

It is also important to master the methods of statistical processing of results and economic evaluation of microbiological leaching of metals.

3.1. LEACHING TECHNIQUE

3.1.1. Amenability study

The amenability of an ore to bioleaching is conditioned by its mineralogical and chemical composition. The known chemical elements likely to be toxic to **Thiobacillus ferrooxidans** and their toxicity levels are reported in the literature [72]. The knowledge of the chemical composition of the ore is therefore a preliminary step which enables to predict any difficulties the process might come up against. It is well known, for example, that certain types of sphalerite contain high concentrations of mercury which may exceed the toxicity level in the leach solutions.

The mineralogical composition of the ore, on the other hand, provides information on the mineral species present. On the basis of the mineralogical and chemical composition, the percentage of mineral species contained in the ore can be estimated. In this regard the base-metal and iron sulfides, carbonates and clay minerals as well as their intergrowths are of major importance. Once the base-metal sulfides contents are known, the maximum amount of metal values recoverable by bacterial leaching can be estimated. The knowledge of the base-metal oxides contents allows to predict the additional metal values produced by leaching supported by the sulfuric acid generated by bacterial activity. The base-metal oxides and carbonates contents combined, provides information on the effect that sulfuric acid consumption may have on the process as a whole. Finally, the amount of clays present allows to evaluate,

although only qualitatively, the importance of any change in rock mass permeability likely to take place during the commercial process.

The presence of different base-metal sulfides may also considerably modify the leaching kinetics of the desired metal due to electrochemical effects. It is well known, in fact, that in addition to galvanic effects, resulting from two different metal sulfides being in close contact, base-metal sulfides solubility is affected by the differences in their electrode potentials. Several papers have been published on this subject and electrochemical nobility scales have been proposed [11, 45, 46, 62, 64, 73]. The relative locations of the metal sulfides in this scale furnish general information on the predictable effects on dissolution rates. However, it should be pointed out that natural doping of the metal sulfides by trace elements – which usually differ from one orebody to another – can lead to significant deviations from this scale. Furthermore the measurement of electrode potentials requires rather sophisticated equipment not usually available in industrial laboratories.

The most convenient way of assessing the amenability of a sulfide ore to bioleaching – once its chemical and mineralogical composition is known – is bench-scale testing. These tests are simple, relatively inexpensive and in most cases yield reliable information.

3.1.2. Shake flask testing technique

Test equipment consists of pyrex-glass Erelenmeyer flasks and of a shaker, which can be more or less sophisticated, consisting basically of a platform with several flask clamps, to which a reciprocating or rotary plane motion is imparted by an appropriate drive mechanism. The device is called "reciprocating shaker" when the motion imparted to the platform is reciprocating and "rotary action shaker" when the motion is rotary. Both devices shake the suspension, ensuring mixing and homogenization as well as the agitation of its surface, thereby enhancing the dissolution of atmospheric oxygen and other gases (for instance carbon dioxide) utilized by microorganisms for their metabolism.

The rotary shaker is preferable to the reciprocating shaker especially if, as in the latest models, stroke and speed are adjustable, since the suspension is uniformly agitated in all directions, assuring a higher degree of homogenization than reciprocating shakers. One drawback of the rotary shaker is that circular streams of suspension are generated inside the flasks; the mutual positions of the mineral particles and the liquid medium change only slightly resulting in unsatisfactory homogenization. One means of solving this problem is to mould the bottom of the flasks so as to form two diametral ridges at right angles with each other which act as baffles.

In order to determine reliable kinetic parameters bioleaching tests in shake flasks must be carried out under carefully controlled temperature and environmental conditions. For this purpose the shaking platform is enclosed in a thermostated, air-tight hood, into which the gas mixtures most suitable to microbial activity (in the case of **T. ferrooxidans** air + CO_2), are introduced at predetermined flow rates.

In current practice shake flasks tests are carried out in 250 cm^3 Erlenmeyer

flasks to which 100 g of pulp, composed of the culture medium, 10 g of ore ground to the desired size and 1–15 ml of inoculum are added. The shaking rate is usually adjusted to between 250 and 300 strokes per minute.

When the ore contains carbonates it can happen that the pH of the pulp rapidly increases from the value of the culture medium (which is usually adjusted to 2.2–2.5) to values at which the **T.ferrooxidans** activity is practically inhibited. In such case, preliminary tests should be carried to determine the amount of acid needed to stabilize the pH at the desired value. These tests can be performed in a 250 cm^3 beaker placed on the plate of a magnetic stirrer. Drops of sulfuric acid solution are added from a burette to a given amount of pulp (ore + culture medium) and the pH is constantly monitored with a pH-meter keeping the electrodes in the pulp. Once the pH of the pulp remains steady within 2.2–2.5 for several hours after the last addition of sulfuric acid, the system can be considered stable and the amount of sulfuric acid added may be taken as a reliable estimate of the highest possible acid consumption of the ore.

In order to assure a minimum of statistical reliability of the results, tests should be carried out on at least three identically prepared flasks.

At the beginning of the test, after determination of the pH of the pulp and sampling for assay, the flask is weighed and the weight recorded on an appropriate data sheet, an example of which is shown in Table 3.1.

The flasks are then clamped to the shaker platform and the apparatus is strated up. Agitation is interrupted at fixed time intervals and the weight of the flasks recorded. The initial weight is restored by adding distilled water

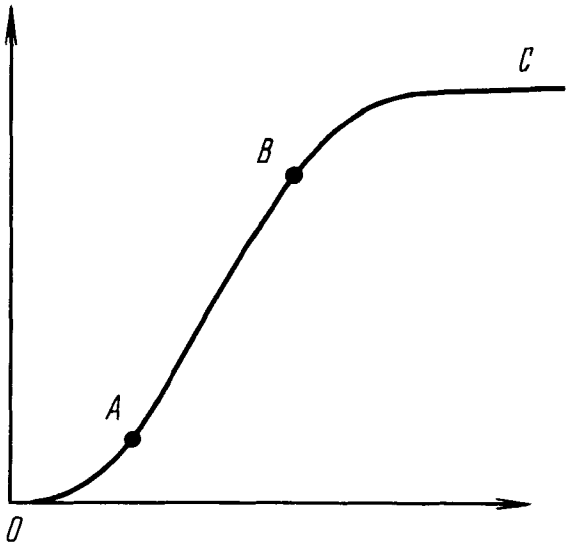

Fig. 3.1. – Shape of metal concentration vs. time in shake-flask metal sulfide bioleaching

Table 3.1

Shake flask test

ore..... particle-size distribution (graph No.....) incoculum strain.....

sample weight..... culture medium..... amount of medium..... inoculum size.....

Flask No.	Year	Month	Day	Hour	T_I °C	T_S °C	Mass*, g	Mass*, after adj., g	pH	Eh	Cu P.P.M.	Zn P.P.M.	Fe P.P.M.	Remarks

*Mass of flask + pulp – under "Remarks" indicate size of sample for assays.

to compensate for evaporation. Then the pulp is thoroughly mixed and allowed to decant on the shaker platform at rest. The pH is measured by dipping the electrodes into the pulp (or, better still, a single combination electrode). A 1 cm^3 sample of the supernatant is then taken and analysed for chemical assay. The results are recorded either in a Table 3.1 or used to plot a metal assay and/or pH vs. time curve which usually exhibits a more or less pronounced "S" shape (Fig. 3.1). Sections OA, AB and BC of this curve correspond to the lag-, exponential- and asymptotic growth phases respectively.

The slope of the straight line tangent to the exponential part of the curve is a measure of the solubilization rate of the metal value represented in ordinates.

It should be pointed out that if the bacteria are not adapted to the ore to be tested, one test of the type outlined above may not be sufficient to characterize the amenability of the ore to bioleaching.

Bacteria are adapted to the ore through a series of inoculations. As a result the lag-phase is considerably decreased, while the rate of bacterial growth and leaching rate increase (Fig. 3.2).

In order to shorten testing times the ore is ground very finely, usually to minus 40 μm. This size bears no relation to the much larger sizes of the broken rock dealt with in in-place, dump and heap leaching.

No information can be gained from shake flask tests on in-place, dump or heap leaching kinetics and/or final metal value recovery, since kinetics and recovery are markedly affected by grain-size distribution. For this reason, it is often preferable to work with concentrates obtained in the laboratory by gravity separation or by flotation. In the latter case flotation reagents residues should be carefully eliminated, since it has been proved that flotation reagents can be determental to bacterial activity [72]. Shake flask tests may last for several days or weeks.

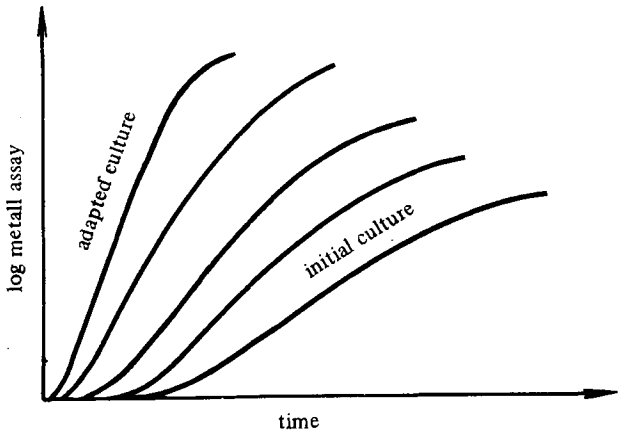

Fig. 3.2. – Change in shape of metal-concentration vs. time curves during adaptation T.ferrooxidans

3.1.3. Air-lift percolation tests

In in-situ, dump and heap leaching the solutions usually percolate through an immobile broken rock mass: laboratory devices which simulate this process – be it on a reduced scale – must be designed and operated such that the leaching conditions are faithfully reproduced.

The air-lift percolator, depicted in Fig. 3.3 can be considered the smallest and simplest of this class of devices. It consists of a 50 mm diameter column 6, ranging in height from 30 to 50 cm, with an open top, divided into two sections by a constriction 8, located near the bottom, which forms the support for a porous glass diaphragm 7. Pulp samples can be periodically drawn off through pipe 12 at the bottom of the column. A glass pipe 13 connects pipe 12 to pipe 10 which runs parallel to the column. The top end of pipe 10 enters the column 6 a few centimetres below the open top, whereas its bottom end is open and can be connected to a compressed air line. An injector 9 is inserted at the junction of pipe 10 with pipe 13. When compressed air is pumped through pipe 10 the leach solution is emulsified and saturated with air or the desired gas mixtures, rises up pipe 10 and returns to the column 6. The section comprised between the porous glass diaphragm and the bottom of the column is filled with glass wool. The ore can be stratified in the column so as to simulate the arrangement of particles in the fragmented rock mass. Several operating variables, among which solid-to-liquid ratio, optimum duration of wet and dry cycles, percolation rate, temperature, gas type and flow rate, acidity, redox potential and composition of leach solution can be accurately

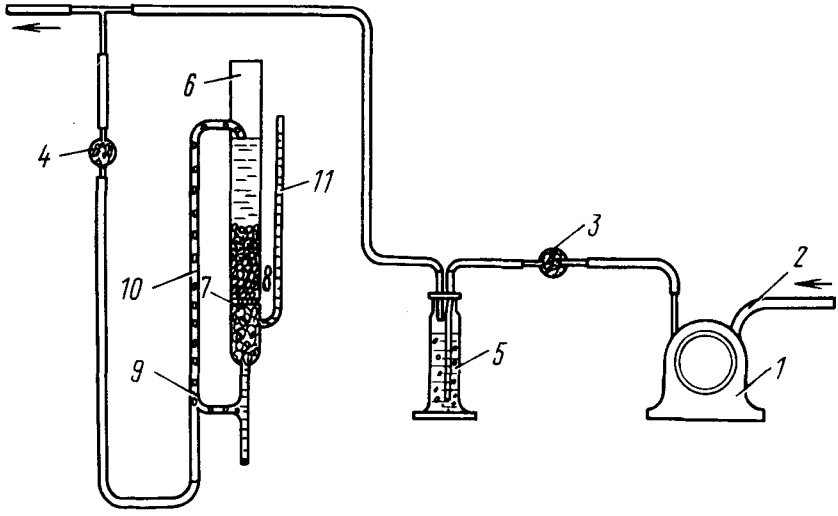

Fig. 3.3. Air – lift percolator:
1 – air compressor; 2 – intake pipe of air compressor; 3, 4 – glass-wool air filters; 5 – air-washing bottle; 6 – column of percolator; 7 – fritted glass diafragm; 8 – construction; 9 – injector; 10 – air-lift; 11 – sidearm

adjusted and controlled. Air-lift percolators can be arranged in series; in this set up the flow of solutions is accomplished for instance by means of peristaltic pumps. The ore charge of a percolator of the type depicted in Fig. 3.3. can range from 150 to 300 g and average grain size from some tenths of a millimetre to 10 mm. Coarser particles are not advisable on account of the fact that the number of particles per cross-section of the column diminishes to such an extent that flow becomes very irregular. This factor, combined with the inhomogeneous composition of each particle, may yield unreliable data. The column can be designed with orifices for inserting thermometers, electrodes, etc.

It goes without saying that correct operation of the device requires the periodic addition of distilled water to compensate for evaporation and that solution sampling should be done only after solution volume has been restored. The device is small enough to be sterilized by autoclaving.

Tests with air-lift percolators yield reliable data on acid consumption, metal solubilization, interactions between different minerals, pH and Eh behaviour, temperature, effect of gas mixture composition and transformations undergone by the run-of-mine ore during leaching. However, it should be mentioned that the results obtained with this technique cannot be used for scaling up to commercial operations, which are carried out under very different conditions. Air-lift percolators cannot simulate commercial operations for a number of reasons, the main one being related to particle-size distribution. The average size of rock fragments involved in the commercial operations often exceeds 50 cm, whereas the diameter of air-lift percolators ranges from 50 to 100 mm. Consequently, the ore used in the tests must be comminuted to a few millimetres average particle size and as is well known particle size is one of the major factors affecting bioleaching kinetics. Hence the column testing is used during the stages of work to follow.

3.1.4. Column testing

In principle, this type of testing does not differ greatly from air-lift percolator testing. The apparatus is quite simple (Fig. 3.4) and its size can vary considerably. The device consists of a vertical column 1, usually of P.V.C. or perspex, supported by a steel trestle. A perforated perspex plate 3, supported by brackets of the same material welded to the inner wall of the column, is located at the bottom end.The ore charge sits on this plate and can fill the column up to a few centimetres from the top. Leach solutions are prepared in a vessel situated under the bottom of the column which also collects the solutions discharged. Leach liquor is circulated by means of a peristaltic pump 2 with adjustable flow rate: the suction tube is placed in the vessel and the discharge tube is connected to spray nozzles for even distribution of the leach solutions over the ore charge, through which the solution percolates. A variable-speed stirrer 4 ensures solution homogeneity in the collecting vessel. Solution pH is measured by electrodes 6 introduced into the vessel 5 and connected to a pH meter 7 which automatically adjusts solution acidity by means of either a control circuit 8 or a computer. The latter actuate the pump 10 which feed acid or alkaline solutions 9 into the

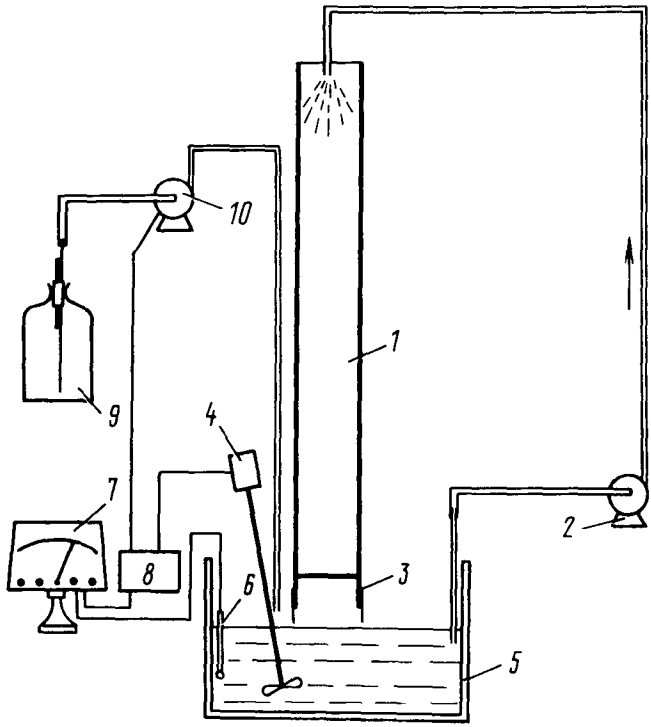

Fig. 3.4. Column testing:
1 – column; 2 – peristaltic pump for leach-solution circulation; 3 – perforated plate; 4 – stirrer; 5 – leach-liquor collecting vessel; 6 – electrode; 7 – pH-meter; 8 – control circuit of computer; 9 – vessel for adjusting pH solution; 10 – peristaltic pump for solution after pH adjustment

vessel, depending on the programmed pH value. Inspection ports can be put in at various heights up the column. These can be used for taking samples of rock and/or leach solutions and for inserting measuring probes into the ore charge, such as, for instance, electrodes and thermocouples.

This technique is better suited to simulating commercial operations such as in-place, dump and heap leaching than air-lift percolators, on account of the fact that the size range of the ore charge can be chosen so as to be representative of that of the commercial operation.

However, experience has shown that the largest lump of the charge should not exceed 20 % of column diameter. In addition, the actual height of the broken rock masses used in commercial bioleaching operations may be as much as several tens of metres, whereas that of a laboratory column does not usually exceed 2–4 m due to the restrictions imposed by laboratory size. The arrangement in series of several columns also allows to simulate dump and heap height with a remarkable degree of similarity and, at least as far as their depth

is concerned, on a real scale. Columns made of glass fibre and stainless steel have also been employed in bioleaching tests. For chemical leaching tests with alkaline solutions, rubber-lined steel columns can also be used. Despite the fact that column geometry undoubtedly affects to a considerable extent process performance, no standardization of column design and operation has yet been attempted. The height-to-diameter ration ranges from 3 to 40, the height of a single unit between 1500 and 6000 mm, the mineral charge from 3 to 1400 kg and the ration of column diameter to average charge particle size from 3 to 40. Data on leach solution circulating loads and flow rates – which depend in any case on specific conditions – are much more scarce. The columns can be thermostated either by means of a suitable heating tape or by keeping them in temperature controlled ambients. The latter solution is preferable especially when the test is performed with several columns, in the first place because temperature is easier to control and secondly because operation is facilitated in conditions of controlled atmosphere. Column bioleaching tests may last as long as four years: this undeniable slowness, which after all is a typical feature of actual commercial operations, is amply offset by the reliability of the results. Moreover, investment costs are relatively low and recourse to much more expensive semi-commercial pilot scale testing on heaps of several hundreds or even thousand of tons of ore can be obviated.

3.1.5. Test heaps

Although column leaching tests usually provide a wealth of information on metallurgical process parameters, often sufficient for designing a commercial-scale operation, no data can be obtained on other aspects of the process such as changes in broken rock mass permeability, evolution of microorganisms and temperature profile patterns within the broken rock mass. It may therefore be advisable, especially when dealing with reserves of run-of-mine ore, considered exploitable by bioleaching, of several million tons, to carry out a final, semi-commercial test prior to embarking on a commercial-scale operation. This test usually consists of setting up a plant, comprised of the heap to be leached, two ponds, one for the preparation of the solutions sprayed onto the heap and the other for collecting the pregnant liquor discharged, as well as a metal recovery section. The latter may consist either of a cementation plant or a pilot solvent extraction plant.

The heap is truncated pyramid in shape, the controlling dimension being its height, which can range from 2 to 3 m when the particle size distribution of the run-of-mine ore is that or a gravel and sand mixture, to as much as 7–8 m when the greater part of the ore is constituted of 100 mm lumps or larger. The volume of the heap can range accordingly from a few thousand to 15.000 tons.

The area where the heap is to be erected must be carefully prepared. In the first place the ground, which should be level and gently sloping (from 4 to 6 % dip) to one side, should have a degree of compaction ranging between 80 and 120 Proctor. This requirement can be met by stabilizing the soil with a vibrating compactor. The flatness of the heap foundation throughout the test is a necessary requisite for the regular and uniform discharge of the pregnant

liquor from the bottom of the broken rock mass and also for preventing stagnant liquor zones forming in the bottom of the heap since these could alter the flow pattern and the regular evolution of the process. A 15–30 cm thick layer of fine sand (flotation-plant tailings are quite suitable) is spread over the level and stabilized soil. A waterproof sheet, for instance of buthyl plastic reinforced with glass fiber from 1 to 2 mm thick, is laid on top of the sand. To avoid punctures, this sheet should then be covered with a layer of fine sand. The permeability of the bottom of the heap is ensured through a final layer of fragmented waste rock composed of 50–150 mm lumps, into which perforated plastic drainage pipes are inserted at regular intervals. The effluent pregnant liquors are collected in a drainage ditch also lined with a waterproof sheet. The liquor flows from the ditch into the collecting pond. No methods have yet been proposed for calculating the volume of the preparation and collecting ponds. As a rule of thumb, which has proven valid in several commercial cases, the volume of the preparation is assumed to be at least twice the daily irrigation flow rate whereas that of the collecting pond should be from 3 to 5 times that of the preparation pond. Both ponds should be lined with a polyethylene sheet. Irrigation of the heap is accomplished by means of spray nozzles inserted at regular intervals in the plastic pipes placed on the flattened top. The selection of nozzle type, distance between each nozzle and each pipe depends on availability of equipment and on the particular test conditions. It is, however, imperative that nozzles be arranged in such a way that the whole of the top of the heap is uniformly irrigated.

Uniform grain-size distribution and uncompacted broken rock are essential for successful leaching. To avoid compaction of the ore during heap construction, the broken rock should be distributed on the heap by means of an overhead conveyor belt and tripper from a bridge unit. Temperature inside the heap, pH and Eh of the leach liquors during the downward flow can be measured by means of small diameter wells (no larger than 100 mm) appropriately located in the heap. Combined with the results of periodic analyses of microorganisms composition at various heights of the heap, these measurements also provide indirect information on the aeration pattern of the broken ore mass. Trenches can be dug, at suitable time intervals, near the two ends of the heap so that the test can continue practically undisturbed. These trenches allow the visual examination of the evolution of the leaching process inside the heap and, in particular, to detect the occurrence of salt precipitations and formation of clay layers which alter (often very detrimentally) the permeability and flow patterns of the liquors. This type of test is also very suitable for determining the most convenient alternation of irrigation and dry periods.

Chemical and mineralogical analyses of rock samples taken at suitable time intervals complete the operation. These data are used to predict the parameters underlying the construction and operation of the commercial-scale heaps.

3.1.6. Tank leaching

When the run-of-mine ore requires comminution down to sizes below one millimetre, to achieve satisfactory liberation and exposure of the mineral

grains, percolation leaching efficiency rapidly deteriorates, since the liquid flows along differential paths whose formation is favoured by the presence of gas bubbles entrapped in the granular mass. In order to achieve contact between the liquid phase and all the mineral grains, the pulp (leach solution + ground ore) is stirred. Microbial tank leaching may probably be successfully applied to highgrade metal-sulfide run-of-mine ores, but no examples of this type of application are reported in the literature. Several attempts have however been made to recover metal values by microbial tank leaching of metal-sulfide concentrates. The process is performed in "stirred tanks" or "reactors" consisting of vessel where the pulp – composed of the sulfide ore or concentrate, the liquid culture medium and the microorganisms – is stirred either mechanically or by gas injection or by both.

Leaching is usually carried out continuously and in several tanks arranged in series. A very convenient feature of tank leaching is represented by that fact that the size of the plant has little effect on results as far as kinetics and metal recovery are concerned. Consequently, the amenability of a run-of-mine ore or a concentrate to microbial tank leaching can be evaluated using small-scale laboratory equipment. The reader is referred to Chapter 4 for further details. It should however be pointed out that the shake flask method (described in Section 3.1.2) is a valid means of preliminary testing for this type of leaching; since it provides very useful information of optimum bioleaching conditions (pulp density, culture medium composition, gas supply) and on the results attainable (solubilization rate and metal value recovery) even though only small amounts of leachable solids are required, of the order of tens of grams.

3.1.7. Scale-up

The objective of the testing is to:

– collect quantitative process data operating with laboratory apparatus or pilot plants and

– develop mathematical models for designing the commercial scale plant.

All of the testing techniques described in this chapter furnish reliable process data. The development of mathematical models can, however, pose some problems to industry technicians on account of the many parameters involved in a bioleaching process. As a matter of fact, the process kinetics are complex, due to the combination of at least two kinetics which interact, i.e. microbial growth and mineral solubilization kinetics.

Air-lift percolators, column and pilot-heap tests, carried out in this order, all lend themselves to scale-up of commercial heaps or dumps. In fact optimum grain-size distribution of the run-of-mine ore, irrigation method and frequency and composition, pH and flow rate of the leach solutions as well as optimum height of the heap are the same as those determined by the tests. Up to the time of writting no scale-up method has been proposed for microorganisms composition and temperature evolution and profiles within the heap. The mathematical scale-up of tank leaching does not appear to be very simple. The exhaustive information provided in Chapter 4 can be considered more than sufficient from the practical point of view, which is, after all, the approach on which the present manual is based.

3.1.8. Extraction of metals from solutions

Extraction of metals from productive solutions in dump, underground and tank leaching can be performed by sedimentation (electrolysis, cementation, crystallization and solid residue formation), as well as sorption and extraction. The above-mentioned techniques are well-known and are not considered in this book. Recently the biosorption methods of metal extraction from solutions are being elaborated (see Chapter 7).

3.1.9. Determination of grain size, grinding of ores, concentrates and leaching products

During laboratory tests in order to characterize the grain size of ores and concentrates they are screened into sizes which is referred to as the grain-size composition. It is usually determined by a screen analysis when a weighed portion of the material in question is sieved through a set of screens with mesh size ranging from 25 to 0.020 mm; finer materials can be subject to a sedimentation (slime) analysis or a microscopic analysis [39], or else, can be sieved through precision screens (100–5 μm). Special devices and instruments can be used such as those produced by the FRITSCH company (FRG) – Analysette-20, a scanning photosedimentograph, designed to measure grain sizes from 1 to 200 μm, Analysette-21, a pippete centrifuge for grain size analysis in the range from 0.05 to 5 μm, and Analysette-22, a laser device for measuring particle sizes from 1 to 900 μm. Granular materials can be subject to dry or wet screening. Dry screening is used when the size of particles is rather large – 2.5–0.5 mm; when finer materials are subject to dry screening, special weighting materials can be placed on screens with a small mesh size (0.038 – 0.020 mm) to speed up the process and break aggregated particles. Wet screening, however, gives more accurate results. In wet screening water is supplied through sprinklers, such as Analysette-3 onto a set of screens placed on a vibrotable. The water breaks aggregated particles. The results of screening are represented as a dependence of the partial/cumulative yield on the mesh size. An example of a concentrate sample screening is given in Table 3.2. The grain-size composition obtained is shown in Fig. 3.5.

Table 3.2

Screening of a concentrate sample

Mesh size, mm	Grain size, mm	Partial yield		Cumulative yield	
		g	%	plus %	minus %
1.6	+1.6	0.2	0.0	0.0	0.0
1.2	–1.6 +1.2	45	15	15	100
0.8	–1.2 +0.8	60	20	35	85
0.4	–0.8 +0.4	90	30	65	65
0.2	–0.4 +0.2	45	15	80	35
–	–0.2	60	20	100	20
Total:		300	100	–	–

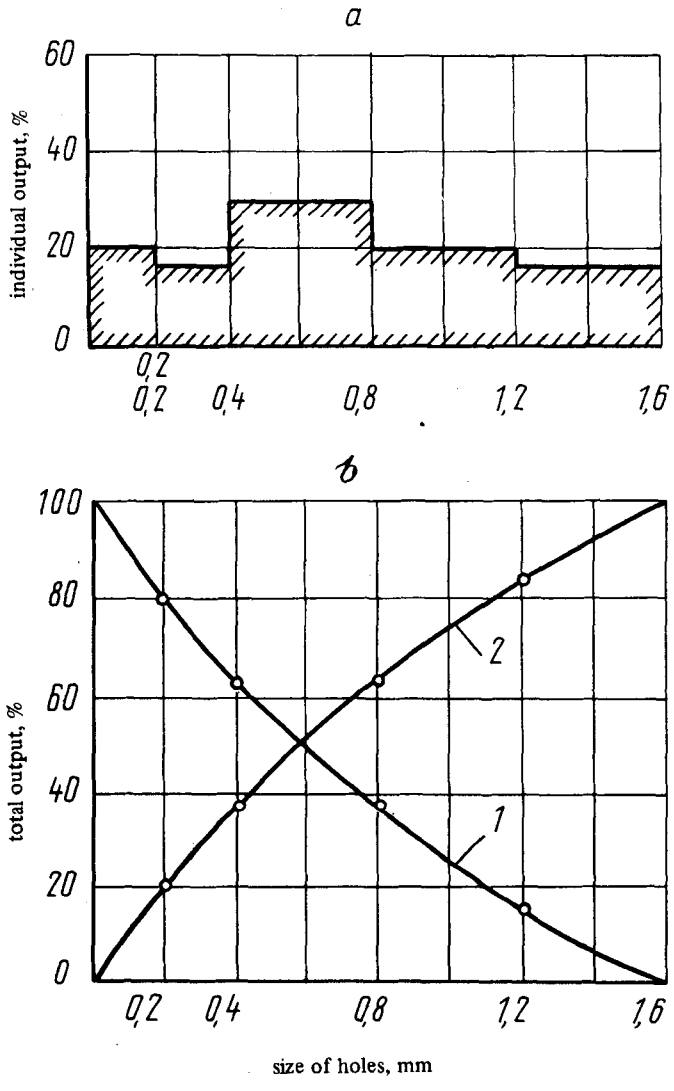

Fig. 3.5. Grain-size composition of the product:
1 – "minus"; 2 – "plus"

The analysis is carried out as follows. A concentrate sample of 300 g is placed on a set of screens with the mesh size (from top to bottom): 1.6, 1.2, 0.8, 0.4 and 0.2 mm, provided with a tray and mounted on a vibroshaker. After the vibroshaker has been switched on, the sample is screened for 30 min. After that the material of grain size –0.2 +0.0 mm is removed from the tray

and the remaining part is screened for another 10 min. If no material is found on the tray after the control screening, the material that has accumulated on each screen is weighed. When the partial yield is determined in per cent of each grain size, the total yield is obtained as well as the grain-size composition of the product in question (Fig. 3.5).

A product as a whole can be characterized by a per cent content of some grain size class, for example, 65 % minus 0.8 mm or 85 % minus 1.2 mm.

The above grain-size analysis which is traditionally used when dealing with granular materials cannot be applied if the products were subject to bacterial leaching. In the process of bacterial leaching bacteria and their metabolites are adsorbed on the mineral surface, which results in the formation of strong aggregates when the pulp sample has been dried. To prevent the distortion of grain-size composition, it is necessary to remove surface-active substances from the surface of the mineral particles. This is done by washing the sample in hydrochloric acid to remove acid-soluble sediments followed by washing in water and an alkali (for example, KOH). The sample is then rinsed in water till it becomes neutral and dried in the air. Then the grain-size composition is determined as described above.

If the grain-size composition of the product is not fit for the experiments to be carried out, **the product sample may be subject to additional grinding.**

Preliminary crushing of lumpy material in laboratory conditions is carried out by means of roll and jaw crushers, while fine grinding is done in ball and planetary mills like Pulverisette by the FRITSCH company (FRG). The grinding is effected as follows: the mill drum is loaded with a certain amount of balls and material to be ground. The amount of the balls and the material is determined experimentally in each particular case. The material is then ground for 10 min. After that the grain size of the product is determined to specify more accurately the duration of grinding. When necessary, the product may be subject to wet grinding at the solid phase content of the pulp of 50–70 %.

On a semi-industrial and industrial scale, the materials are subject, as a rule, to wet grinding in ball mills operating in connection with a classifying apparatus – either a spiral classifier or a hydrocyclone. If a desirable grain size is not obtained in the discharge from the classifier after one cycle of grinding, the material obtained is thickened, if necessary, and subject to additional grinding.

It should be noted that in tank bacterial leaching the products used are finely milled – 90 % minus 0.030 mm and even 90 % minus 0.015 mm. It is very difficult to obtain these classes by means of grinding in ball mills, therefore mechanoactivating devices, gas jet mills and various attritional grinders with hard-alloy fittings have been used of late for fine and super-fine grinding [22].

3.1.10. Analytical method of determining the chemical composition of ores, concentrates and leaching products

Different instrumental methods are used nowadays to analyse the elements, i.e. molecular spectroscopy, electron diffraction and X-ray diffraction analysis.

Many companies, for example the Swedish Perkin-E., advertise in their catalogues the atomic absorption, mass spectrometric, X-ray, plasma analysers and other equipment.

3.2. METHODS OF STUDYING THE ORE AND MINERAL COMPOSITION

As noted in Section 3.1.1, the knowledge of chemical composition of ores enables us to solve a number of important technological problems. Of no less importance is the knowledge of oxidation mechanisms of sulfide minerals and their association. This is achieved by studying the physico-chemical properties of these minerals.

Below is the brief description of sulfide minerals and methods of their studying.

3.2.1. Characteristics of sulfides

An ore deposit usually contains ore and non-ore minerals. The term "ore deposit" as a rule suggests a deposit of non-ferrous metals in which the ore portion is represented chiefly by sulfides, arsenides and sulfoarsenides. The sulfide fine structure (chemical bond type, valency of anions and cations, defects, etc.), semiconductor properties and electrochemical characteristics have a strong effect on the activity of microorganisms contacting with sulfides. Dissolved non-ore minerals (usually quartz, carbonates, sulfates) influence the chemical characteristics (Eh, pH) of aqueous solutions inhabited by bacteria. Particularly strong effect is produced by carbonates such as a calcite, dolomite, and magnesite present in the primary ore. They are relatively easily dissolved at low pH ($CaCO_3 + H_2O \rightarrow [CaHCO_3]^+ + OH^-$) thus resulting in an increase of pH of the leaching solutions and the death of microorganisms [78].

Textural and structural characteristics of ores are important for the development of bacteria. Textures resulting from minerals' crushing and attrition enhance the bacterial activity. The formation of suspensions which is important for bacterial oxidation of sulfide minerals owes not only to the tectonic and technical crushing of ore but also to physico-mechanical properties of minerals, particularly their brittleness. Brittle minerals more readily form natural suspensions which have an enormous surface area of particles and hence a high chemical activity. Highly illustrative in this respect is the following row of minerals arranged according to their growing specific surface (m^2/g) of the same fraction obtained after the same time of wet grinding on a planetary mill [40]: chalcocite (2.0 – bismuthine (2.4) – pyrrhotite (2.7–3.0) – cubanite (3.4) – antimonite (3.6) – bornite (3.7.) – pentlandite (3.7) – galena (4.3) – pyrite (4.4–4.9) – arsenopyrite (5.0 – chalcopyrite (5.6– cinnabar (7.3) – sphalerite (7.6– 9.7) – molybdenite (9.0)

Of no less importance are chemical (admixture distribution) and physical (doubles, etc.) heterogeneities of the sulfide grain surface, as well as the pattern of grain intergrowth. All these heterogeneities result in formation of local microgalvanic pairs which significantly affect the processes of electrochemical and bacterial corrosion of sulfides.

A detailed description of sulfide minerals' structure can be found in crystallochemical studies among which particularly noteworthy is the monograph by Below [6]. We shall briefly discuss only those structural characteristics of sulfides that are directly related to their biogenic and abiogenic oxidation. To illustrate, the formation of certain types of bonds in pyrite, FeS_2, pyrrhotite, $Fe_{1-x}S$, and pentlandite, $(Ni, Fe)_8{}^{IV}Fe^{VI}S_8$ is considered below.

Sulfides are semiconductor compounds with predominance of the covalent or, more precisely, the donor-acceptor type of bond. The energy level distribution of electrons in the atoms of Fe, Ni and S is as follows:

Fe^0: $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$

Ni^0: $1s^2 2s^2 2p^6 3s^2 3p^6 3d^8 4s^2$

S^0: $1s^2 2s^2 2p^6 3s^2 3p^6$

It can be seen that the atoms of Fe and Ni have incomplete subvalent electron shells (complete ones have 18 electrons): the Fe atom has 6 d-electrons instead of 10 and the Ni atom has 8 d-electrons.

Pyrite FeS_2. Fe^{2+} cation in pyrite lacks 12 electrons, e.e. 6 pairs to fill the 18-electron shell. Sulfur as the S^{2-} ion can give the metal ion only 4 electron pairs $[:\ddot{S}:]^{2-}$. However, two S^{2-} anions joined together form the so-called dumbbell sulfur $S_2^2 = [:\ddot{S}:\ddot{S}:]^{2-}$ which provides 6 electron pairs to the Fe cation. Such a pairing of sulfur atoms produces sufficiently strong bonds in the pyrite structure, which seems to account for the high pyrite resistance to oxidation.

In the sulfides where the metal atom needs more or less electrons than a sulfur atom can provide a slightly different type of interatomic bonding takes place.

Pyrrhotite $Fe_{1-x}S$. In pyrrhotite the Fe^{2+} cation needs 6 pairs of additional electrons (the acceptor electrons), and is located inside an octahedron, but each S^{2-} anion can provide only 4 electron pairs. The Fe-octahedrons, therefore, are arranged in columns so that two octahedrons have one common face (Fig. 3.6). The metal atoms thus become closer to each other and make up for the missing sulfur electrons by exchanging their own ones. The metal bond plays a more important role in this sulfide, which significantly affects its physical properties and makes it sufficiently susceptible to oxidation.

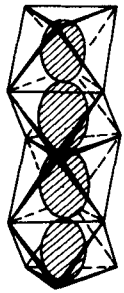

Fig. 3.6. Principal of pyrrhotite structure: columns of octahedrons with common faces through which Fe cations are linked by direct metal bond

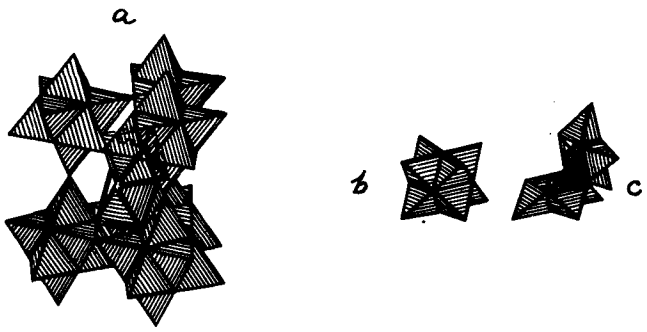

Fig. 3.7. Pentlandite structure:
a – NaCl-like packing of 8-member groups of Ni-tetrahedrons; b, c – a cubical cluster consisting of 8 Ni atoms inside a 8-member group

Pentlandite $(Ni, Fe)_8^{IV} Fe^{VI}S_8$. In pentlandite the nickel and some of iron atoms are surrounded by four S^{2-} anions, i.e. occupy a tetrahedral position. But the nickel needs five electron pairs (it has 8 d-electrons) while iron needs as much as six (see the electron configuration above). The lack of electrons in each atom is offset by joining of the (Ni, Fe)-tetrahedrons into eight-member groups ("stars"), the cluster pattern accounting for shorter distances between metal atoms and resultant increased electron exchange (Fig. 3.7). In pentlandite such 8-member "stars" alternate with Fe-octahedrons. The pentlandite electron structure, therefore, has sites with enhanced metal bonds and with a greater number of free, non-localized electrons. As a result, electrons are more readily released, as in the case of pyrrhotite, and the resistance to oxidation is reduced.

3.2.2. Electrochemical properties of minerals and methods of their study

The chief factors determining the intensity and direction of oxidation processes, including biogenic oxidation, are the electrode potential (EP) of an ore mineral and the redox potential (Eh) of the electrolyte (solution). The EP of a mineral (the potential jump at the mineral-electrolyte interface) is directly proportional to the work of electron exit from the mineral's crystalline structure and is a measure of its susceptibility to oxidation. The so-called electrochemical series of minerals in which the latter are arranged according to the EP values, are in general consistent with their resistance to oxidation and may, therefore, be regarded as a criterion of minerals' behaviour in a natural process. A mineral with a high Ep is less susceptible to oxidation than a mineral with a low EP under the same conditions, and when the two are in contact the latter becomes the anode. In the series that follows, for example, the sulfides are arranged according to their oxidability found experimentally: galena (0.30)–chalcocite (0.35)–sphalerite (0.35)–chalcopyrite (0.40)–bismuthite (0.40)–stannite (0.45)–pyrrhotite (0.45)–tetrahedrite (0.45)–arsenopyrite (0.50)–pentlandite (0.55)–pyrite (0.55–0.60) [79, 80].

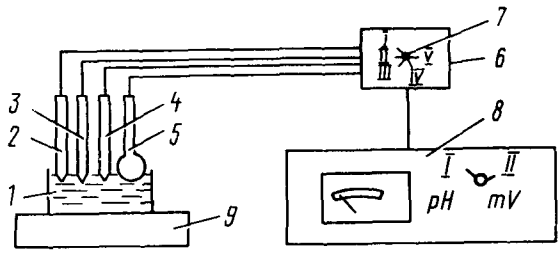

Fig. 3.8. A schematic diagram of installation for measuring electrode potential of minerals:
1 – galvanic cell; 2 – glass electrode; 3 – AgCl electrode; 4 – platinum electrode; 5 – mineral electrode; 6 – cable box; 7 – multiposition switch; 8 – pH-meter; 9 – magnetic stirrer

Besides, the study of EP of minerals under particular physico-chemical conditions offers quite realistic forecasts as to the initial oxidation products – the solution and early hypergenic products.

The measurement of EP of semiconductor minerals is carried out on a special installation which includes (Fig. 3.8):

1) a galvanic cell with the selected electrolyte and immersed electrodes (platinum, glass, silver chloride and mineral ones), placed on a magnetic stirrer;

2) a pH-meter, e.g. pH-340;

3) a radiotechnical type multiple switch for connecting the various electrodes to the pH-meter and for contacting mineral electrodes to each other.

4) a mineral electrode which is a polished section pressed into a cylindrical cement case 10-15 mm in diameter and 5-8 mm high, and in which the surface areas not intended for measurements are covered with paraffin. The section is connected to the pH-meter system with a platinum wire.

The installation can simultaneously measure the pH and Eh of a solution, as well as the EP of a mineral.

EP of minerals is measured in distilled water, and the recorded values, besides the EP, include variation of pH and the time in which the potential becomes stabilized. The values obtained for a number of minerals can then be compared. The experience of studies of this kind has been documentated in a number of works [74 –77].

A more widely used method of electrochemical research consists in measuring EP of an individual mineral-electrode at different pH values. The variation of pH is achieved by adding an alkali (NaOH) solution to the acidic solution (of sulfuric acid). Intervals of EP measurements are 5-10 min, the accuracy of measurements is ±10 mV. The measurements are done relative to the hydrogen half-cell.

The results are presented as EP-pH graphs to be plotted against the equilibrium diagram of the elements in H_2O at 25 °C [26, 74, 75]. For each section of the curves with the same slope, probable reactions of sulfide oxida-

tion are formulated to match the H^+/electron ration with the experimental data (the slope). The calculations are based on the well-known Nernst's equation:

$$Eh\ (EP) = Eh_0 + \frac{0.059}{n} \lg \frac{A_0}{A_y},$$

where n is the number of electrons in the reaction;

A_0 and A_y are the activity products of ions formed and participating in the reaction, respectively.

The drawing of the oxidation reactions should be made by taking into account the modern concepts of the forms in which the elements occur in diluted sulfuric acid electrolytes and the data of mineralogical observations. Such an approach will help make the choice of oxidation reactions less arbitrary, thus offering quite a realistic prediction of oxidation products at early stages of the process and a good basis for the understanding of chemical aspects of the bacterial oxidation of ores, minerals and concentrates. A more detailed account of the electrochemical method for drawing up mineral oxidation equations can be found in the works by R.M. Gurrels [26], R.M. Gurrels and C.L. Kraist [27], M.N. Pourbaix [59], L.K. Yakhontova and A.P. Grudev [74–76].

To illustrate equations for the oxidation of chalcopyrite ($CuFeS_2$), a very common sulfide in copper ores, are drawn below.

The experimentally obtained EP-pH graphs for chalcopyrite are plotted against the equilibrium diagram of Cu-Fe-S in H_2O at 25 °C (Fig. 3.9). The EP-pH curve can be divided into 3 sections (pH 1–3, pH 3–7 and pH 7–9) with different slopes. The oxidation process has different characteristics in each of these sections. Using statistical methods (the least square method), it is necessary to calculate the slope of each of the sections, i.e. the tangent of the slope angle for the section. This requires solving a system of two equations with two unknown quantities (see Section 3.4).

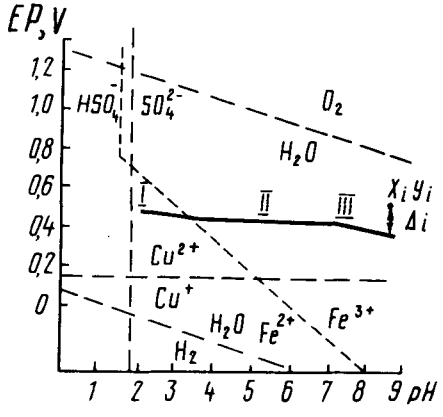

Fig. 3.9. An EP-pH graph for chalcopyrite plotted against the equilibrium diagram for Cu, Fe and S in H_2O at 25 °C

The calculation for the chalcopyrite curve slope in section I (pH 1–3) will give the value of about –0.020, in sections II and III (pH 3–7 and pH 7–9) the slopes will be 0 and –0.017, respectively. The theoretical Nernst's equation in our case will read as follows:

$EP = E^0 - \frac{0.059\ m}{n}$ pH (for individual activities of ions entering the reaction and produced in it), where:

E^0 is the standard electrode potential;

m is the number of H^+ ions;

n is the number of electrons

In this equation the value $\frac{0.059\ m}{n}$ pH will be the one describing the slope of the curve: $a = tg\alpha = \frac{0.059\ m}{n}$ pH.

3.2.3. Semiconductor properties of sulfides. The zone theory of crystal structure

The majority of sulfides belong to semiconductors by their electric properties. The zone theory of crystal structure offers a general approach to considering their structure in the energy aspect. The corresponding scheme of a semiconductor crystal is shown in Fig. 3.10. The lower zone, completely filled with electrons is called the valent zone. A zone of higher energy which is incompletely filled with electrons is called the conductivity zone. Between these two there is an energy gap, the forbidden zone.

The real crystals have defects of their crystal lattice in the form of foreign atoms, empty points, etc., the so-called admixture centers. The energy levels of admixture center electrons are located in the forbidden zone of energies [35, 43, 60]. They may supply electrons to the conductivity zone (the donor levels) or capture excited electrons from the valent zone (the acceptor levels). In the former case the electron (n-type) conductivity is manifest, and in the latter, the hole (p-type) conductivity. To illustrate, pyrite FeS_2 is consi-

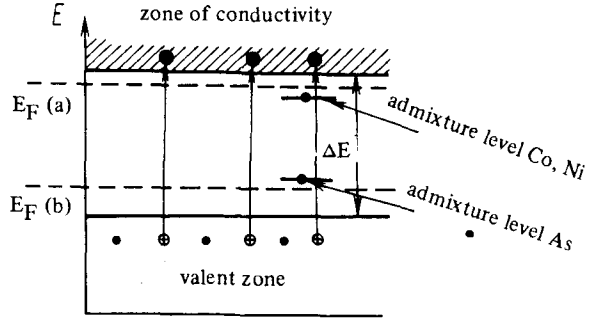

Fig. 3.10. A zone scheme of a semiconductor crystal; E_F (a) – Fermi level for electron-type semiconductor; E_F (b) – Fermi level for hole-type semiconductor; ΔE – forbidden zone

dered. The electron conductivity (n-type) of this sulfide is usually associated with cobalt and nickel impurities, which isomorphously replace iron [61, 63]. Iron has 6 d-electrons, cobalt, 7 d-electrons, and nickel, 8 d-electrons. Thus cobalt and nickel supply to the conductivity zone one or two free electrons, respectively, which take part in the conductance. The admixture levels of these metals are located near the pyrite conductivity zone (Fig. 3.10). The hole type of conductivity (p-type) in sulfides is due to incorporation of arsenic into their crystal lattice. Arsenic isomorphously replaces sulfur ($S^{6+} \rightarrow As^{5+}$), which produces an acceptor level located near the ceiling of the valent zone.

The type of conductivity in semiconductor crystals determines the position of the Fermi level, or the electro-chemical potential, a most important characteristic of sulfide semiconductors. In minerals with n-type conductivity the Fermi level is located near the conductivity zone or within it, while in minerals with hole conductivity, within or near the valent zone (Fig. 3.10). The Fermi level determines the thermodynamic work of exit of an electron from the substance. The work of electron release for a p-type semiconductor is greater than that for an n-type semiconductor nearly by the width of the forbidden zone. Therefore, oxidation of n-type sulfides should proceed more activity. The difference in the electron relase work between sulfides with different conductivity is important in bacterial processes of sulfide oxidation.

To illustrate these postulates, several examples are given below.

Fig. 3.11 shows results of experimental electrochemical oxidation of n- and p-type pyrite specimens. The EP-pH curves for the n-type pyrite (samples 1–6) are located below the curves for the p-type pyrite (samples 7–8). The general tendency, therefore, is that n-type pyrite may be oxidized at a markedly lower Eh of the solution as having lower EP values than the p-type pyrite.

Experiments on pyrite oxidation using **T.ferrooxidans** also showed a difference in the oxidation rates of n- and p-type pyrites. The n-type pyrite

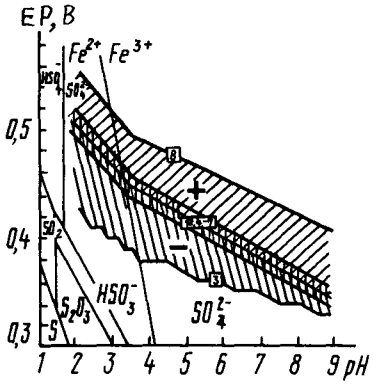

Fig. 3.11. EP-pH graphs for pyrite plotted against the equilibrium diagram for Fe and S in H_2O at 25 °C. Vertical shading shows an area where the majority of EP-pH graphs are located. Oblique shading shows the area of possible location of EP-pH graphs for the hole- (+) and electron-type (–) pyrite

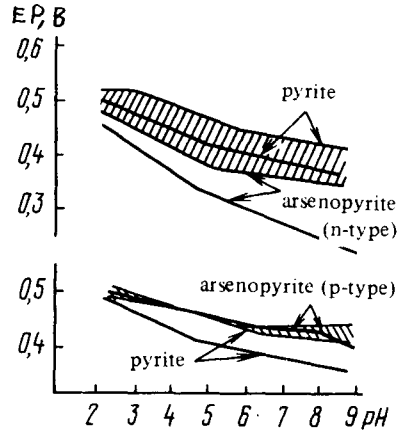

Fig. 3.12. EP-pH graphs for arsenopyrite with electron (n-type) and hole (p-type) conductivity in contact with pyrite. The potential difference in the pairs is shaded

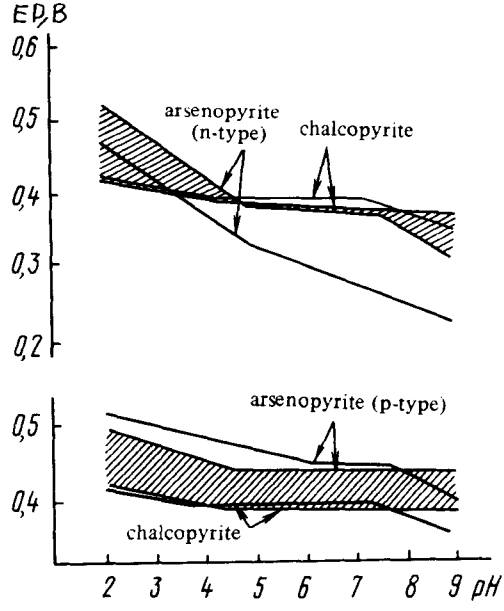

Fig. 3.13. EP-pH graphs for arsenopyrite of the electron (n-type) and hole (p-type) conductivity in contact with chalcopyrite. The potential difference in the pairs is shaded

as having a lower electron release energy, is oxidized at an initially high rate but the process rapidly slows down (as the conductivity zone electrons are exhausted). Pyrite of the p-type slowly enters the oxidation process, but the process lasts longer and is more stable.

Type of sulfide conductivity is particularly important in the case of ore oxidation. This allows forecasting the direction of oxidation, including bacterial oxidation, its intensity and possible products. For example, examination of EP-pH diagrams for a combined oxidation of n-type pyrite with n-type arsenopyrite under favorable conditions for the bacterial activity (pH<3) revealed that arsenopyrite as having a lower EP (0.45 V) compared to 0.55 V of pyrite will be the first to undergo oxidation. For p-type arsenopyrite the presence of pyrite has mainly a stabilization function, the potentials of the two minerals at pH<3 being rather close (Fig. 3.12). For the same pH range arsenopyrite of n- or p-type of conductivity in a combination with chalcopyrite will play the role of the cathode, thereby stimulating the chalcopyrite oxidation (Fig. 3.13). More complex (e.g. arsenopyrite-pyrite-chalcopyrite) combinations are of course possible, and will produce somewhat different results. Problems of this kind must be solved in each case individually, which should be taken into consideration when devising technological schemes for bacterial leaching of ores and concentrates.

3.2.4. Methods of investigating bacterial oxidation of sulfide minerals

Sulfide minerals are oxidized by microorganisms by an electrochemical (corrosion) process. The sulfide semiconductor plays the role of the anode. The process can be controlled by the electrode and redox potential and pH.

The method of studying bacterial oxidation of sulfides with the view of obtaining electrochemical parameters is a standard procedure, but has some specific features.

The initial sulfide mineral is ground to powder, and about 0.5–1 g of it is placed into a glass cell. To the latter 50 ml of a nutrient medium and 4 ml of a culture fluid with 10^5-10^8 cells/ml are then added. The initial pH should be equal to 2.5. A mineral electrode connected to the pH-meter system is also immersed into the cell. The cell is placed into a shaker kept in a thermostat at about 28 °C. Measurements of EP of the mineral and Eh and pH of the solution are made daily. With the same intervals a quantitative analysis of the solution for elements contained in the mineral is done. The experiment is carried out usually for 7–12 days. At the end, count of bacterial cells is done. In order to assess the role of bacteria, a parallel control experiment without bacteria is run, and the same parameters are monitored. The results are presented in the form of a table or a graph with EP(Eh) and experiment duration (days) as the coordinates. Data on solubilization of elements contained in the mineral are plotted on the same graph. Fig. 3.14 shows results of an experiment with chalcopyrite oxidized by **T.ferrooxidans**. Decomposition of chalcopyrite by bacteria was intensive. The greater part of Cu and Fe extraction at the 1:1 ratio occurred in the course of first 6 days, amounting to about 25 % of the total respective metal content

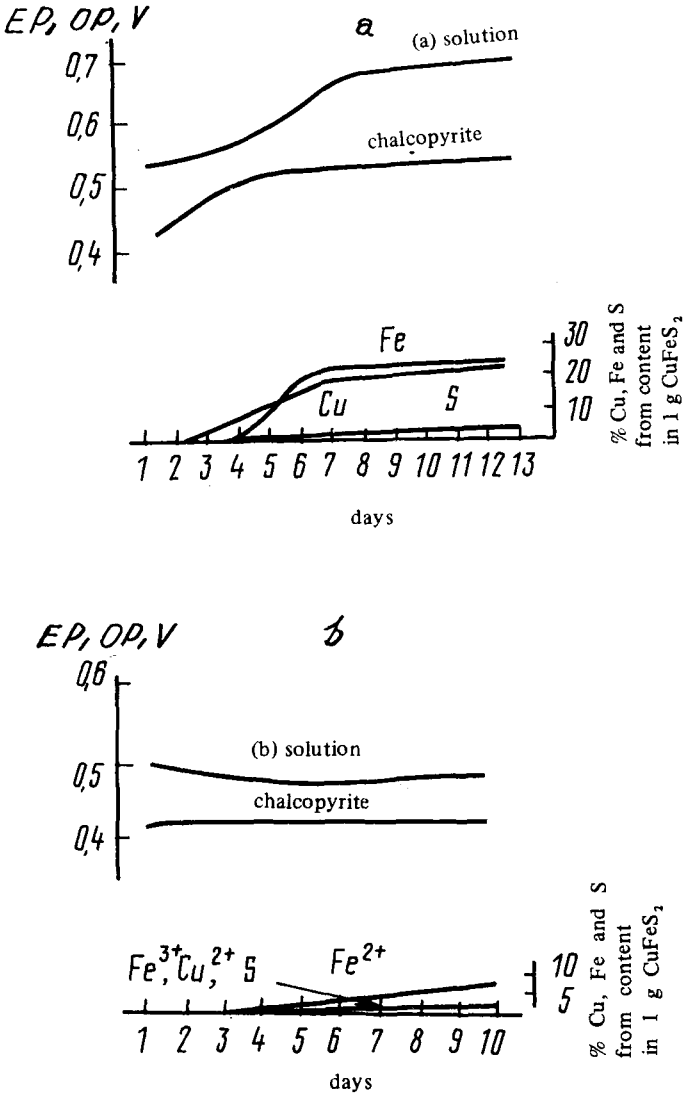

Fig. 3.14. Variation of the chalcopyrite electrode potential and the solution redox potential with time, and graphs of leaching copper, iron and sulfur from chalcopyrite in the experiment with bacteria (a) and in a control experiment (b)

in the sulfide. During the extraction, the EP and Eh changed from 0.4 to 0.5 V and from 0.5 to 0.7 V, respectively. The end of extraction of Cu and Fe was indicated by reaching the stable levels of EP and Eh (0.5 and 0.7 V, respectively). The changes in Eh of the medium and in EP of the mineral occurred almost simultaneously, which is indicative of a close relationship between electrochemical characteristics of the mineral and medium with those of solution, and hence with the course of metal leaching.

In the control experiment chalcopyrite was virtually unchanged. Accordingly, the EP of chalcopyrite remained constant (about 0.4 V) and was in equilibrium with the Eh of the medium (0.5 V). The changes in EP and Eh during bacterial leaching thus have a regular character and may be used for monitoring the process.

3.2.5. Oxidation of contacting sulfides

In sulfide deposits intended for bacterial leaching, one has to deal usually with polymineral associations – ores, rather than with individual minerals. Oxidation of each sulfide under these conditions becomes a much more complex process; it is directed and controlled by particular characteristics of electrochemical reactions between contacting minerals. A mineral having a higher EP plays the role of the cathode and accelerates the oxidation of a mineral with a lower EP, the anode. The greater is the difference between the EP of the two minerals, the higher is the rate of the anode-mineral decomposition. In order to make a correct evaluation of the direction and rate of bacterial leaching of ores, as well as of the composition of resultant solutions, it is advisable to study the combinations of minerals in the ore, to identify the ore types, and to carry out an experiment on oxidation of those pairs of sulfide minerals that simulate the major types of ore associations. In a pyrite copper ore deposit, for instance, these pairs will be pyrite-chalcopyrite, pyrite-chalcocite, pyrite-bornite, chalcopyrite-chalcocite.

The advised method of electrochemical experiment consists in placing the two mineral electrodes into a galvanic cell filled with electrolyte solution. Using the installation shown in Fig. 3.8 the two electrodes are connected to form a galvanic couple. The measurements of pH and Eh of the solution and EP of the minerals are as usual done at 5 min intervals. The EP-pH groups are plotted against the equilibrium diagrams of the elements in aqueous solutions. Our experiments on combined oxidation of pyrite (FeS_2) with copper sulfides such as chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) and bornite (Cu_5FeS_4) indicated that the EP of pyrite was always higher than that of any mineral it contacts. Therefore, pyrite always acted as cathode and was protected from the corrosion process, while the copper sulfides acting as anode were decomposed. These results suggest that during bacterial leaching of this type of ores pyrite acting as cathode will, along with the bacterial cell, stimulate destruction of copper sulfides (at the anode). Indeed, experiments on bacterial leaching of sulfide pairs having pyrite as one of the members, the extraction of copper from copper sulfides-chalcopyrite, chalcocite and bornite, was more intensive than in the case of monomineral ores. Solubilization of copper was more than twice as high as without pyrite (Table 3.3).

Table 3.3

Solubilization of copper in experiments with the monomineral and mixed samples during 8 days

Monomineral samples	Solubilization of copper in per cent of content in 1 g of mineral sample	Mixed samples	Solubilization of copper in per cent of content in 1 g of mineral sample
Chalcopyrite	25	Chalcopyrite-pyrite	60
Chalcocite	12	Chalcocite-pyrite	50
Bornite	30	Bornite-pyrite	50

3.2.6. Methods of studying phase transitions on the surface of sulfide minerals

In the process of bacterial leaching the surface of sulfide minerals is transformed, producing, e.g., oxides, elemental sulfur, etc. Besides, the above data show that individual minerals are selectively leached from the polymineral mixture under study, depending on the value of the electrode potential and the crystallo-chemical structure. Thus, the nature and state of the surface of sulfide minerals determine the character of their interaction with bacteria and their metabolites, which in turn determines the leach-ability of the polymineral product under study. Reliable data on the mineral surface state is also necessary for a viable thermodynamic analysis of possible reactions and mechanisms of bacterial leaching. As is known, that a preliminary estimation of the mineral surface can be made through the analysis of the crystallic structure and of bonds revealed when the crystal is destroyed [69]. If the mineral composition changes considerably during bacterial leaching and depends on pH and Eh, the **thermodynamic analysis technique** is widely used to this end. Crystallo-chemical peculiarities of the mineral structure are used primarily to explain differences in the structure and strength of compounds that form on the surface of the minerals [69]. When considering the equilibrium relations in the thermodynamic analysis of possible reactions of the mineral surface the equilibrium constant formulae is used as well as the dependence of the standard free energy of formation on the equilibrium constant or the standard electrode potential, the dependence of the reaction constant on the electrode potential changes.

According to the mass action law, the equilibrium constant K characterizes the relationship between concentrations of reagents and reaction products. For example, in the reaction:

$$bB + cC \rightleftharpoons dD + eE \qquad (3.1)$$

$$K = \frac{[D]^d \cdot [E]^e}{[B]^b \cdot [C]^c}$$

where: [B], [C], [D], [E]— the activity of the components.

The activity of solids and pure liquids is assumed to be a unity. The value of K can be calculated if the standard free energy of reaction ($\Delta F^0_{reac.}$) is known, since they are bound by the relation:

$$\Delta F^0_{reac.} = -RT\ln {}^oK \qquad (3.2)$$

where: R – the universal gas constant, T – the absolute temperature.
At 25 °C

$$\Delta F^0_{reac.} = -1.364 \lg {}^oK \qquad (3.3)$$

The standard free energy of a reaction is the accumulated free energies of the formation of the reaction products in their standard state minus the accumulated free energies of the formation of the initial substances in their standard state.

$$\Delta F^0_{reac.} = \Sigma \Delta F^0_{reac.\ prod.} - \Sigma \Delta F^0_{reagents} \qquad (3.4)$$

As the standard free energy of the hydrogen half-element is assumed to be zero, any redox reaction can be expressed in the form of two half-reactions – reduction and oxidation. When the activity of all substances is qual to unity the standard free energy and the standard electrode potential of the reaction ($F^0_{reac.}$) are bound by the relation:

$$\Delta F^0_{reac.} = E^0_{reac.} \cdot n \cdot F \qquad (3.5)$$

where:

n – the number of electrons involved in the reaction;

F – the Faraday constant 23.06 Kcal/V·g-eq.

If the activity of the substances is not equal to unity, the following relations hold:

$$\Delta F_{reac.} = \Delta F^0_{reac.} + RT\ln {}^oK \qquad (3.6)$$

$$E = F^0_{reac.} + \frac{RT}{nF} \ln {}^oK \qquad (3.7)$$

In order to determine the equilibrium concentrations of the ionic or molecular components under the boundary conditions of conversion of one compound into another, it is necessary to carry out full chemical calculations of the system [1, 66]. To this end, a system of equations is obtained and solved which comprises the expressions of the equilibrium constants of the corresponding reactions of dissociation of compounds, water and possible hydrolysis of ions, as well as balance and electric neutrality equations.

Consider the application of the thermodynamic analysis for the determination of the phase transition boundary conditions, taking by way of example oxidation of lead sulfide. Oxidation to anglesite – lead sulfate – gives:

$$PbS + 4H_2O \rightleftharpoons PbSO_4 + 8H^+ + 8\bar{e} \qquad (3.8)$$

$$\Delta F^0_{reac.} = +55.02 \text{ Kcal}$$

$$E^0_{reac.} = +0.299 \text{ V}$$

$$E = +0.299 - 0.059 \text{ pH}$$

Oxidation to lead thiosulfate gives:

$$2PbS + 5H_2O \rightleftharpoons PbS_2O_3 + Pb(OH)_2 + 8H^+ + 8\bar{e} \qquad (3.9)$$

$$\Delta F^0_{reac.} = +93.15 \text{ Kcal}$$

$$E^0_{reac.} = +0.504 \text{ V}$$

$$E = +0.504 - 0.059 \text{ pH}$$

In the Pourbaix coordinates (Eh–pH) the obtained phase transition equations have the same slope of 0.059 pH and at all pH values the oxidation reaction to anglesite begins earlier while the free energy of this reaction is less. It seems that the oxidation reaction of thiosulfate might be excluded as metastable; however, it should be taken into account that the thermodynamic approach determines only the basic possibility of a chemical reaction. In real chemical systems, a thermodynamically predetermined reaction may not take place because of the kinetic parameters of the system under study. Therefore, the theoretical thermodynamic dependences obtained should be experimentally tested. To this end, **electrochemical research methods** are used most frequently, since measurements can be taken under conditions close to industrial ones. As a rule, an electronic control system is used in such electrochemical studies to maintain the polarization current in the circuit of the electrochemical cell at such a level that the potential drop at the surface of the mineral under study corresponded to the specified one. This is done by means of potentiostats, registering potentiometers and oscillographs are used as control systems, audio-signal generators are used to apply high-frequency alternating voltage. The design of electrochemical installations and methods of making electrodes are described in numerous manuals [1, 16, 19].

The potential value of phase transitions of sulfides to corresponding oxidized compounds can be determined, for example, by **the curves of charge of mineral electrodes.** If a mineral covered with an oxide film is immersed into a solution chemically inert with respect to the film and the cathode polarization is switched on at a constant current value, the electrode then begins to have a potential value at which reduction of the oxidized compound is possible. The electrode potential does not practically change during reduction since the electric charges are consumed to reduce the oxidized compound. The application of this method at different pH values may verify the dependence obtained as a result of the thermodynamic analysis. **The potential drop method,** on the contrary, is based on the study of oxidation of pure mineral

surfaces. To this end, the mineral electrode is dressed when it is immersed in water and kept there for 30 min at the potential values 0.3–0.2 V lower than the values calculated for the assumed oxidation reaction. Then the polarization circuit is opened and the curves of the potential drop are registered. As a rule, the oxidation products of sulfide minerals do not have a sufficient ionic or electronic conductivity. This explains why the potential values at the beginning of the sulfide surface oxidation coincide when it is electrochemically passivated and they can be determined by **registering potentiodynamic polarization curves.** To do so, an increase of the potential at a specified rate is ensured on the mineral electrode with a simultaneous registration of the current value in the cell circuit. The use of potentiostats allows to scan the potential to both the cathode and anode regions on the dressed or oxidized electrode. The course of the chemical reaction at this or that potential value is determined by minimum and maximum peaks. When a mineral electrode is placed into solutions under study there begins ionic exchange between the electrode and the solution. The process, however, goes in the two ways at different rates. As a result, the potential becomes distributed at the electrode and a double electrical layer of oppositely charged ions is formed. The capacity of this double electrical layer changes if there is sorption of molecules and oxidation of the mineral electrode surface. Therefore, phase transitions can be detected by measuring **the differential capacity of the double electrical layer.** Bacterial leaching, as a rule, is effected in the pulp which is a mixture of polymineral particles. Therefore, it is necessary, as it was noted above, to take into account the mutual effect of the minerals on their oxidation. The process as a whole is electrochemical in character and corrosive in essence. The mutual effect of sulfide minerals on their oxidation can be estimated by representing spontaneous processes as polarization curves [1, 66]. When two minerals are oxidized together, one of them (the anode) is oxidized more rapidly than the other (the cathode). The process is intensified when there are bacteria in the pulp. The degree of galvanic protection of the cathode mineral can be either partial or complete [1, 66], depending on the slope of the polarization curves and the electrode potential value.

3.3. METHODS OF OBTAINING T.FERROOXIDANS BIOMASS

The concentrated biomass needed to intensify the oxidation processes can be obtained by different methods. Yet obtaining a large biomass of acidophilic chemolithoautotrophic bacteria for industrial purposes using the classical methods is very complicated. An example is the iron-oxidizing bacteria **T.ferrooxidans.**

In order to obtain 1 g of biomass (absolutely dry weight) the bacteria should oxidize about 26 g of ferrous iron to the ferric state, even if its biosynthesis efficiency is 100 %. The real efficiency for these bacteria is 25–30 % at best [32], and hence about 100 g of ferrous iron is to be oxidized to produce 1 g of biomass. The amount of biomass that can be grown in a unit volume of the nutrient medium equals, according to the Monod-Ierusalimsky model for a continuous culture,

$$X = \frac{S_0 - S}{a}, \qquad (3.10)$$

where:

S_0 and S are the substrate concentrations at the inlet and outlet of the cultivating vessel, and a is the consumption coefficient, i.e. the amount of substrate consumed by the culture per unit of biomass grown. The maximum possible value of S_0 for the ferrous ion in the medium is about 50 g/l since at higher concentrations the growth of bacteria is almost completely inhibited. Assuming that S may be close to zero and a = 100 g/g, the maximum bacterial biomass concentration for this method of continuous cultivation according to equation (3.10) cannot exceed 0.5 g/l, which is equivalent to about 10^{10} cells/ml. In fact, however, the biosynthesis efficiency at such a high concentration of total iron in the medium is several times lower, and the attainable cell concentration is about 10^9 cells/ml.

The currently used media proposed for cultivation of iron-oxidizing bacteria by Leathen (Chapter 2) No. 1 and Silverman and Lundgren (Chapter 2) No. 2 have lower levels of iron and maximum attainable concentration of bacteria for these media is between 10^9 and 10^{10} cells/ml.

Thus, the use of classical method for cultivation of chemolithoautotrophs, e.g. **T.ferrooxidans**, does not permit to produce bacterial cultures of the density high enough and hence making the process highly efficient. Besides, waste material of the process per one gram of bacterial cells produced amounts to no less than 100 g of oxidized iron or about 400 g of $Fe_2(SO_4)_3$ in the form of an acidic solution (pH = 2) over 10 l in volume. In these circumstances, production of kilograms of biomass would proportionally, 1000-fold increase the consumption of iron and sulfuric acid as well as the amount of wastes.

Described below are the most technologically acceptable methods of obtaining the active bacterial biomass.

3.3.1. Method of Biomass Production by Using the Electric Energy

One way to increase the cell concentration in the culture and at the same time to overcome all the above-mentioned drawbacks of the classical method is offered by the method proposed by Kinsel and Umbreit [34]. Its idea is combining two processes, namely, the microbiological oxidation of Fe^{2+} and electrochemical reduction of Fe^{3+} to Fe^{2+}.

Thus, a new method of supplying energy for the process has been developed, applicable only to chemolithoautotrophic microorganisms. The method comprises the electrochemical reduction of the energy-providing substrate directly in the culture.

Fig. 3.15 shows a scheme of reactions taking place in an electrochemical cell that provides **T.ferrooxidans** cells with energy. The energy for cell growth in this case is provided by the electric energy supplied to the electrochemical cell's electrodes, while the energy-providing substrate, in this case the ferrous ion, serves merely as a carrier of energy from the cathode to the bacterial cell. The Fe^{2+} is not supplied from outside but is formed in the culture on the anode surface.

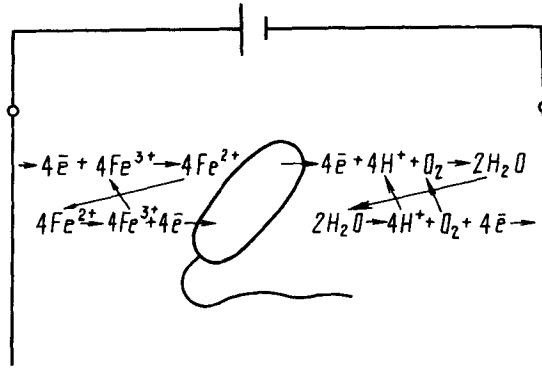

Fig. 3.15. A scheme of reactions taking place at electrolytic cell electrodes and at the cell

3.3.2. Control of Continuous Cultivation of T.ferrooxidans

The concept of controlling continuous cultivation of **T.ferrooxidans** is the automatic correlation of two reverse processes – bacterial oxidation of Fe^{2+} and electrochemical reduction of Fe^{3+}. Most useful for practical application is the method of maintaining the constant cathode potential [12].

For maximum reduction rates and hence maximum reactor productivity the cathode potential is maintained in the plateau region of the cathode

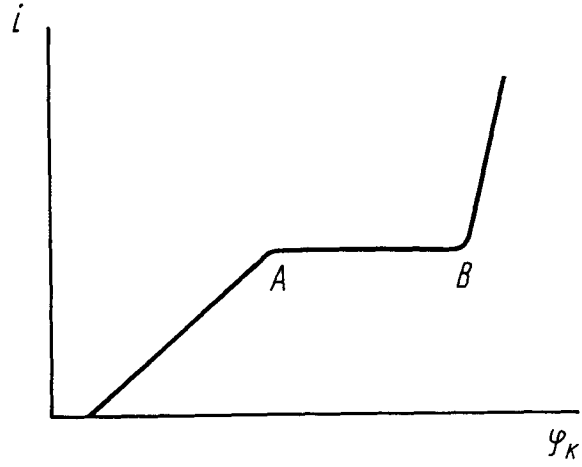

Fig. 3.16. Current through the electrolytic cell as a function of voltage at the cell's electrodes. The A-B region corresponds to maximum current of Fe^{2+} reduction, to the right of B is the region of hydrogen liberation at the cathode

potential vs. reactor current curve (Fig. 3.16). This provides for reactor functioning in a mode of current maximum for ferric iron reduction. Any variation in the oxidative capacity of the culture would result in a change of ferric iron concentration and hence in a change of its electrochemical reduction rate. As a result of this, the rates of bacterial oxidation and electrochemical reduction would be automatically kept equal.

3.3.3. Control of Mineral Nutrition of the Culture

The rate of mineral components supply to the culture with the medium flow should be correlated with the rate of energy substrate supply by electrochemical reduction. Under this condition it is possible to retain the limiting role of the energy source. The turbidostat cultivation method implies a positive relationship between biomass production and medium influx, and for this reason it is suitable for growing chemolithoautotrophs. However, the realization of the densitostat cultivation method for iron-oxidizing bacteria is hampered by the absence of a biomass concentration sensor, without which the entire system of automated coordination between the medium influx and biomass increase is impossible.

A chemolithoautotrophic culture, however, allows easy measuring and recording of the introduced energy since it is proportional to the electric current. If the biosynthesis efficiency is constant in time (and this is the case when the medium parameters in the reactor are kept constant), a meter of the electric current passed through the reactor, may replace the biomass density sensor [13]. Besides, instability of the biosynthesis efficiency is not a significant problem for controlling mineral nutrition. The rate of mineral components feeding is calculated for the maximum attainable efficiency, and a drop in the efficiency, if it occurs for some reason, would result only in an increase of background concentrations of the elements but not in a change of the limiting factor or inhibition of culture growth.

The culture dilution D (h^{-1}), efficiency η, biomass concentration X (g/l) and the specific current through culture I (A/l) are related as follows:

$$D = 0.0785\, I_{\eta}/X \qquad (3.11)$$

It can be seen that culture dilution is proportional to the current if η and X are constant, if the cultivation conditions remain unchanged. The coefficient of the equation was calculated for the dry biomass calory content of 5 kcal/g and the energy yield of the $Fe^{2+} \rightarrow Fe^{3+}$ reaction of 11 kcal/mol.

The growing bacterial cells consume two gaseous components – molecular oxygen and carbon dioxide. The former is a reagent involved in the iron oxidation by the cells, the latter is the source of carbon for production of biomass. These two gases are needed in greatly differing quantities. If the biosynthesis efficiency equals 25 %, production of 1 g of bacterial biomass is accompanied by consumption of about 10 l of oxygen for substrate oxidation; the amount of CO_2 assimilated as the carbon source is about one liter. When the

biosynthesis efficiency drops, the O_2/CO_2 ratio increases while the CO_2 and biomass ratio remains virtually the same.

The high demand of oxygen by **T.ferrooxidans** does not affect the economic characteristics of the process as all required oxygen is produced at the anode of the same reactor where the bacteria are grown.

It should be noted that **T.ferrooxidans** can oxidize iron for a long time (10 days and more) without carbon dioxide at the same rate as in its presence. However, growth of biomass does not occur in this case.

3.3.4. Equipment for Continuous Cultivation

This section deals with a cultivation vessel for continuous cultivation of **T. ferrooxidans** coupled to electrochemical reduction of ferric iron.

The same cultivation vessel can be used for batch cultivation of the same microorganisms, but some of the cultivation vessel systems are not used in this case. The general design of the cultivation vessel is shown in Fig. 3.17 [38].

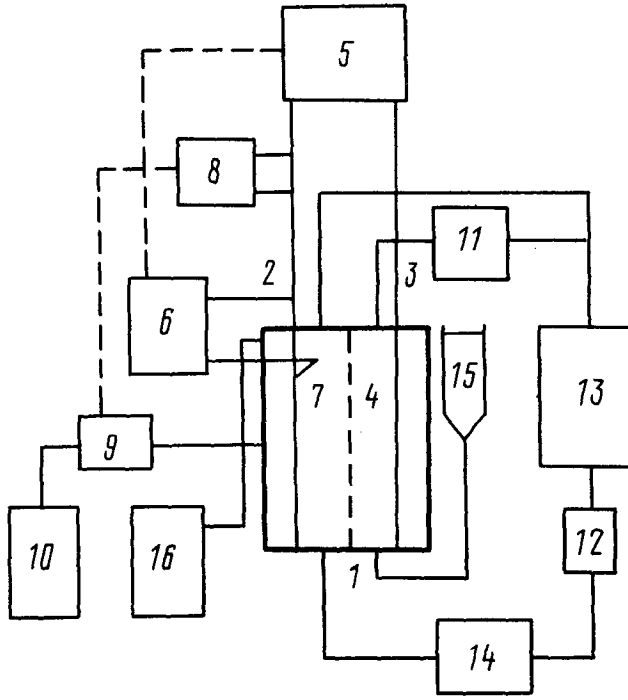

Fig. 3.17. A block diagram of the cultivator for continuous growing T.ferrooxidans: 1 – reactor; 2 – cathode; 3 – anode; 4 – ion-exchange membrane; 5 – adjustable DC power source; 6 – potentiostat; 7 – cathode potential sensor; 8 – current integrator; 9 – nutrient medium dosator; 10 – nutrient medium tank; 11 – oxygen meter; 12 – compressor; 13 – gas holder; 14 – gas consumption meter; 15 – distilled water tank; 16 – harvest collector

Reactor. The design of the reactor is affected by the fact that it plays the role of an electrochemical cell. The design should provide optimal conditions for the growth of bacteria such as temperature, pH, chemical composition and aeration of the medium on the one hand, and optimal conditions for the electrochemical process, such as minimum ohmic resistance, and high current density, on the other. These requirements do not always coincide, so that the technical solutions employed are chosen by compromise and call for further improvement.

The maximum reactor efficiency is determined by its cathode area since the cathode current density is limited. The culture volume is of little relevance to efficiency, only the amount of biomass per cathode area unit (g/m^2) is important. This allows creating reactors with a relatively thin culture layer on the cathode surface. In the reactor designs created and tested by the authors the cathodes were rectangular flat platinum plates, and the culture layer on the cathode was 10 to 20 mm thick.

The cathode space is separated from the anode space by a cation-exchange membrane, which prevents oxidation of the reduced iron at the anode, thereby increasing the electrochemical efficiency of the process. The anolyte layer thickness is not essential for cultivation. The anode like the cathode has a

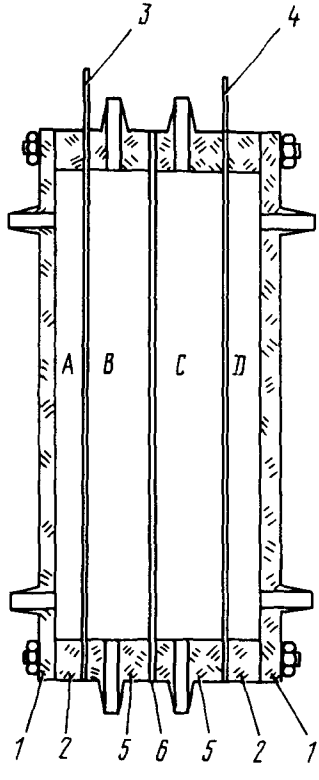

Fig. 3.18. Schematic section of the reactor:
1 – outer wall of water jacket; 2 – water jacket frame; 3 – cathode; 4 – anode; 5 – frame of anode and cathode spaces; 6 – ion-exchange membrane
A, D – water jacket; B – cathode space; C – anode space

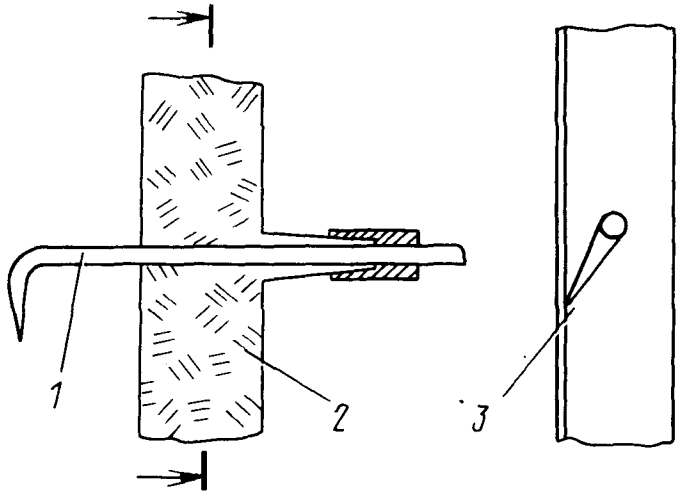

Fig. 3.19. Insertion of the cathode potential capillary sensor into the reactor:
1 – sensor; 2 – cathode space wall; 3 – cathode

platinum surface. The need to stabilize the culture temperature necessitates the existence of cooling jackets at the anode and cathode. A section of the reactor is shown schematically in Fig. 3.18. Structurally, the reactor is a divisible packet of parts braced together by studs located at the perimeter; leakage is prevented by rubber gaskets. The cathode compartment is equipped with connections for inlet and outlet of the culture, for aerating gas mixture, for mounting thermometers and temperature sensors, as well as for an electrode for measuring the cathode potential. The anode compartment wall is equipped with connections for inlet and outlet of the anolyte, and for outlet of oxygen produced at the anode.

The walls of all reactor compartments are made of material resistant to the corrosive media inside the reactor, e.g. of plexiglass.

Thermostabilization System. In this reactor thermostabilization of the culture is accomplished by the flow of water from a thermostat through the reactor jackets. The contact thermometer of the thermostat is mounted into the cathode space to measure the bacterial medium temperature. The temperature optimum **T.ferrooxidans** growth and oxidation is rather broad, so that stabilization of temperature with the accuracy of ±1 – 2 °C is quite satisfactory.

Electric Feeding System. To maintain the stable cell growth the cultivation is performed under the stable cathode potential.

A glass capillary used as a sensor is inserted into the reactor as shown in Fig. 3.19. The capillary is connected by means of a silicon rubber hose with a plexiglass beaker containing sulfuric acid solution (pH 1), which is interconnected by an agar bridge with the similar beaker containing saturated KCl.

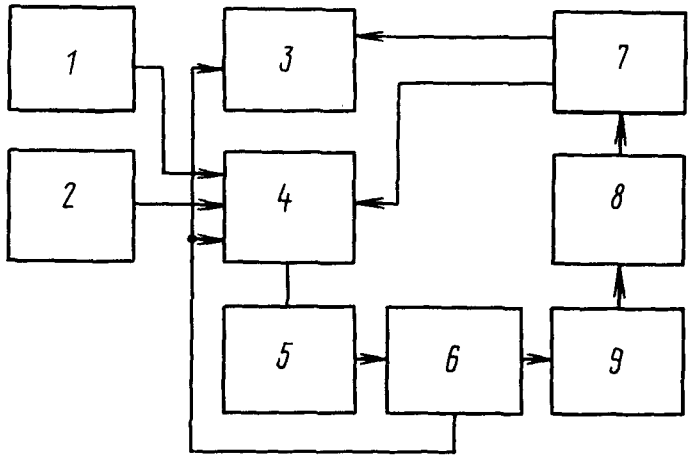

Fig. 3.20. Block diagram of electric power supply to the reactor:
1 – external generator; 2 – potential and current setting unit; 3 – cathode potential and current recorder; 4 – error amplifier; 5 – controllable current source; 6 – current sensor; 7 – buffer stage of cathode potential amplifier; 8 – cathode potential sensor; 9 – reactor

A silver chloride electrode from which the voltage relative to the reactor cathode is taken, is immersed into the KCl solution.

The constant cathode potential can be maintained by a powerful potentiostat with output from 0 to 30 V and current from 0 to 30 A.

If such a powerful potentiostat is not available, the size of the electrochemical reactor should be decreased so that a less powerful potentiostat should maintain the needed potential or a power amplifier should be used. The block diagram of the system is shown in Fig. 3.20.

The function of setting the cathode potential and amplifying the difference signal is fulfilled by a commercial P5848 potentiostat. As a controllable DC power source a thyristor unit ATEZ-50/460p-2u4 complete with a matching transformer TS2.5-380/27.5 is used. Signal generator G6-26 provides for the external display of potential. A ShSM75-100-0.5 shunt serves as a current sensor. The buffer stage consists of a 140UD8A operational amplifier connected as a voltage follower. The current and cathode potential values are recorded on a KSP-4 automatic potentiometer.

Mineral Nutrition and Harvesting System. This system includes a nutrient medium tank (calculation of the nutrient medium supply is explained below), a dosator, a current integrator, i.e. a meter of the current that passed through the reactor, and a harvesting tank.

The medium and harvesting tanks have no special features except that they must be made of a corrosion-resistant material (the medium is acidic, $pH \leqslant 2$). The dosator is a hose pump (Fig. 3.21) consisting of an electric motor and inbuilt pressure regulator. The integrator, i.e. a meter of electric current, switches on the dosator after a specified amount of electricity has passed

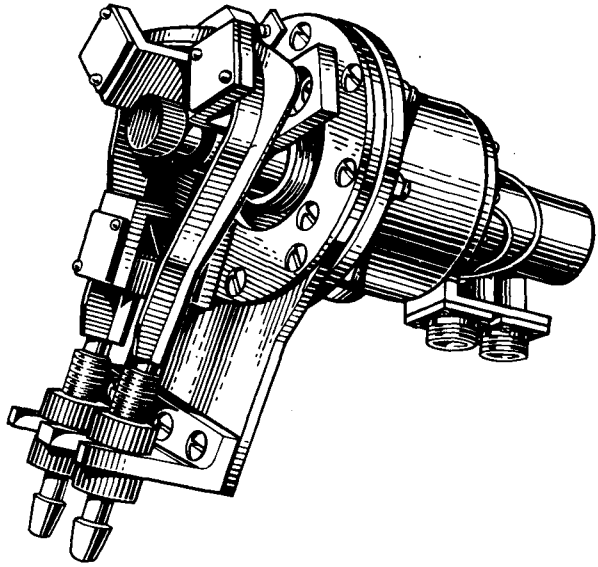

Fig. 3.21. Nutrient medium dosator with D-60G motor

through the reactor. The dosator is activated for a definite time period to feed a certain (precalculated) amount of the nutrient medium to the culture [13].

In order to explain the choice of parameters for matching reactor current and the nutrient medium flow, let us consider the equation (3.11) after rewriting it in the following form:

$$D = \frac{V_l}{V} = 0.0785I \frac{\eta}{X} \qquad (3.12)$$

or

$$V_l = 0.0785I\, V \frac{\eta}{X} \qquad (3.13)$$

where:

V_l is the nutrient medium consumption by the reactor in l/h;

V is the culture volume in the reactor in liters; the efficiency of biosynthesis η can be assumed to be 0.25.

Since I is the current per culture volume, the product of I and V is the total current through the reactor. Then the amount of electricity equal to 1 A·h (or 3600 coulombs) should correspond to the following medium volume that should be fed to the reactor:

$$V_l = 0.0785 \frac{\eta}{X} \qquad (3.14)$$

Assuming dry biomass concentration of 4 g/l and biosynthesis efficiency of 0.25, the V_l value will be about 5 ml/A·h. Such a rate of feeding can easily

be ensured by means of the above-mentioned current integrator, for example, by setting the dosator triggering at 1 A·h and by adjusting the dosator operation time to correspond to the injected medium volume of 5 ml. This is not the only combination of the values possible, and the integrator may be set at 2 A·h and the injected medium portion at 10 ml, etc.

The excess of culture fluid is discharged at the level of the overflow sleeve in the cathode wall of the reactor. It should be noted that the volume of the harvested biomass will be greater than the volume of nutrient medium fed to the reactor since oxidation of iron is accompanied by the production of water at the amount of 0.336 ml per A·h of the current. At the anode, water is electrolyzed, and its volume at the anode diminishes by the amount equal to that synthesized at the cathode. For this reason the design of the cultivating vessel includes a tank with distilled water from which the anolyte, a solution of H_2SO_4 with pH 1–1.5, is continuously replenished with water by the communicating vessels principle.

Culture Aeration System. For laboratory cultivating vessels a closed circuit system for culture aeration (Fig. 3.17) is quite convenient. Since the gas mixture used for aeration is different from the atmospheric air, it is necessary to mix the gases in a required proportion in the gas holder and then to use the mixture for a certain time interval for which the gaseous composition in the reactor can be predicted.

The closed aeration system consists of a gas holder, a compressor, a gas consumption gauge, a foam separator, and a cooler for discharged gas.

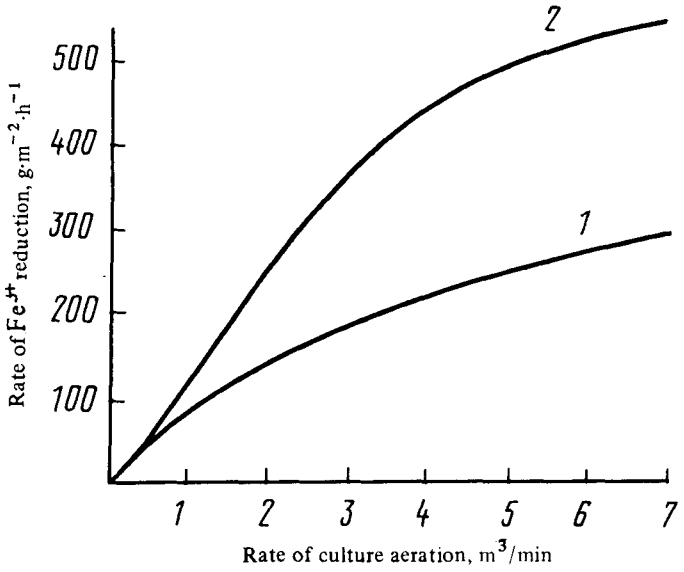

Fig. 3.22. Fe^{3+} electroreducing rate as a function of culture aeration intensity:
1 – electroreduction rate for the +300 mV cathode potential which corresponds to the beginning of a plateau; 2 – the same at the end of the plateau

The compressor may be of any design that ensures airtightness of the aeration system. Non-piston compressors are preferable since they do not contaminate the gas by oil vapours and require no gas purification. The authors used a compressor of their own design with a rubber membrane. Rotatory gas blowers and other clean-gas designs can be used.

The aeration not only provides the bacteria with oxygen and carbon dioxide required for growth but also creates turbulence of the culture at the cathode surface, thus reducing the diffusion layer thickness at the cathode. It can be seen from Fig. 3.22 that the saturation current increases with increasing aeration intensity up to 8 – 10 m^3/min per m^2 of the horizontal section area of the cathode compartment (m/min).

Foam separator consists of a glass flask 1/4 to 1/2 of the reactor volume (Fig. 3.23). The gas output of the reactor is fed to the upper part of the flask and the liquid fraction of the discharged gas mixture runs off into the reactor through the lower outlet of the flask, the gaseous fraction being fed into the cooler. Glass coolers cooled with tap water are preferable since the culture medium that may get into the cooler can cause corrosion of metal components. The condensate formed in the cooler is discharged into the reactor. Without the cooler the condensate will be accumulated in the gas holder and the gas communications of the cultivation vessel.

The **T.ferrooxidans** culture growing under normal physiological conditions produces little foam, and the above design is quite sufficient for foam breaking. An increased foaming of the culture is an accurate indicator of some unfavourable factors in the medium (low pH, elevated temperature and other factors leading to the death of cells in the culture).

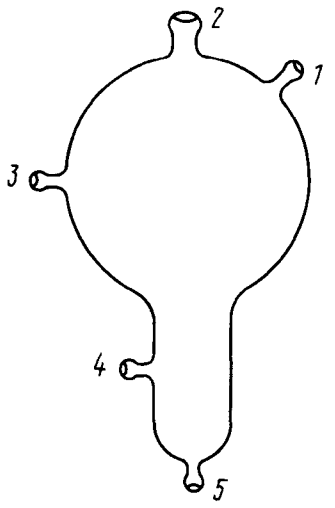

Fig. 3.23. Foam separator:
1 – inlet of the gas-liquid mixture from the reactor; 2 – gas outlet to the cooler; 3 – inlet of oxygen from the reactor anode space; 4 – culture drainage into the harvest collector; 5 – liquid return to the reactor

3.3.5. Nutrient Medium Composition for Continuous Cultivation

In the case of cultivation coupled to electrochemical reduction of ferric iron, the bacterial concentration can reach dozens of grams per liter. For the continuous cultivation of **T.ferrooxidans** by this method it is necessary to apply balanced media [14]. This is based on the following regularities.

As follows from the mathematical model devised for continuous cultures by Monod and Ierusalimsky [67], the concentration of an element consumed by microorganisms in the nutrient medium, in the background medium and the biomass concentration are related as follows:

$$S_0 = S + aX \qquad (3.15)$$

Therefore, no nutrient medium is versatile, suitable for continuous cultivation at any biomass density. It is only the calculation method based on equation (3.15) that is versatile, for the medium calculated according to this equation is balanced with regard to the needs of bacterial cells. As follows from equation (3.15) to calculate the concentration of any element in the nutrient medium (S_0) one should know the consumption coefficient of this element for production of biomass (a) and its minimum background concentration (S) that does not limit the cell growth.

However, very high concentrations of elements, even if they do not inhibit cell growth, are not economical.

Table 3.4 summarizes the consumption coefficients of principal mineral elements for the biosynthesis of bacterial biomass and their sufficient background concentrations found experimentally.

Table 3.4

Values of the consumption coefficient a and minimum non-limiting background concentrations S for principal biogenic elements in the nutrient medium

Parameter, mg/l	Biogenic elements			
	N	P	K	Mg
S	⩽30	⩽10	⩽25	⩽4
a	100±10	14±2	5±1	2±0.5

Calcium was not added to the nutrient medium. It is either not required by the cells or the requirement is negligible and is met by calcium-containing impurities in the reagents used to prepare the medium.

The components for the nutrient medium include ammonium, potassium and magnesium sulfates, phosphoric and sulfuric acids.

The pH optimum for cell growth of **T.ferrooxidans** is usually between 1.8 and 2.1. Iron is added to the medium as ferrous sulfate, the optimum concentration of the iron ion ranges from 4 to 8 g/l.

In the course of continuous cultivation iron is predominantly in the ferric state, for this reason the pH of culture is higher than that of the fresh medium, and in order to have an optimal pH value in culture the initial medium must be acidified to pH 1.6 – 1.65.

3.3.6. Effect of Carbon Dioxide and Molecular Oxygen

If the aeration rate is high, e.g. 7–10 l/min, a carbon dioxide content of 0.5 % in the gas mixture is sufficient to support the bacterial growth [37]. At lower carbon dioxide content the culture grows slower while the rate of iron oxidation remains the same even in a complete absence of carbon dioxide, but diluted cultures can be grown with the atmospheric air.

The upper limit of CO_2 concentration is quite high, above 50 %.

An increase in O_2 content of the aeration mixture to 30 % reduces the growth rate of cells, while at 60 % O_2 the growth is sharply inhibited and the cells start to die.

The effect of the both gases is naturally a function of the rate of culture aeration.

3.3.7. Biomass Concentration in Culture

The maximum efficiency of the unit is a function of the rate of energy substrate (Fe^{2+}) regeneration (electrochemical reduction) and of the culture biosynthesis efficiency.

In order to attain the maximum efficiency of the unit the ferric iron concentration should be as high as possible, ensuring the maximum reactor current.

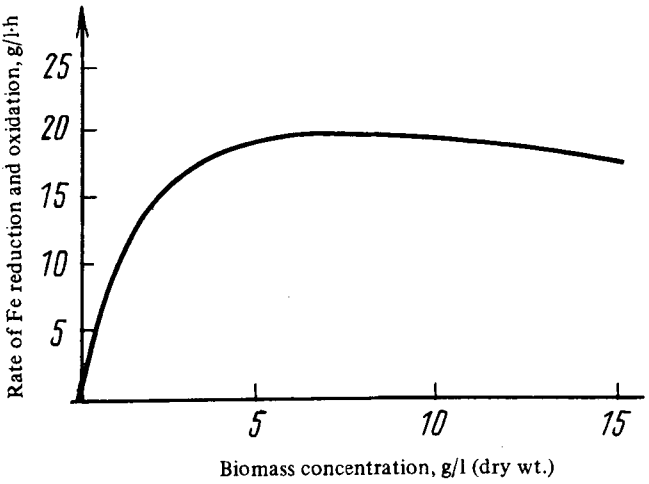

Fig. 3.24. Rate of ferrous iron oxidation by T.ferrooxidans as a function of biomass concentration (total iron concentration of 4 g/l and aeration rate of 7 l/min)

However, the total iron concentration in culture is limited to 8 g/l, since at higher concentrations jarozite precipitate forms at the cathode, reducing its working area and the current. To increase the rate of iron reduction in such conditions the $Fe^{2+} \rightleftharpoons Fe^{3+}$ equilibrium must be shifted to the right as much as possible, and the culture should oxidize the reduced iron at the same rate at which it is reduced at the cathode.

Fig. 3.24 shows an experimentally found relationship between the rate of ferrous iron oxidation by a culture of **T.ferrooxidans** and the biomass concentration in the reactor with a synchronous reduction of oxidized iron. It can be seen that the optimum biomass concentration (absolute dry weight) is between 5 and 10 g/l, in this case the culture layer at the cathode is about 15 mm. For a different layer thickness the optimum biomass concentration will also be different.

Wet biomass concentration in the medium is determined by means of centrifugation (see Chapter 2).

A unit of the design described above can produce as much as 100 g dry weight biomass per 1 m^2 cathode area per day when the main cultivation parameters are maintained within their optimal ranges. The current density under these conditions is about 250 A/m^2 and the culture efficiency is about 20 % (250 A·h/g of dry biomass).

As shown above, the optimal bacterial biomass concentration during continuous cultivation ranges from 5 to 10 g/l (dry weight). Such high concentration (10^{12} cells/ml) can be obtained in the same cultivating vessel as a result of batch cultivation. In this case the flow of nutrient medium should be switched off, and the culture may be grown starting at any low concentration and reaching the desired concentration. All nutrients are added to the medium at the beginning of the process except for phosphorus which is added as it is consumed since high levels of phosphorus in the medium induce the formation of jarosite precipitate on the cathode surface.

Apart from the platinum electrodes the use of lead anodes and high-alloy stainless steel cathodes is possible, although their life will be limited because of corrosion.

Experiments showed that bacteria grown in the electrochemical cultivator do not lose their ability to oxidize sulfide minerals. They were successfully tested in leaching arsenopyrite gold-containing and copper-zink concentrates [24, 57].

3.3.8. Other Methods of Biomass Production and Fe^{3+} Regeneration

Tank leaching seldom allows to obtain bacteria concentration in the pulp exceeding 10^9 cells per 1 ml, which limits the maximum rate of oxidation of sulfide minerals in the dense pulp. It is possible to harvest active biomass from pregnant solutions prior to the precipitation of metals and recirculate it. The biomass is obtained by centrifugation or separation. Such biomass isolation [58] allows to attain cell concentration as high as 10^{10} to $0.5 \cdot 10^{11}$ cells of bacteria per ml in the leaching system.

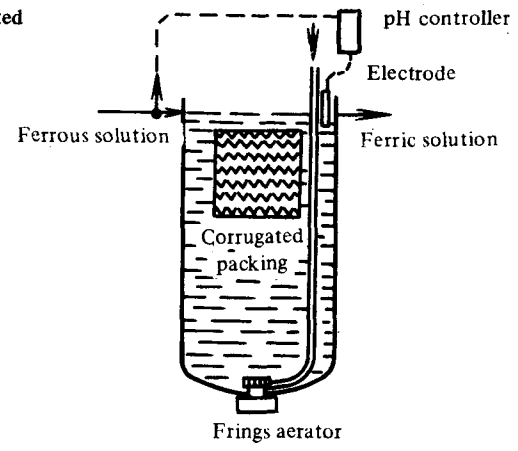

Fig. 3.25. Submerged corrugated pack unit – forced aeration

A method called bacterial Film Oxidation (BACFOX Process) [42] is proposed to raise the number of bacteria in liquors during heap and underground leaching. The method consists in the following. **T.ferrooxidans** are placed in the form of a film on a surface submerged into Fe^{2+} solution saturated with air. Oxidation of Fe^{2+} takes place in the flow. Bacteria bind with precipitated jarozite; the film containing bacteria and jarozite may be originated on various materials (glass, plastics). Livesey-Goldblatt et al. [42] report that the best results are obtained in the media where bacterial film was fixed on corrugated plastic (Fig. 3.25). Maximum specific rate of Fe^{2+} into Fe^{3+} oxidation is 7.5 g/h per 1 m^2 of bacterial film.

3.4. METHODS OF STATISTICAL PROCESSING OF RESULTS

Microbiological studies under laboratory or industrial conditions may yield data on the biomass, products of primary and secondary metabolism, the substrate transformed in a certain way, etc. Experimental results may also include various kinetic characteristics of the growth and development of microorganisms, obtained on the basis of data on the accumulation and transformation of substances in the system in question, and data supporting or changing our understanding of the regulatory mechanisms of the system. The results expressing quantities of certain substances, the number of cells, etc., may be used further to calculate kinetic characteristics which are necessary for obtaining a physiobiochemical model of the process under study (Fig. 3.26).

As a rule, measurements obtained in a certain number of trials are considered to be random values since too many unaccountable factors may influence them. Naturally, a greater number of trials would enhance the validity of our inferences. In microbiological studies, however, to increase the number of replications is a very difficult and expensive matter. The methods of mathe-

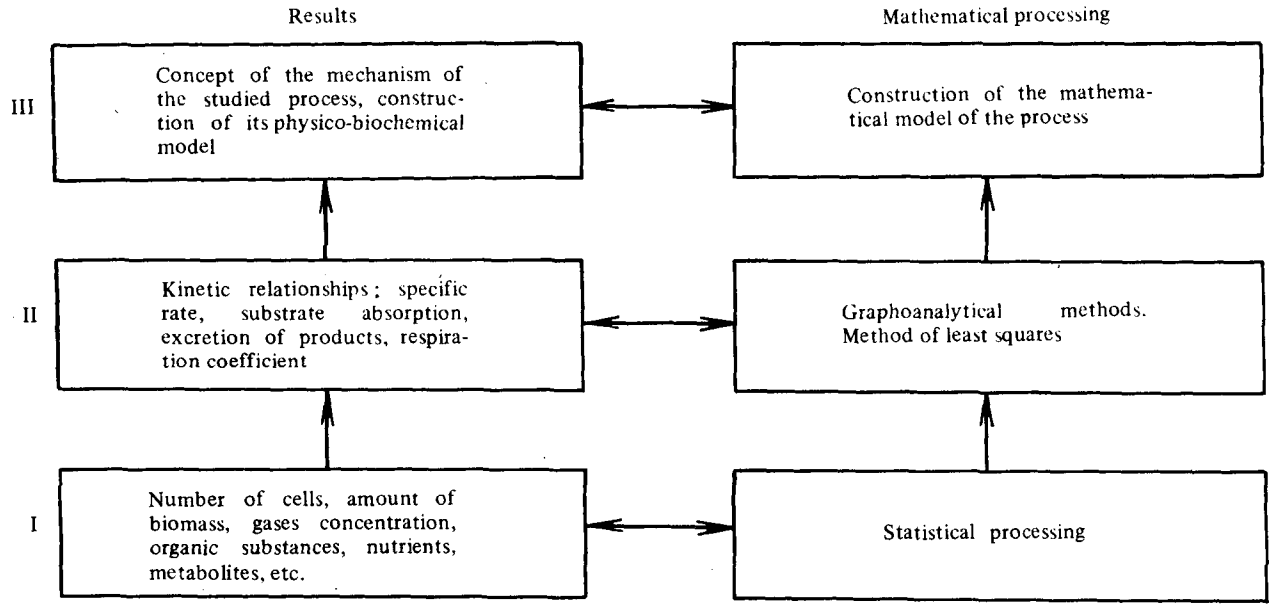

Fig. 3.26. Scheme of mathematical processing of microbiological data

matical statistics allow to estimate the reliability of the experimental material, draw reasonable conclusions and make predictions.

This problem is dealt with in numerous manuals, monographs and articles written specially for biologists [4, 5, 9, 55]. Therefore we shall consider here only some of the statistical methods that are most frequently used in experiments on bacterial and chemical leaching of metals from ores and concentrates.

3.4.1. Calculation of the Mean and its Confidence Interval

Consider the results of a certain experiment consisting of $n - x_i$ observations (the sample of size n). Let us assume that the parent population has a normal distribution. It is then possible to show [5, 9, 55] that the best estimator of the population mean is the sample mean $-x$ (the arithmetic mean) calculated as:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \tag{3.16}$$

Different samples with the same mean, however, may have different spread of the values with respect to this mean. Therefore, the standard variance defined as:

$$S = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2 \tag{3.17}$$

is an important statistic since it serves as a measure of the sample variability or dispersion.

An estimator of the random variable x is a random variable as well and it is distributed in a certain way around its true value. Let us determine an interval within which the true value will be found with a specified probability γ – the confidence interval. The interval is determined as follows:

$$I_\gamma = t_{\gamma, n-1} \cdot \frac{S}{\sqrt{n}} \tag{3.18}$$

where:

$t_{\gamma, n-1}$ – the value of Student test. The latter is found in Table 3.5 at the crossing of the column corresponding to the specified probability γ, and row number $n - 1$ (number of trials minus one);

S is calculated as in (3.17).

The interpretation of the confidence interval is as follows: for the confidence degree of 95 % (or 0.95) the true value of the mean will fall within this interval in 95 per cent of cases and outside it – in 5 per cent of cases. The result obtained after this procedure can be represented as:

$$[\bar{x} - I_\gamma; \bar{x} + I_\gamma] \quad \text{or} \quad \bar{x} \pm I_\gamma \tag{3.19}$$

Table 3.5

The values of $t_{\gamma, n-1}$ of the Student test

K	Confidence degree		K	Confidence degree	
	95 %	99 %		95 %	99 %
1	12.71	63.66	18	2.10	2.88
2	4.30	9.92	19	2.09	2.86
3	3.18	5.84	20	2.09	2.84
4	2.78	4.60	21	2.08	2.83
5	2.57	4.03	22	2.07	2.82
6	2.45	3.71	23	2.07	2.81
7	2.36	3.50	24	2.06	2.80
8	2.31	3.36	25	2.06	2.79
9	2.26	3.25	26	2.06	2.78
10	2.23	3.17	27	2.05	2.77
11	2.20	3.11	28	2.05	2.76
12	2.18	3.06	29	2.04	2.76
13	2.16	3.01	30	2.04	2.75
14	2.14	2.98	40	2.02	2.70
15	2.13	2.95	60	2.00	2.66
16	2.12	2.92	120	1.98	2.62
17	2.11	2.90			

It is also possible to find the 95 % confidence interval without using Table 3.5, i.e. without determination of $t_{\gamma, n-1}$ of the Student test. The interval is then calculated as:

$$I_{95\ \%} = 2\sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n(n-3)}} \qquad (3.20)$$

which holds for $n>4$.

Example. T. ferrooxidans oxidizes Fe^{2+} on the medium 9K (No. 2). To calculate the mean for ten measurements of the oxidation rate and its confidence interval it is necessary to fill in Table 3.6. The first column of the table contains the measured values of Fe^{2+} oxidation, the second one – deviation from the mean, and the third one – squared deviation. The second column is filled in after $\bar{x}$ has been claculated as in (3.16), the third column is filled next to the second. The eleventh row of Table 3.5 contains the sums of all ten values in corresponding columns.

Let us calculate $\bar{x}$ as in (3.16):

$$\bar{x} = \frac{2312}{10} = 231.2\ (\frac{mg}{l \cdot h})$$

Calculation of the standard variance using (3.17):

$$S = \sqrt{\frac{5743.6}{9}} = 25.26\ (\frac{mg}{l \cdot h})$$

For the 0.95 confidence degree we find the value in Table 3.5, at the crossing of row 9 with column 95 % that:

$$t_{95\,\%,\,9} = 2.26$$

Thus, for the confidence probability $\gamma = 95$ % the confidence interval (3.18) equals:

$$I_{95\,\%} = 2.26 \cdot \frac{25.26}{\sqrt{10}} = 18.19 \left(\frac{\text{mg}}{\text{l} \cdot \text{h}}\right)$$

Table 3.6

Calculation of the Mean $\bar{x}$ and Standard Variance S

Measurement number N	Oxidation rate of Fe^{2+} (mg/l·h)	Difference $x_i - \bar{x}$	Squared difference $(x_i - \bar{x})^2$
1	259	27.8	772.84
2	253	21.8	475.24
3	263	31.8	1011.24
4	251	19.8	392.04
5	239	7.8	60.84
6	228	−3.2	10.24
7	210	−21.2	449.44
8	214	−17.2	295.84
9	199	−32.3	1036.84
10	196	−35.2	1239.04
$\sum_{i=1}^{10}$	2312		5743.6

and in 95 per cent of cases the true value of the mean oxidation rate of Fe^{2+} will fall in the interval (213.01; 249.39) or

$$\bar{x} = 231.2 \pm 18.19 \left(\frac{\text{mg}}{\text{l} \cdot \text{h}}\right)$$

Calculation of the confidence interval without using the Student test, i.e. as in (3.11) gives:

$$I_{95\,\%} = 2\sqrt{\frac{5743.6}{10.7}} = 18.12 \left(\frac{\text{mg}}{\text{l} \cdot \text{h}}\right),$$

which agrees well with the confidence interval obtained with the help of Table 3.5.

Estimation of the Number of Trials Necessary for Obtaining Specified Accuracy. Suppose that for a series of preliminary experiments the standard variance S and the confidence interval I_γ for the specified probability γ have been determined. The obtained confidence interval, however, does not satisfy the researcher. To calculate a new confidence interval I_p, other n trials are made, where n is a number of required trials determined as:

$$n = \left(\frac{t_{p,\,n_1-1} \cdot S}{I_p}\right)^2 \qquad (3.20)$$

where $t_{p,\,n_1-1}$ is found in Table 3.5 for the new confidence degree p in the row corresponding to the number of preliminary trials minus one: $(n_1 - 1)$.

Example. Determine the number of replications necessary to obtain the accuracy: $I_{95\,\%} = 30\ \frac{mg}{l \cdot h}$ in determining the oxidation rate of Fe^{2+} or other substrates.

According to the preliminary data considered in the above example, for 10 replications S = 25.26 and $t_{95\,\%,\,9} = 2.26$ (Table 3.5).

Thus:

$$n = \left(\frac{2.26 \cdot 25.26}{30}\right)^2 = 3.61$$

As the number of replications is necessarily an integer, the obtained value should be rounded off to the nearest greater integer (necessarily to the greater one). Conclusion: it is necessary to have not less than four replications in the experiment or to make four consecutive experiments.

Exclusion of Outliers. Frequently, the sample obtained should be checked for the so-called outliers. Outliers are usually due to some measurement errors that have escaped the attention of the researcher. There are several tests for outliers. One of them is based on the estimation of the difference between the two extreme values in a given sample, which, however, requires the use of a great number of tables [4]. Another method is to compare the difference between the outlier and the sample mean with a certain critical value:

$$(x_{out} - \bar{x}) \ \vee \cdot S \cdot f \qquad (3.21)$$

($\vee$ stands for comparison), where S – the standard variance obtained for the sample with the exclusion of the outlier, while f is taken from Table 3.7 in the row having a number of trials (the sample size) without the outlier. If the difference is greater than the product S·f, the outlier should be excluded from the sample as an error, otherwise it should be included into the sample, and new calculations of x, S and I_γ are to be made.

Example. The specific sulfur oxidizing activity of the enzymes of **T. ferrooxidans** was measured 5 times:

0.7; 0.4; 2.1; 0.9; 0.6 (n mole S^0/min·mg of protein).

Calculations for the four values (the "suspicious" 2.1 being excluded) give:

$\bar{x} = 0.65$ n mole S^0/min·mg of protein, S = 0.21 n mole S^0/min·mg of protein.

The difference $|x_{out} - \bar{x}| = |2.1 - 0.65| = 1.45$

Table 3.7 gives for the four measurements: f=6.54. Thus the product $S \cdot f = 0.21 \cdot 6.54 = 1.37$. Comparison gives $1.45 > 1.37$.

Conclusion: the value 2.1 should be excluded from the sample as erroneous.

Table 3.7

Values of f for Determining the Outliers

n	f	n	f
2	77.96	18	2.98
3	11.46	19	2.95
4	6.54	20	2.93
5	5.04	21	2.91
6	4.36	22	2.90
7	3.96	23	2.88
8	3.71	24	2.86
9	3.54	25	2.85
10	3.41	26	2.84
11	3.31	27	2.83
12	3.23	28	2.82
13	3.17	29	2.81
14	3.12	30	2.80
15	3.08	40	2.74
16	3.04	60	2.68
17	3.01	120	2.63
		∞	2.58

Determination of significant difference between the means of two samples. In order to estimate whether the difference of two groups of experimental results obtained independently under different or similar conditions is significant or not, it is necessary to compare the difference between the two means with a critical value:

$$|\bar{x}_1 - \bar{x}_2| \sqrt{\quad} t_{\gamma, n_1+n_2-2} \cdot \sqrt{\frac{1}{n_1}+\frac{1}{n_2}} \cdot \sqrt{\frac{\sum_{i=1}^{n_1}(x_i-\bar{x}_1)^2 + \sum_{i=1}^{n_2}(x_i-\bar{x}_2)^2}{n_1+n_2-2}} \tag{3.22}$$

where:

n_1 and n_2 – the sizes of the first and second samples respectively;

t_{γ, n_1+n_2-2} – the value of the Student test for the confidence degree γ, and the number of the row equal the sum of the sizes of the two samples minus two (Table 3.5). Under the second radical in the numerator is the sum of the squared differences of the first and second samples.

If the difference of the means exceeds the critical value calculated as in the right hand side of (3.22), the difference is considered significant for the confidence degree γ, otherwise not.

Example. The oxidation rate of Fe^{2+} by **T.ferrooxidans** on two media containing ZnS and $CuFeS_2$ respectively was measured. To determine whether the difference between the means is significant it is necessary to fill in a table similar to Table 3.6 for the two samples.

For the medium with sphalerite we obtain (see Table 3.6):

$$n_1 = 10, \bar{x} = 231.2 \text{ mg/l·h}, \sum_{i=1}^{10}(x_i - \bar{x}_1)^2 = 5743.6$$

For the medium with chalcopyrite we obtain:

$$n_2 = 10, \bar{x}_2 = 231.3 \text{ mg/l·h}, \sum_{i=1}^{10} (x_i - \bar{x}_2)^2 = 8828.10$$

For the confidence degree of 95 % we find in row number 18 of Table 3.6:
$t_{95\,\%,\,18} = 2.1$.
The difference of the means $|231.2 - 231.3| = 0.1$

The right-hand side of (3.22) is:

$$2.1 \cdot \sqrt{\frac{1}{10} + \frac{1}{10}} \cdot \sqrt{\frac{5743.6 + 8828.10}{18}} = 2.1 \cdot 0.45 \cdot 28.45 = 26.88.$$

Comparison gives $0.1 < 26.88$.
Conclusion: there is no difference in the oxidation rates of Fe^{2+} on media with chalcopyrite and sphalerite.
When the sample size is rather large ($n > 20$) the expression (3.22) can be simplified:

$$|\bar{x}_1 - \bar{x}_2| \; V \; \sqrt{(I_\gamma)_1^2 + (I_\gamma)_2^2} \qquad (3.23)$$

The right-hand side of the expression represents the square root of the sum of squared confidence intervals of the two samples.

The correlation coefficient. The correlation coefficient $r_{x,\,y}$ is a measure of the degree and character of correlation between two random variables X and Y. It is calculated as:

$$r_{x,\,y} = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{S_x S_y (n-1)} \qquad (3.24)$$

where S_x and S_y – standard variances of the random variables X and Y respectively.

Note, that the positive value of the correlation coefficient ($r_{x,\,y} > 0$) is indicative of a positive or direct correlation – the increase of the random variable X results in the increase of Y. A negative correlation coefficient ($r_{x,\,y} < 0$) implies that the increase of X will result in the decrease of Y. Besides, depending on the value range, the correlation may be specified as:

weak if $0 \leqslant |r_{xy}| < 0.3$
moderate if $0.3 \leqslant |r_{xy}| < 0.7$
strong if $0.7 \leqslant |r_{xy}| \leqslant 1$

The correlation coefficient is used in regression equations to obtain predictions on the value of one random variable on the basis of the known value of another:

$$\overline{Y} - \overline{Y} = r_{xy} \frac{S_y}{S_x} (x - \overline{x}) \qquad (3.25)$$
$$\overline{x} - \overline{x} = r_{xy} \frac{S_x}{S_y} (y - \overline{y})$$

Example. Determine the existence and character of correlation between the oxidation of iron and sulfur by **T.ferrooxidans** on the medium with chalcopyrite [25]. Experimental data and the results of intermediate calculations are shown in Table 3.8.
Calculate then:

$$S_x = \sqrt{\frac{8828.1}{9}} = 31.32; \; S_y = \sqrt{\frac{7.66}{9}} = 0.92$$

$$(3.24) \text{ gives } r_{xy} = \frac{231.89}{31.32 \cdot 0.92 \cdot 9} = 0.88$$

Conclusion: A strong positive correlation is found between the oxidation of iron and sulfur by **T.ferrooxidans** on the medium with chalcopyrite.
Method of Least Squares. Kinetic studies of microbiological processes, including the determination of the growth rate and substrate consumption dependence on changes in environmental conditions and calculation of the growth yield and maintenance coefficient, require the determination of the slope of straight lines and their portions cut off by some straight lines on the corresponding axes. From the point of view of the regression theory it is necessary to determine the coefficients a and b in the regression equation:

$$y = ax + b \qquad (3.26)$$

where:
y is a random variable which lineary depends on the other random variable x.

A possible solution is to determine the correlation coefficient and make use of the regression equation (3.25). For the example considered above:

$$y - 3 = 0.88 \frac{0.92}{31.32} \cdot (x - 31.3)$$

Thus: $y = 0.026x + 2.2$; $a = 0.026$; $b = 2.2$.

Another approach is based on the least squares method. It is based on the minimization of the sum of squared deviations of the experimental values from the sought for theoretical straight line. The following formulae are used in the method of least squares:

$$\begin{cases} bn + a \sum\limits_{i=1}^{n} x_i = \sum\limits_{i=1}^{n} y_i \\ b \sum\limits_{i=1}^{n} x_i + a \sum\limits_{i=1}^{n} x_i^2 = \sum\limits_{i=1}^{n} x_i y_i \end{cases} \qquad (3.27)$$

Table 3.8

Calculation of the Correlation Coefficient r_{xy}

Group number	x – oxidation rate of Fe^{2+}, mg/l·h	$x_i - \bar{x}$	$(x_i - \bar{x})^2$	y – oxidation rate of S^0, mg/l·h	$y_i - \bar{y}$	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$
1	266	34.7	1204.09	4.39	1.39	1.93	48.233
2	275	43.7	1909.69	4.12	1.12	1.25	48.944
3	257	25.7	660.49	3.54	0.54	0.29	13.878
4	251	19.7	388.09	3.25	0.25	0.06	4.925
5	220	−11.3	127.69	3.19	0.19	0.03	−2.147
6	243	11.7	136.89	3.05	0.05	0.00	0.585
7	200	−31.3	979.69	2.73	−0.27	0.07	8.451
8	214	−17.3	299.29	2.44	−0.56	0.32	9.688
9	182	−49.3	2430.49	1.78	−1.22	1.49	60.146
10	205	−26.3	691.69	1.51	−1.49	2.22	39.187
$\sum_{i=1}^{n}$	2313		8828.10	30		7.66	231.89

Linear system of equations (3.25) has the only solution which is determined after the four variables of the experimental data: $\sum_{i=1}^{n} x_i$; $\sum_{i=1}^{n} y_i$; $\sum_{i=1}^{n} x_i^2$ and $\sum_{i=1}^{n} x_i y_i$ have been found.

As the method of least squares requires less preliminary calculations than the regression equation of type (3.25), the former is more widely used in microbiological studies.

Example. In [25] data is given on growth and consumption of the substrate **T.ferrooxidans** in a continuous culture:

Growth rate (dilution rate)	μ [1/h]	0.01	0.02	0.04	0.06	0.08	0.09	0.1
Substrate consumption	$q\left[\frac{g \cdot a \cdot Fe^{2+}}{g \cdot C \cdot h}\right]$	0.33	0.407	0.63	0.9	1.126	1.205	1.36

In Pert's equation [48]: $q = \frac{\mu}{Y_{max}} + m$ the substitution of x for μ and of y for q gives $y = ax + b$, where $a = \frac{1}{Y_{max}}$; b=m. (3.28)

Calculations by the method of least squares require filling in Table 3.9 where a row in each column contains the sums of all values in the column. Substitution of these sums in (3.27) gives:

$$b \cdot 7 + a \cdot 0.4 = 5.958$$

$$b \cdot 0.4 + a \cdot 3.02 \cdot 10^{-2} = 0.4251$$

Solution of the system of equations gives: a = 11.85, b = 0.198.

$$\text{Thus, } Y_{max} = \frac{1}{a} = 0.0844 \left[\frac{g \cdot C}{g \cdot a\ Fe^{2+}}\right] \qquad (3.29)$$

$$m = b = 0.198 \left[\frac{g \cdot a \cdot Fe^{2+}}{g \cdot C \cdot h}\right] \qquad (3.30)$$

Apparently, the statistical processing of the results obtained requires numerous calculations. A wide spread of computers has made it possible to effect the statistical procedure on computers, for which standard programmes have been developed including those for the above methods. We must note that frequently statistical processing of results in microbiology gives reliable data when the sample size is considerable. Therefore, it is recommended to resort to mathematical modelling of experiments to reduce the number of required replications of experiments.

Table 3.9

Calculations by the Method of Least Squares

No.	$x_i=\mu$ [1/h]	$y_i=q_i$ [$\frac{g\cdot a\cdot Fe^{2+}}{g\cdot C\cdot h}$]	x_iy_i	x_i^2
1	0.01	0.33	0.0033	$0.01\cdot10^{-2}$
2	0.02	0.407	0.0081	$0.04\cdot10^{-2}$
3	0.04	0.63	0.0252	$0.16\cdot10^{-2}$
4	0.06	0.9	0.0540	$0.36\cdot10^{-2}$
5	0.08	1.126	0.0901	$0.64\cdot10^{-2}$
6	0.09	1.205	0.1084	$0.81\cdot10^{-2}$
7	0.1	1.36	0.1360	$1\cdot10^{-2}$
$\sum_{i=1}^{7}$	0.4	5.958	0.4251	$3.02\cdot10^{-2}$

3.4.2. Study and Optimization of Biogeotechnological Processes by Means of Mathematical Modelling

At the present time there are numerous mathematical models developed for physical, chemical and microbiological processes that are important in biogeotechnology [7, 17, 18, 33, 36, 41, 56].

To solve the problem of increasing the output of the final product and optimize production processes formal models of the "black box"-type are used most frequently. In these models a variable to be optimized (for example, the total volume of the product obtained, the rate of its output, consumption of certain substrates, etc.) is represented as a function of process variables, the coefficients of which have no real physiological sense. Such models are instrumental in understanding how certain process variables (like the size of particles and the concentration of bacterial cells, pH, etc. affect the target function, and allow to optimize the process. They, however, do not provide an answer why their effect is such as it is. Formal models are constructed without taking into account the mechanisms of processes involved and how they are realized (for example, dump, in-situ or tank leaching).

The advantage of formal models lies in their simplicity while their limitation is the impossibility of realizing them under conditions different from those of preliminary experiments.

To make use of another type of models – mechanistic – that are constructed and studied by means of target-oriented experiments, the researcher should have a preliminary clear-cut hypothesis on the interaction of the chosen process variables, and on the kinetics and stoichiometry of corresponding reactions.

The advantages of such models are obvious. Their construction results in a better understanding of the studied process. Besides, such models allow to optimize and make prediction for the studied process on the basis not of a single target function, as is done in formal models, but of a number of them (for example, the dilution rate, the substrate consumption rate, etc.). These models can also forecast other operational modes of the process (for example, the transition from periodic to continuous leaching of metals). There are, however, both objective and subjective factors that impede the introduction of these methods into biological practice.

As is noted above, mechanistic models require for their construction certain experimental and theoretical material that would allow to formulate a preliminary hypothesis on the character and conditions of the process. Besides, unlike many of the considered above methods of mathematical processing of results, which an experimenting microbiologist may have a good command of, mathematical modelling requires his or her joint work with a mathematician. The formalization of preliminary hypotheses formulated by a microbiologist, their presentation in the form of mathematical equations (most frequently, differential and non-linear ones), the model analysis, determination of the model constants and its solution by means of a computer – all this requires special training in mathematics.

To work together, the microbiologist and mathematician should have "a common language", i.e. each of them should have some kind of understanding of what the other can do.

Formal models of leaching. The simplest way of obtaining a formal model is to represent the experimental curve of metal leaching obtained for different values of a certain variable (time, concentration of the reagent or bacteria) as a function of this variable. This method was first used to describe the process of chemical leaching of copper and uranium from low-grade ores [70]:

$$m(t) = 1 - e^{-k(t+c)} \qquad (3.31)$$

where:

$m(t)$ – the amount of recovered metal;

t – time; k, c – constants determined specifically for heap leaching. The model describes an experimental curve of the hyperbolic type, however, any change in conditions, e.g. the heap geometry, the acid strength or the size of ore particles, necessitates a change of k and c, and equation (3.31) would no longer predict the course of the process.

A similar approach was used to describe the rate of chemical leaching as a function of the main process parameters [56]:

$$\frac{1}{m}\frac{dm}{dt} = k(T, H)\, S^h \qquad (3.32)$$

where:

$k(T, H)$ – the kinetic coefficient the value of which is determined by the temperature and hydrodynamics of the medium,

S – the reagent concentration,

h – an empirical exponent.

A power dependence of the form:

$$\frac{dm}{dt} \begin{cases} A_1 m^n + A_2; & m > m_{crit.} \\ A_3 m^n & m < m_{crit.} \end{cases} \qquad (3.33)$$

where:

A_1, A_2, A_3 and $m_{crit.}$ – empirical coefficients, was used to describe hydrometallurgical processes. [illegible] The application of this model made it possible to obtain optimum flow charts for tank leaching of copper-zinc concentrate.

Formal models of the regression-type have been widely used in practical work. For them, the mathematical model is represented in the form of a regression equation (or a power series):

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i,j=1}^{n} a_{ij} x_i x_j + \sum_{i=1}^{n} b_i x_i^2 + \ldots \qquad (3.34)$$

where:

Y – studied function called a response surface, and x_i, x_j – variables (factors) which determine the value of Y. In many cases it is possible to use a simpler linear dependence:

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i \qquad (3.35)$$

or a quadratic dependence:

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i,j=1}^{n} a_{ij} x_i x_j + \sum_{i=1}^{n} b_i x_i^2 \qquad (3.36)$$

There are different methods of estimating the regression coefficients in equations (3.34), (3.35) and (3.36). Some of them, pertaining to linear regression, are dealt with above (the determination of the correlation coefficient, the method of least squares). For more complex cases, there are methods of obtaining a design for many factors which serve as a basis for calculating the values of a_{ij} and estimating their significance [29]. These methods allow to vary, after the coefficients have been determined, the level of the factors to find the optimum value of Y on the response surface. These methods can be successfully used to optimize many factors of processes of leaching metals from ores and concentrates.

In [32] the methods of mathematical design of experiments were used to determine the optimal conditions of bacterial leaching of non-ferrous metals from ores. In the equation of linear regression four factors were varied: x_1 – pH, x_2 – KH_2PO_4 (mg/l), x_3 – concentration of the cells of **T.ferrooxidans** in 1 ml, x_4 – concentration of Fe^{2+} (mg/l). The oxidation rate

of Fe^{2+} was taken to be the optimization parameter Y. The equation obtained

$$Y = 172 + 7.25\,x_1 - 1.75\,x_2 + 15.25\,x_3 + 9.25\,x_4 \qquad (3.37)$$

allowed to make new series of experiments to find the optimum value of Y on the response surface. As a result, the oxidation rate of Fe^{2+} was increased by 2.5 times in comparison with the oxidation rate on Leathen's medium.

In [32] there are examples of how linear regression equations were used to optimize process conditions for leaching with respect to three factors – x_1, x_2 and x_3, which made it possible to increase the rate of extraction of copper into the solution by 2 times and that of zinc by 1.5 times.

Singh and Torma [65] made use of a similar method to analyze data on leaching of uranium from ores. The amount of uranium extracted over a period of 30 min was taken as Y while the strength of HCl (x_1), $NaClO_3$ (x_2), temperature (x_3), the pulp density (x_4) and agitation rate (x_5) were taken as factors determining its value. The results of 29 experiments made it possible to obtain a quadratic regression equation:

$$10^6 Y = -43 + 20.9x_1 - 24.6x_1^2 - 637.5x_2 + 2.35 \cdot 10^5 x_2^2 + 0.2x_3 - 0.003x_3^2 +$$

$$+ 0.1x_4 - 0.001x_4^2 - 221.5x_1x_2 - 0.1x_1x_3 + 1.3x_2x_3 + 0.1x_2x_5 + 0.0003x_3x_5 \qquad (3.38)$$

which allowed to predict the leaching rates for the studied range of the variables.

Methods for Obtaining Adequate Mechanistic Models. Schematically, the construction of an adequate mechanistic model can be represented as in Fig. 3.27. Prior to modelling as such, the researchers (a microbiologist and a mathematician) should formulate certain ideas concerning the object of their study. On the basis of preceding experiments carried out with **T.ferrooxidans,** and reported data, the process of oxidation of Fe^{2+} is described in words, i.e. a verbal physiobiochemical model is first obtained. Simultaneously, model variables are identified.

To make it clearer, let us consider, by way of an example, a verbal physiobiochemical model of the oxidation of lower iron [41].

The following assumptions served as a basis for the model:

1. The growth of **T. ferrooxidans** is controlled by the oxidation of lower iron, which is a kind of "a bottle-neck" of the process.
2. Fe^{2+} is a limiting substrate of the "bottle-neck" reaction.
3. The kinetics of the "bottle-neck" (dependence of the specific reaction rate on the concentration of the limiting substrate) is similar to Monod's kinetics.
4. The microbial growth yield per unit of consumed substrate is constant.
5. Fe^{2+} and biomass are chosen as process variables.

More complex physiobiochemical models are naturally based on a greater number of assumptions. For example, the physiobiochemical model of chalcopyrite leaching contains both explicit and implicit assumptions on the

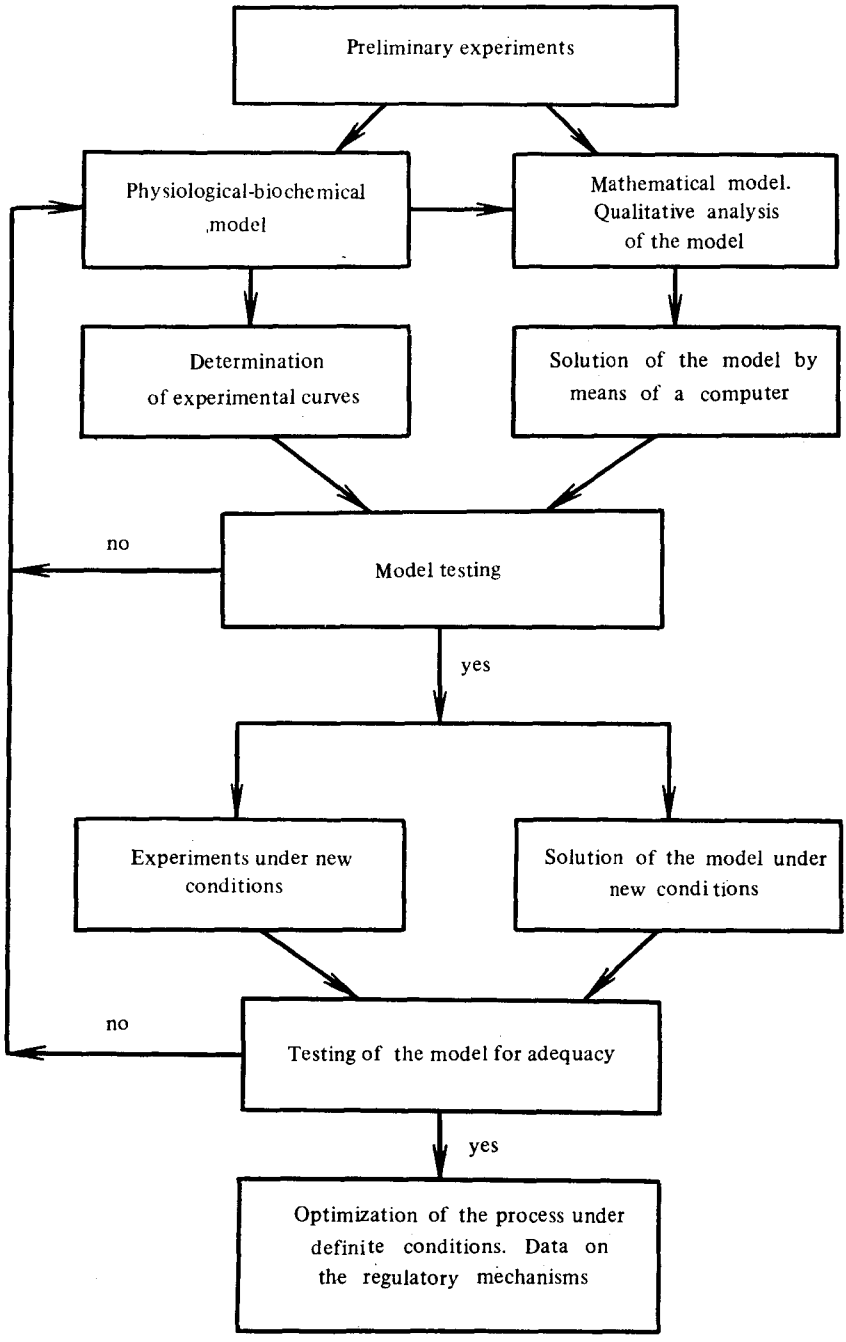

Fig. 3.27. Principal scheme of process study by mathematical modelling method

stoichiometry of chemical and bacterial leaching, on direct and indirect effects of **T. ferrooxidans**, on the recovery of iron and kinetics of the above process stages etc. [31].

When such a model is formulated, the microbiologist comes to the fore because his or her knowledge and intuition may help correctly determine "bottle-necks" of the process. It often happens that the kinetics of the interaction of certain variables cannot be determined in principle or their determination requires considerable time and means. In such a case an assumption is made on the most probable kinetics of that process stage, which can be later either supported or rejected.

The difference between a verbal and a mathematical model can be made clear by means of the following example. The statement: "The growth rate of **T. ferrooxidans** increases with the increase of Fe^{2+} concentration up to the maximum", is a very simple verbal model while the formalization of this statement in the form of an equation:

$$\mu = \frac{\mu_m Fe^{2+}}{K_s + Fe^{2+}} \qquad (3.39)$$

makes it possible to determine the numerical value of constants μ_m and K_s, and to predict accurate values of the growth rates for specified concentrations of lower iron.

The physiobiochemical model serves as a basis for a mathematical model which is constructed by the mathematician who takes into account both the process variables and the mass balance of the system. The mathematician should also carry out a qualitative analysis of the mathematical model – to determine the stability of possible stationary states, the domains of feasible and bifurcation values of the parameters, as well as to study the phase-plane portraits of the system. Such a study would allow to determine the range of external conditions of experiments aimed to test the quality of approximation and adequacy of the model, and to set guidelines for further experiments.

Preliminary values of certain parameters and coefficients of the model can be determined in independent experiments or taken from reported data.

In compliance with the physiological model obtained, the microbiologist makes an experiment (for example, at a constant temperature, agitation, pressure, etc.) to obtain the temporal dynamics of the model variables. The mathematician solves the obtained system of differential equations of the process mathematical model by means of a computer. The solutions obtained and the experimental curves (e.g., those of biomass or product accumulation, substrate consumption, etc.) are compared for different values of the model parameters to test the model for the quality of approximation.

The system of differential equations of deterministic models can often be represented in a general form as:

$$\frac{dy_i}{dt} = f_i(Y_1, Y_2, \ldots, Y_n; X_1, X_2, \ldots, X_m; a_{i1}, a_{i2}, \ldots, a_{ik}) \qquad (3.40)$$

$i=1, 2, \ldots, n.$

where:

$\{x_1, \ldots, x_m\} = \vec{x}$, $\{y_1, \ldots, y_n\} = \vec{y}$, $y_i(0) = \vec{y}_0$ – input and output coordinates, and coordinates of the origin respectively;

$\{a_{i1}, \ldots, a_{ik}\} = \vec{a}_i$ – the equation parameters.

The closeness of the calculated and experimental (marked with "e") data can be estimated by a function of the form:

$$\phi = \sum_{i=1}^{n} \sum_{\lambda=1}^{d} \sum_{j=1}^{l} (Y_{i\lambda j} - Y^e_{i\lambda j})^2 \qquad (3.41)$$

where:

n – the number of equations,

d – the number of series of experiments,

l – the number of points at $\bar{x}(t_j) = x^e(t_j)$;

$y_i(0) = y_i^e(0)$, $0 \leqslant t \leqslant T_k$.

The identification of unknown parameters of the model comes to the determination of the minimum of the function ϕ with respect to k variables $\bar{a}_i$. This task can be solved only by a mathematician-programmer who knows how to determine the constants by means of a computer, i.e. who has a command of methods pertaining to an independent and quite complex field of studies.

The testing of the model for the quality of approximation may give two results (Fig. 3.27): a) "no" – the calculated results do not coincide with the experimental curves obtained for all considered values of the model parameters; b) "yes" – the value of the ϕ test is satisfactory, i.e. the mathematical model describes the process quite well for the given range of external conditions.

The result a) implies that some assumption in the physiobiochemical model is false. The model then should be reconsidered and tested again for the quality of approximation till a positive result is obtained. In the course of this study adequate kinetic characteristics may be determined for certain stages of the process without direct experiments in this field. Regretfully, the majority of the published models on leaching of metals do not provide information on such a cyclic model testing. The authors usually simply note discrepancy of the calculated results and certain segments of the experimental curve and make assumptions as to how this discrepancy can be overcome, without describing the second cycle – the actual testing and correcting of the model.

Assume that an adequate mathematical model is obtained after several cycles of testing. This model can be used then to optimize the studied process, to adapt it to continuous culture and to study how changes in external conditions will affect the process.

It should be noted, however, that changes in the external conditions of the process may bring about changes in the interaction of certain factors (e.g., a deficit of O_2 or excess of metal ions may appear), i.e. not only the numerical

values of certain parameters may change, but the model itself too. The calculated results are then again compared to the experimental ones for the new external conditions.

The process of obtaining an adequate mathematical model may enlarge our knowledge of the regulatory mechanisms and kinetic characteristics of the process. Besides, the model itself can be used for the optimization of different production processes (periodic, continuous, semi-periodic and fed-batch).

Example of a Study of the Oxidation of Fe^{2+} to Fe^{3+} by means of Mathematical Modelling. The oxidation of Fe^{2+} to Fe^{3+} by **T. ferrooxidans** is an important stage of many biohydrometallurgical processes and at the same time it lends itself to modelling because the process is rather simple (there is no multiphase medium, the process is limited only by the concentration of the single substrate which is the source of energy, the stoichiometric characteristics of the process are known, etc.).

Table 3.10 contains the results of a study of the oxidation of Fe^{2+} to Fe^{3+} by **T. ferrooxidans** according to the above principles.

Proceeding from the well-known model of Monod [41], which turned out to have a very limited application in the description of the oxidation of Fe^{2+}, several models were consecutively obtained for an ever increasing range of external conditions with respect to the temperature, acidity of the medium and the initial substrate concentration [20, 21, 49, 51, 53, 54]. All the model modifications had one thing in common – they all were based on the assumption of the possibility to consecutively alter "bottle-necks" [52] that presumed the oxidation of Fe^{2+} and hydrolysis of $Fe_2(SO_4)_3$ (Table 3.10, Model II).

$$2FeSO_4 + 1/2\,O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O \qquad (3.42)$$

$$Fe_2(SO_4)_3 + 6H_2O \rightleftarrows 2Fe(OH)_3 + 3H_2SO_4 \qquad (3.43)$$

The desire to broaden the range of applicability of the obtained model to higher and lower substrate concentrations compared with the original ones, as well as to higher and lower temperatures resulted in the necessity to take into account the reversability of the hydrolysis reaction. It was assumed that under certain conditions part of the oxidized iron obtained in the above reaction precipitates while under different conditions all ferric oxide is in soluble form (Table 3.10, Model IIa):

$$Fe^{3+}_{prec} + Fe^{3+}_{dissol.} = Fe^{3+} \qquad (3.44)$$

Besides providing more accurate information on the change of kinetic characteristics of the oxidation of Fe^{2+} and hydrolysis of $Fe_2(SO_4)_3$ reaction with temperature Model IIa drew the attention of the researchers to an important regulatory function of hydrolysis – slight variations in the balance of $Fe_2(SO_4)_3$ hydrolysis and $Fe(OH)_3$ solution considerably change the rate of the oxidation process as a whole. Thus, a new direction of the experimental work was found.

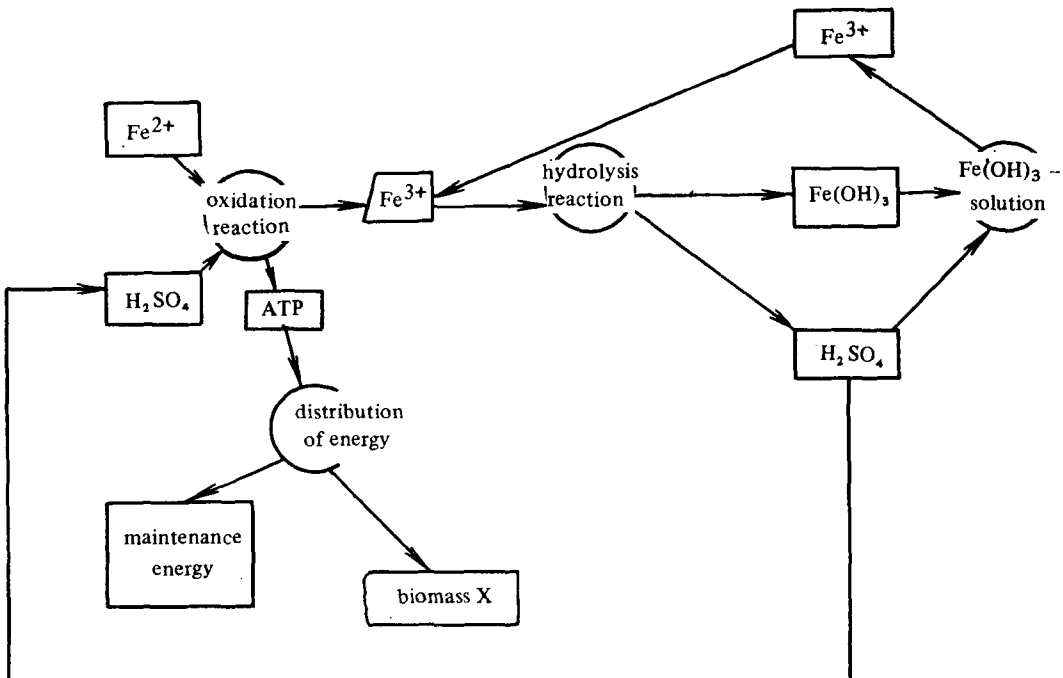

Fig. 3.28. Block diagram of T. ferrooxidans growth and Fe^{2+} oxidation (corresponds to model IIb, Table 3.11)

The attempt to describe, besides the oxidation of iron, the growth of the biomass of **T.ferrooxidans** resulted in the necessity to take into account the maintenance energy (Fig. 3.28, Table 3.10, model IIb).

Note, that every subsequent model incorporates, in a natural way, the preceding one. For example, the equation $Fe^{3+}_{dissol} = Fe^{3+}$ holds for the external conditions of Model II, since all iron obtained in the oxidation reaction is dissolved, and Model IIa transforms then into Model II. When the model is used in practice to optimize production processes this implies that whenever process conditions may coincide with the external conditions of the model IIa, its equations can be used appropriately. If such conditions are excluded, it is better to use the equations of Model II as more simple ones. Model IIb should be used when the biomass of **T. ferrooxidans** is considered to be one of the variables of the cultivation process, for example, in processes with a turn-over of the biomass.

Besides the models described in Table 3.10, there were certain stages in the cyclic testing of the models, not presented in this table, that provided negative results. These results, nevertheless, contributed to our knowledge of the process mechanism. For example, the testing of the models based on the assumption of an inhibitory effect of ferric oxide, resulted in the rejection of this assumption for models II and IIa, while for Model IIb this assumption was shown to be valid.

Thus, the use of mathematical modelling and the cooperative work of microbiologists and mathematicians in studying iron biooxidation gave the following results:

1) the following hypotheses on the regulatory mechanisms of the process were confirmed: existence of two reactions that control the process, and dependence of their rates on the substrate concentrations, the acidity of the medium and temperature (without taking into account the adaptation of bacteria);

2) a new direction for further experimental work was determined – to study the effect of the reversability of the hydrolysis reaction to better understand the process regulatory mechanism;

3) kinetic characteristics of the oxidation and hydrolysis reactions were found together with their dependence on temperature;

4) a range of the medium acidity was determined, within which the Fe^{2+} oxidation attains a maximum rate, as well as dependence of this range on temperature;

5) the model obtained can be used for the optimization and control of oxidation processes of different types with respect to various controlled parameters.

Modelling of Leaching by T. Ferrooxidans. Leaching of metals from ores and concentrates by means of microorganisms is the most complicated of all the processes considered above. Correspondingly, it is much more difficult to construct and test its mechanistic models. Therefore, a few attempts have been made so far to consider theoretical concepts of bacterial and chemical leaching of metals from concentrates in connection with the physical regularities of periodic processes.

Table 3.10

A Study of Ferrous Iron Oxidation by T. Ferrooxidans by means of Mathematical Modelling

Brief description of the model	Ref.	Mathematical model	Model adequacy domain	Applicability for process control and optimization	Data on the regulatory mechanisms
One "bottle-neck" — the oxidation reaction with the kinetics of Monod (Model I)	[11]	$\frac{dx}{dt} = \frac{\mu m\, Fe^{2+}}{K_s + Fe^{2+}} \cdot x$ $\frac{d\,Fe^{2+}}{dt} = -\frac{1}{Y}\,\frac{\mu m\, Fe^{2+}}{K_s + Fe^{2+}} \cdot x$	It describes only the exponential phase of the curves x(t) and Fe^{2+}(t) at $20\ ^{o}C \leq T \leq 31\ ^{o}C$		The oxidation rate corresponds to the kinetics of Monod only in a limited range of the substrate concentration and temperature values
Two "bottle-necks" – the oxidation and hydrolysis reactions. The biomass production is proportional to the formation of Fe^{3+}. No maintenance energy (Model II)	[20, 51, 52, 53]	$\frac{dFe^{2+}}{dt} = -\mu_1 (Fe^{2+}, pH, T) \cdot Fe^{3+}$ $\frac{dFe^{3+}}{dt} = \left[\frac{1}{Y_1}\mu_1 (\ldots) - \mu_2 (Fe^{3+}, pH, T)\right] Fe^{3+}$ $\frac{d\,pH}{dt} = -\left[-\frac{1}{Y_2}\,\mu_1 (\ldots) + \frac{1}{Y_3}\mu_2 (\ldots)\right] Fe^{3+}$	It fully describes the curves Fe^{3+} (t), Fe^{2+}(t) and pH(t) at T=28°, 12 °C at the initial concentration of $Fe_0^{2+} \sim$ 5 g/l	For the studied T and Fe_0^{2+} a range of pH values is determined within which the two reactions are optimal, that considerably increases the process rate at lower temperatures. It is possible to optimize continuous leaching	Kinetic dependences of the rate of oxidation and hydrolysis on Fe^{3+}, Fe^{2+} and pH are obtained. Their dependence on temperature is studied
In addition to the conditions of Model II, the	[21, 49]	$\frac{dFe^{2+}}{dt} = -\mu_1 (Fe^{2+}, pH, T) \cdot Fe^{3+}$	Fully describes the curves Fe^{3+} (t), Fe^{2+} (t) and pH (t)	For all practically possible temperature and initial substrate	Kinetic curves μ_{max} (T) and K_S (T) are obtained; the

reversability of the hydrolysis reaction is indirectly taken into account (Model II a)		$\frac{dFe^{3+}_{sol.}}{dt} = [\frac{1}{Y_1}\mu_1(\ldots)\cdot Fe^{3+} - \mu_2(Fe^{3+}, pH, T]\cdot Fe^{3+}_{sol.}$ $\frac{dpH}{dt} = -[-\frac{1}{Y_2}\mu_1(\ldots)\cdot Fe^{3+} + \frac{1}{Y_3}\mu_2(\ldots)\cdot Fe^{3+} + \frac{1}{Y_3}\mu_2(\ldots)\cdot Fe^{3+}_{sol.}]$, $\cdot Fe^{3+} = Fe^{2+}_0 + Fe^{3+}_0 - Fe^{2+}$	for different initial temperature and concentration values	concentration values optimum intervals of the pH are determined. It is possible to optimize a continuous culture	effect of the hydrolysis reaction reversability at different concentrations of the imitating substrate is estimated
In addition to the conditions of Model IIa, the maintenance energy is accounted for	[50]	$\frac{dFe^{2+}}{dt} = -(\frac{\mu_1(\ldots)}{Y_{max}} + m)\cdot x$ $\frac{dFe^{3+}_{sol.}}{dt} = \frac{1}{Y_1}\mu_1(\ldots)\cdot x - \mu_2(\ldots)Fe^{3+}_{sol.}$ $\frac{dpH}{dt} = -[-\frac{1}{Y_3}\mu_1(\ldots)\cdot x - \frac{1}{Y_4}\mu_2(\ldots)\cdot Fe^{3+}_{sol.}]$ $\frac{dx}{dt} = \mu_1(\ldots)\cdot x$	It accurately describes the curves of Fe^{3+} (t), Fe^{2+} (t), pH (t) and that of the biomass – x (t)	It is possible to optimize the continuous culturing with respect to the biomass, multistage processes and processes with recirculation	The maintenance energy is determined together with its dependence on external conditions, and the maximum growth yield

Table 3.11

Study of Continuous Bacterial Leaching of Concentrates by means of Mathematical Modelling

Brief description of the model	Reference	Mathematical model	Model adequacy domain	Applicability for process control and optimization	Data on the regulatory mechanisms
Conditions of the model in [15] are supplemented by a possible growth of cells immobilized on the solid phase. The growth rate is constant	[23]	$\overline{x} = \frac{K}{a(D/\mu_m - 1)} + \frac{\mu_m}{D \cdot a} S$ K – the ratio of adsorption to desorption rates; S – area of the available surface of the concentrate; a – share of the surface area occupied by bacteria	Substrate – finely divided zinc concentrate $0.0171 \leqslant D \leqslant 0.1038$ $[h^{-1}]$ $2.0 \leqslant pH \leqslant 2.5$ T = 36 °C Concentration of bacteria does not limit the leaching rate	Optimization and control with respect to the model equation variables are possible	The values of many physiological growth constants have been determined
Conditions of the model in [23] are supplemented by a possible growth of cells in a liquid medium. Kinetics of the growth is similar to the kinetics of Monod	[10]	$\overline{x}_s = \frac{kx_b}{a(1 + kx_b)}$ x_b – concentration of bacteria in the solution; x_s – concentration of bacteria adsorbed by the solid phase	Substrate – finely divided pyrite. $0.012 \leqslant D \leqslant 0.22$ $[h^{-1}]$ pH = 2.8 T = 28 °C	Process optimization and control in a wider range of external conditions are possible	The rate of bacterial leaching is found to be a function of many external conditions. The assumption of a predominant effect of cell adsorption is supported

Mochev [44] developed a model of bacterial leaching of ore based on the assumption that the development of the population from a single bacterium is a branching Markovian process and the effective size of ore particles has a continuous distribution from 0 to a certain maximal value. The introduction of an empirical dependence of the component recovery on time and taking into account the laws of diffusion allow to calculate the amount of processed solid phase in the ore mass.

When describing the periodic processes of copper leaching from chalcopyrite and nickel leaching [30, 56] the assumed physiobiochemical model was expressed as a system of quasi-chemical equations. After certain assumptions had been made on the kinetics of these reactions, a mathematical model of the process took the form of non-linear differential equations describing the change rates of the process variables.

Comparison of the computed model solutions with the experimental data allowed to improve the physiobiochemical model – it showed that the effect of changes in the acidity and in the redox potential of the medium on the reaction kinetics should be taken into account. Regretfully, the second version of the mathematical model with necessary corrections and ammendments has not been obtained so far.

For a steady-state stationary process of continuous culturing, many of the variables of periodic culturing can be regarded as constants.

The fact that in this case the growth rate of the microorganisms equals the dilution rate makes mathematical modelling considerably simpler.

Table 3.11 contains results of a study of continuous bacterial leaching of concentrates by means of mathematical modelling. Gormely [23] modified Erickson's model [15] for the growth of periodic culture on hard soluble substrates. He introduced additional assumptions that the cells grow only in contact with the solid phase and that the growth rate is a constant value. In his equation for the steady-state biomass concentration, the only factor limiting the substrate growth includes "the available surface area", which, however, varies in the leaching process.

The model of Chang [10] contains an additional assumption on the possibility of the cells growth in the liquid phase, with the growth kinetics satisfying the equation of Monod. This allowed to obtain an adequate model applicable to the optimization and control of the process in a wider range of external conditions and at the same time supported the initial assumptions of Gormely and Erickson. The modelling also yielded certain kinetic regularities of the bacterial development on a solid medium, including the dependence of the bacterial leaching rate on various variable factors of the environment. Note, that in principle such regularities can be determined only by means of modelling.

Thus, continuous microbial leaching of finely divided concentrates and regeneration of iron have been most fully studied so far by means of mathematical modelling.

The methods of mathematical modelling described and illustrated in this work require of research workers who wish to use them strict adherence to

certain principles. To reject any of the stages of the diagram shown in Fig. 3.27 means to considerably diminish the efficiency of the model applications.

If the results of the model and the experiment have not been compared or their comparison has given an unsatisfactory result, and the second stage of the cycle – testing of the model – is lacking, it is impossible to verify the initial assumptions and to optimize the process.

If the comparison with the experimental data is positive but the model has not been tested for adequacy for different conditions, the model then only supports the initial assumptions and facilitates process control and optimization within a limited range of external conditions.

The use of mathematical modelling in process control and optimization gives satisfactory results only when joint studies are carried out according to the complete diagram presented in Fig. 3.27.

A wider use of this method in studying bacterial leaching of metals has been limited so far by a shortage of experimental data on the kinetics of the "key reactions" of this process and, in particular, by scarce experiments aimed at determining the dynamics of possible model variables. Some of the published works contain valuable information for modelling. Panin et al. [47] described the oxidation kinetics for sulfide minerals; Imai [28] provided experimental curves for the growth of bacteria, changing acidity of the medium, leaching of sulfide minerals and accumulation of ferric oxide on media with and without bacteria for different initial concentrations of sulfides. In order to obtain an adequate mathematical model, however, microbiologists, mathematicians and engineers should work in close cooperation.

In conclusion it should be noted that in the last few years researchers working in the field of mathematical modelling of biotechnological processes have rejected the idea of obtaining a universal model, though attempts to do this were made when the possibilities of computers seemed so fascinating. At present the focus of attention is on the models that serve to describe, study and optimize specific processes. This direction seems to be the most rational one in studying biohydrometallurgical processes by means of mathematical modelling.

3.5. METHODS FOR EVALUATING ECONOMIC PARAMETERS OF BACTERIO-CHEMICAL LEACHING

The economic efficiency of bacterio-chemical leaching processes depends primarily on the character of the processes (dump, in-situ or tank leaching), on the metal strength in the ore or product to be leached, on the quality and quantity of products obtained, on capital and operational costs.

Let us briefly consider a method of estimating the economic efficiency based on a number of material and cost indices.

Dump leaching. In dump leaching initial data for the determination of economic indices of the process include:

– metal content of heaped ore;

– amount of ore to be leached;

– total and annual metal recovery over the period of leaching;
– duration of leaching;
– technique of metal recovery from the pregnant solution;
– self-cost of the commercial product obtained.

First of all, capital cost is estimated, which includes the costs of ore extraction and storage, as well as those of reagents used in the process and of other auxiliary materials. Sometimes the ore extraction cost is considered when mining cost is estimated. In our case the ore extraction cost is not taken into account in the estimation of the metal recovery cost and the self-cost of the metal.

The capital cost of heap leaching is not high. It includes investments on the preparation of the leaching site, piling of ore on it and expenditures on technical means used such as bulldozers, boring rigs, pumps, the piping of the cementators, loaders and devices for loosening the heaps. In intensive bacterial leaching, the cost of a compressor station and regeneration pool is to be taken into account as well. Besides, the capital cost should cover expenditures on:

– construction of a pilot project, when necessary;
– delivery of various reagents and auxiliary materials;
– store houses and the like;
– instruments of analytical and microbiological control;
– conducting research.

Other types of expenditures are referred to as operational costs. Operational costs usually cover expenditures on:

– wages and salaries of the personnel;
– electric power;
– water;
– sulfuric acid;
– salts used in the preparation of nutrient media and added to solutions (if this is required by the process technology);
– air;
– auxiliary materials such as iron used for cementation;
– transportation;
– depreciation charges.

If, for example, the total expenditures on the recovery of 1t of copper by heap leaching together with the mining and metallurgy process are taken as 100 %, their general profile will be as follows:

mining – 25 %;
sulfuric acid – 20 %;
iron for cementation – 17 %;
metallurgy process – 31 %;
labour, electric power, water, etc. – 7 %.

The expenditures on sulfuric acid may amount to 40 and more per cent of the total copper recovery cost as the consumption of sulfuric acid depends on the mineral composition of the leached ore, primarily on the content of pyrite carbonates, and on the intensity of microbiological processes. If necessary conditions are created in the leached heap to sustain the life of microorganisms, the consumption of sulfuric acid diminishes 5–10 times, which considerably decreases the total cost of copper recovery.

The organization of heap leaching in Bore (Yugoslavia) where the copper content of the ore is 0.2–0.4 % and the annual copper output amounts to 1000 t may serve as an example of the distribution of expenditures in the cost profile of 1 t of cement copper (Table 3.12).

Table 3.12

The Cost Profile of 1 t of Cement Copper

Items of expenditures	Share in the cost structure, %
Electric power	22.3
Sulfuric acid	31.98
Cementation	8.46
Total	65.84
Labour	24.48
Miscellaneous	9.68
Total	100.0

Transportation and metallurgy process amount, as a rule to 40–50 % of the cost of 1 t of cement copper.

Thus, in heap leaching the main expenditure items are sulfuric acid, cementation and recovery of pure copper by metallurgy process [1, 2].

In-situ leaching. When estimating the economic efficiency of in-situ leaching, it is necessary to take into account the fact that the cost of the site prospecting, stripping and preparation of the orebody for exploitation, as well as of water drainage and ventilation is determined for commercial grades of ore only, i.e. low-grade and residual ore is not taken into account when the production expenditures are estimated. Therefore, the self-cost of metal leached from such ores is determined as a sum of actual expenditures related to the leaching process as such, i.e. preparation of blocks for solution mining, consumption of reagents, depreciation of the process equipment and labour costs.

The economic efficiency of solution mining is determined by the following:

– the cost of a solution mining project is considerably lower than the cost of a traditional mining complex of the same capacity with subsequent ore dressing at ore preparation mills;

– solution mining does not require a number of processes inherent to traditional mining techniques – driving and maintenance of workings, handling of ore within stopes and its transportation to the surface, etc.

– lower capital and operating costs of solution mining allow to develop deposits and orebodies where the metal content is several times lower than that of commercial grades of ore.

The economic feasibility of solution mining is determined on the basis of the capital cost estimation, which includes preparatory mining, drilling and insulation costs.

The economic efficiency of solution mining is estimated on the basis of the following initial data:

– ore reserves in the blocks;

– amount of leach liquors;
– duration of the impregnation of the orebody or block by leach liquor;
– productivity of the blocks in terms of pregnant solutions when the leaching process is in a steady state;
– acid strength or the pH of leach liquors;
– rated duration of leaching (in months);
– rated metal recovery;
– metal value in leach liquors;
– rated solution losses;
– total amount of solution, including those in receptacles and the piping;
– temperature of leach liquors;
– content of iron and microorganisms in solutions.

The capital cost of solution mining projects covers, first of all, preparation of leaching blocks, construction of drive dams, installation of solution receptacles, driving of drainage workings for the outflow of pregnant solutions, as well as installation of leakage protection devices and so on.

The capital cost also includes expenditures on drilling boreholes for the injection of leach liquors into the leaching blocks and blast fracturing. Equipment used in solution mining consists mainly of pumps that supply leach liquors into the wells and pump pregnant solutions to the surface for subsequent metal recovery. Besides the pumps, the capital investments cover the price of piping (of polyethylene, polypropylene or fiber glass), the compressor station, regeneration pool and of the installation for metal recovery by precipitation, sorption or solvent extraction, and purification works.

The total cost of in-situ leaching of copper amounts to 75–85 US dollars per ton and depends primarily on the consumption of acid and scrap iron (Table 3.13).

Table 3.13

The Cost Profile of 1 t of Cement Copper at the Annual Production Rate of 8 000 t of Copper

Item of expenditures	Share in the cost profile, %
Labour	10.5
Sulfuric acid	36.3
Cementation	37.5
Solution pumping	0.9
Electric power	3.6
Miscellaneous	11.2

Tank leaching. While studying the economic feasibility of bacterial tank leaching it is necessary to compare its technico-economic parameters with those of competitive techniques and processes [3].

To calculate technico-economic indices, it is necessary, first of all, to elaborate a production programme for the bacterial leaching workshop or installation on the basis of the adopted flowchart and parameters obtained.

These calculations are based on the following initial data:
- the annual amount of processed concentrates;
- metal content in concentrates;
- duration of leaching (h);
- metal content in leaching products;
- equipment necessary according to the adopted flow chart;
- process parameters;
- the mode of work of the leaching workshop;
- the amount of overheads at the construction site location;
- the consumption and price of the main reagents.

The capital investments on the construction of a leaching workshop are calculated with due regard to the price-setting system in the locality chosen, means of transportation; overheads of general construction works and assembly of metallic structures, regional wage rates and the amount of planned revenues.

The calculation of the process and handling equipment cost is carried out in accordance with the requirements of the adopted flow chart. Table 3.14 gives an example of calculations of the process equipment cost with due regard to unspecified auxiliary equipment together with assembly and transportation expenditures.

Table 3.14

Calculation of the Process and Handling Equipment Cost

Items of equipment	Quantity	Price, thous. roubles	Total price, thous. roubles
Leaching installations	6	55	330
Pumps, installations for filtration	1	—	970
Total			1300
Piping			260
Metal structures			200
Equipment assembly			120
Rubberizing			110
Transportation means and overheads			130
Total			2120
Miscellaneous and unspecified equipment			530
Total cost of the process and handling equipment			2650

The cost of the main process equipment amounts to 60 % of the cost of the whole equipment.

Then the cost of the construction of the buildings and auxiliary structures of the leaching workshop is estimated (Table 3.15).

Thereupon the total capital investments on the construction of the bacterial leaching workshop, process and handling equipment are determined.

Table 3.15

Calculation of construction cost of buildings and auxiliary structures of the leaching workshop

Item	Volume, m^3	Cost of 1 m^3, roubles	Total cost, thous. roubles
Bacterial leaching section	2000	15	30
Compressor station	4300	16	70
Section for grinding, filtration and preparation of reagents			200
Thickeners			240
Miscellaneous			60
Total			600

In order to estimate operating costs, it is necessary, first of all, to determine the cost of the reagents used and then add to it the wage fund for the personnel, the cost of materials used, of electric power and water, as well as depreciation charges. Annual operating costs include:

- wages and salaries;
- bonuses and safety measures;
- materials;
- electric power;
- water;
- depreciation charges;
- routine repair;
- waste piling.

In tank bacterial leaching the main items of operating costs are depreciation charges (30–35 %), routine repair works (15–20 %) and waste piling (10–15 %).

The results of the capital and operating cost estimation are used to determine economic parameters of the concentrate processing as a whole (Table 3.16).

Table 3.16

Main economic parameters of concentrate processing by bacterial leaching

Parameters	%
1. Capital investments, total	100
including:	
the bacterial leaching section	42
the concentrate processing section	53
waste piling and storage	5
2. Annual operating cost, total	100
including:	
bacterial leaching	35
concentrate processing	59
waste piling and storage	6

To estimate the economic feasibility of bacterial leaching, it is necessary to compare the main technico-economic parameters of the process with those of techniques used in the industry for the processing of such concentrates. Thus, gold-arsenic concentrates are frequently subject to roasting with subsequent cyanidation. Therefore, the technico-economic parameters of bacterial leaching-cyanidation should be compared with those of the roasting-cyanidation technique. The results of this comparison are given in Table 3.17.

Table 3.17

Main economic parameters of the processing of gold-arsenic concentrates by different techniques, %

Parameters	Techniques	
	roasting-cyanidation	bacterial leaching-cyanidation
1. Capital investments	100	58
2. Annual operating costs	100	82
3. Profitableness	100	170
4. Annual revenues	100	215

These data show that the bacterial leaching is economically justified in this case as it allows to obtain higher revenues and profitableness with lower capital and operating costs.

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Chapter 4

BACTERIAL TANK LEACHING TESTS ON SULFIDE CONCENTRATES

4.1. GENERAL

Bacterial tank leaching is a process whereby metals are leached from ores and concentrates is special tanks utilizing bacteria. Leaching is performed in Pachuca tanks, reactors, conditioning tanks or similar equipment where pulps can be stirred, aerated and thermostated.

Mesophilic strains of the genus **Thiobacillus**, belonging mainly to the species **T. ferrooxidans**, in association with other syntrophic species of chemolithoautotrophic bacteria isolated from sulfide deposits are currently used in commercial bacterial tank leaching [7, 8, 9, 16, 17].

This technique can be applied for recovering metals from several complex metal sulfide concentrates for which no suitable and environmentally sound process has yet been developed (Table 4, 1).

The test procedure followed in bacterial tank leaching may be divided into the following steps, according to the desired objectives and type of sulfide involved: bench scale testing, process optimization and development of scale-up criteria, pilot plant operation and, finally, semi-industrial plant operation.

It should be pointed out that to assess the feasibility of tank leaching ore of a new deposit, the procedure outlined below should be followed:

Table 4.1

Types of Sulfide Concentrates Amenable to Bacterial Tank Leaching

Metal sulfide concentrate	Objective of leaching	References
Gold-arsenic Gold-pyrite	Selective recovery of arsenic, liberation of gold finely intergrown with sulfide minerals	[8, 11, 14, 17, 18, 20]
Tin-arsenic Tin-copper-arsenic Copper-arsenic	Selective recovery of arsenic	[8, 12, 15, 17, 19, 20]
Copper-zinc Lead-zinc	Selective recovery of zinc	[4, 8, 17, 23]
Copper-molybdenum	Selective recovery of copper	[1, 5, 8, 17]
Copper-nickel	Selective recovery of nickel	[6, 8, 13, 22]

Table 4.2

Steps in Development of Bacterial Tank Leaching Tests

Step	Objective	Operation
1. Sample preparation	To provide the required amount of sample representative of the sulfide concentrate	Chemical, mineralogical and grain size analysis; comminution to appropriate grain size; preparation of concentrate batches for testing
2. Acquisition of bacterial strains	Preparation of bacterial cultures which can actively oxidize the sulfide concentrates	Assessment of sulfide concentrate amenability to leaching; adaptation of bacterial strains; preparation of required amounts of bacterial cultures for laboratory testing
3. Bench testing	Assessment of feasibility of bacterial tank leaching. Preliminary quantitative evaluation of technological efficiency parameters (metal solubilization kinetics, metal recovery). Determination of evolution of ionic composition of pulp; estimation of biomass concentration and activity during various leaching stages; assessment of effect of pulp composition on leaching efficiency	Bench-scale batch and continuous leaching tests. Preparation of suitable amounts of adapted bacteria for further, longer scale testing
4. Scale-up	Determination of: a) specific and maximum flow rates; specific bacterial growth rates at various leaching stages; b) reagents, CO_2 and air requirements; c) type of leach liquor regeneration and recycling; composition of pregnant solutions; d) possibilities of metal recovery from pregnant liquors; e) development of models and criteria for scale-up purposes	Performance of continuous leaching tests
5. Pilot-plant testing	Development of process flowsheet and assessment of validity of scale-up procedures. Acquisition of operation, technological and economic data	Simulation of a continuous commercial plant on pilot-plant scale
6. Development and operation of a semi-industrial plant	To check applicability of process on an industrial scale	Operation of a semi-industrial plant

Bacterial tank leaching can be performed under non-sterile conditions, because the low pH (1.5–2.5) and high concentrations of heavy metal ions render the leaching medium elective to a certain extent.

Conditions, objectives and operations of each investigation step of bacterial tank leaching technology are discussed in the sequel (Table 4.2).

4.2. SAMPLING PROCEDURE

Selection and preparation of metal sulfide samples are crucial in the development of tank bacterial leaching technology. Reliability of results depends on sampling accuracy [2].

4.2.1. Sampling Requirements

Sample representativeness is a necessary requisite for successful leaching tests and the main conditions in this respect are:

– the ore samples from which the concentrates are produced must be representative of the orebody as a whole;

– during sampling the ore-dressing plant must be operating in closed circuit. At the preliminary investigation stage, when only the basic beneficiation flowsheet is developed, samples can be taken from an open circuit concentration operation;

– only samples of concentrates obtained with the recommended ore dressing process should be used.

Other essential requirements for sample representativeness are that its percentages in:

– major and minor components;

– impurities;

– non-metallic minerals

do not differ from the effective percentages of the crude ore.

For scale-up the sample should be a concentrate or other sulfide product obtained from a closed-circuit operation developed and recommended for the beneficiation of the ores from the deposit under study. Sizeable samples are required, of the order to several tens of kilograms.

For semi-industrial tests, the samples should be purposely produced by a pilot concentrator or semi-industrial plant according to the recommended ore-dressing process.

As for investigations on concentrates produced by a mill already in operation, sampling should be done in coordination with the research and development organizations responsible for the investigations. In this case at least 1–5 t of sample are needed [2].

When the process is to be applied to complex metal sulfide ores, e.g. copper-zinc-pyrite ores, the chemical and mineralogical composition of concentrates should comply with the requirements of maximum metal extraction.

The sample should be dried to 3–5 % humidity, packed in sacks, batches not exceeding 60 kg in weight should be boxed and prepared for shipment to the organization in charge of testing. The sample should be provided with

a sampling certificate and a label indicating its mass, chemical composition and other relevant data.

4.2.2. Sample Homogenization

For laboratory and scale-up tests, the original sample must be reconstituted and appropriate size batches are then prepared in order to comply with test conditions and technical characteristics of the test equipment.

Homogenization should be carried out according to guidelines accurately describing each step of the procedure.

The weight of the original sample is then reduced to the desired size without affecting its representativeness. This can be done by mixing (homogenization) according to the ring-and-cone method and by subsequent quartering or by means of special sample splitting devices [10].

Mixing by the ring-and-cone method is described below. The sample is poured onto a hard, smooth surface, e.g., a table, so as to form a cone (Fig. 4.1). The material from the centre of the cone is transferred to the base by repeated scooping to form a ring (Fig. 4.1a, then another cone is scooped and flattened into a disk by rotary and translational (downward) movements using a metal or wooden spatula. The disk is then formed into a ring again. This procedure is repeated at least three times. It should be noted that mixing by the ring-and-cone method is only used in conjunction with reduction by the quartering method when the segregation effect does not impair reduction accuracy.

In the quartering procedure the thoroughly mixed sample is shaped into a disk and divided by a spider into four quadrants. Two opposite quadrants are rejected. The remaining two are mixed by the ring-and-cone method. The procedure is repeated until the required mass is obtained.

Mixing with the rolling technique is used when the mass of finely ground material is relatively small (about 1 kg). The sample is spread over the middle

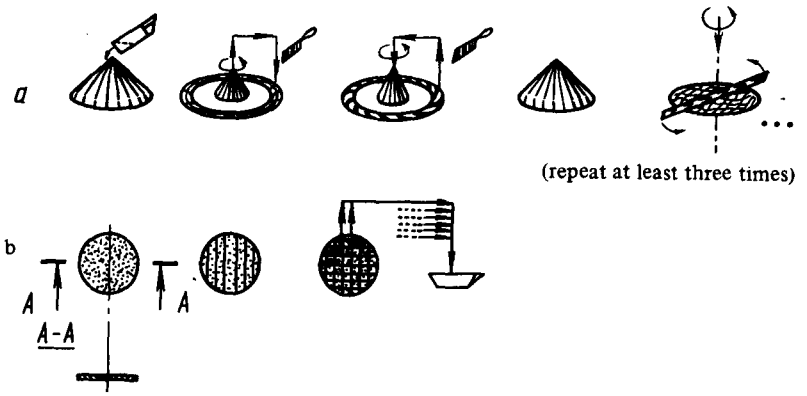

Fig. 4.1. Homogenization of a sulfide concentrate sample:
a – mixing by the ring-and-cone method; b – reduction by quartering

of an oil-cloth, 0.5–1.5 cm thick, so as to occupy 1/5 of its surface. Then by lifting up each corner of the oil-cloth the sample is moved to the opposite corner and back to its original position. This procedure is repeated at least three times. The sample mixed in this way is reduced by the quartering method.

Quartering is usually used when selecting small samples for direct use in the experiments. When reducing with the quartering method the sample, thoroughly mixed by rolling, is flattened with a spatula into a disk and a mesh of perpendicular lines traced onto it. Then, from line intersections or from the centre of a square the required sample is selected, preferably covering the entire disk surface (Fig. 4.1b).

Grain size distribution is determined by sieve analysis according to ISO-TC guidelines which prescribe sample size and sieving procedure (dry or wet, manual or mechanical) and duration.

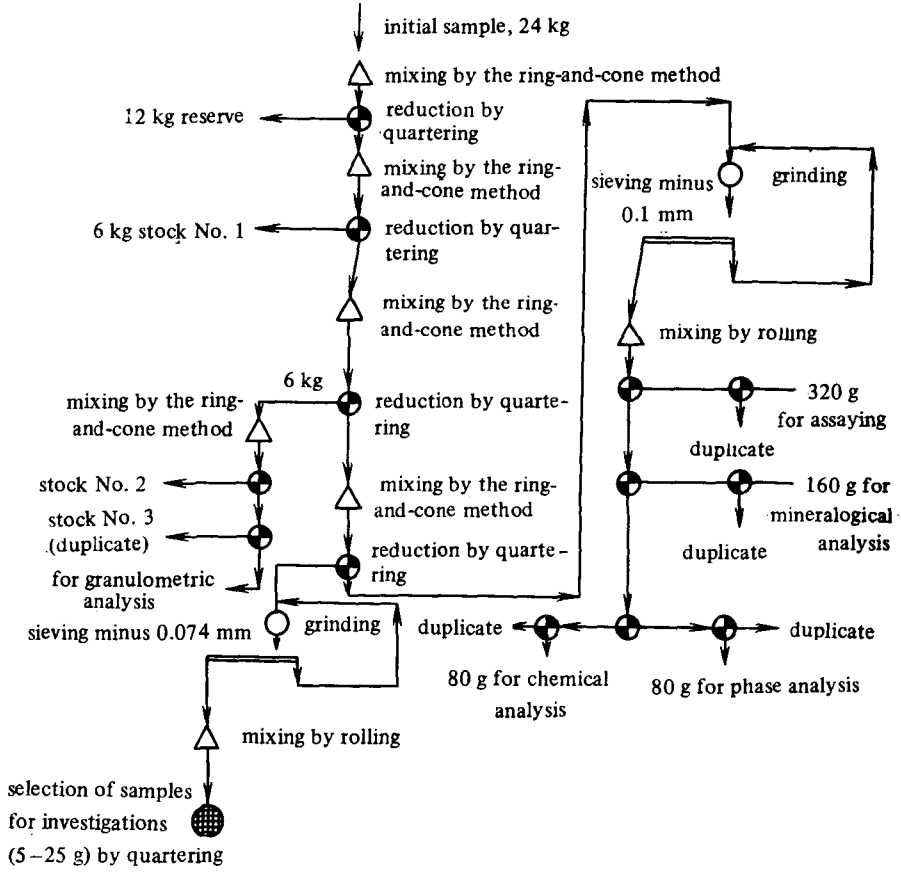

Fig. 4.2. Scheme of preparation ot an average sample of concentrate, mass 24 kg:
△ = mixing; ◓ = reduction

The size fractions thus obtained are weighed and the percentage composition of the total weight of the sample is calculated. If the subsieve content of the sample is to be determined, the – 0.044 mm fraction is further analysed, for instance, by a standard sedimentation method.

Fig. 4.2. shows a rough diagram of reducing a homogenized sample of sulfide concentrate weighing 24 kg, 100 % – 1.5 mm. The original sample is mixed by the ring-and-cone method and divided into two equal parts by quartering. One part is kept for umpire while the other, homogenized, is divided into two 6-kg lots. In this way, we obtain stock No. 1 and a 6-kg sample which in turn is divided into two 3-kg lots after homogenization. Each of these, after homogenization is also divided into two equal parts. Thus four 1.5-kg samples are obtained. One of the samples is stock No. 2. The second sample after homogenization is divided into two parts: one for size class analysis and the other as a duplicate. The third sample is used for laboratory bacterial leaching tests. The fourth sample is subdivided according to the diagram for chemical, mineralogical and phase analyses as well as assaying. Each sample analyzed should have a duplicate.

The samples weighing from 1 to 1.5 kg to be employed for bacterial leaching are usually ground. The particle size of the feed is one of the major factors affecting the bacterial leaching process. As a rule, for bacterial tank leaching the feed is 100 % – 40 μm.

Grinding serves to increase the specific surface of particles, to liberate mineral grains and create defects in the crystal lattice, etc. which contributes to increasing the accessibility of sulfide minerals to bacteria [8, 17]. Small batches (up to 1 kg) are usually ground in a laboratory wet ball mill.

Samples for semi-industrial tests are ground in continuous closed grinding circuits consisting of a ball mill and suitable classifier, such as a hydrocylone. In this case the ground material is stored as pulp in special tanks and is fed as required into tanks for bacterial leaching. The performance of the grinding circuit and the grain-size distribution of the feed are checked by sieve analyses.

The knowledge of the composition of the sulfide concentrate allows to predict the type of bacterial leaching process and to establish the preparation required for the concentrate to be leached. In this way, once the mineralogical composition of the concentrate as well as the value of the electrode potentials of its mineral components are known, the leachability sequence of the latter can be determined since minerals with the lowest electrode potentials are leached first.

The phase analysis data showing mineral and chemical composition of each size class allow to estimate in advance the required mesh-of-grind of the sulfide.

4.2.3. Major Parameters of the Pulp

In bacterial tank leaching the sulfide concentrate is leached in pulp form, i.e. a liquid suspension of finely ground particles of the concentrate. The major parameters determining the properties and composition of the pulp are: solid-to-liquid ratio, physical properties and chemical composition of the phases, grain size of the solid phase, density of the solid and liquid phases of the pulp. The dilution of the pulp is expressed by the ratio of mass of solid

to mass of liquid contained in a given mass of pulp and can be written either in decimals or in percent:

$$R = \frac{L}{S} = \frac{\%\,l}{\%\,s} \text{ (units)}, \qquad (4.1)$$

where:

R – dilution of pulp (units);
S – mass of solid phase (g), (kg);
L – mass of liquid (g), (kg);
% s – solid content of pulp (% w/w);
% l – liquid content of pulp (% w/w).

The solid phase content of the pulp (% s) is related to its dilution (R) by the ratio [10]:

$$\%\,s = \frac{1}{R+1} \cdot 100 \qquad (4.2)$$

% s and R may only be used if the densities of the solid and liquid phases of the pulp are known, e.g., only at the very beginning of leaching. However, as leaching progresses the densities of both the liquid and solid phases of the pulp continually change as a consequence of the differential leaching of mineral components of the sulfide concentrate. The dilution of the pulp during leaching should therefore be calculated by weighing a sample of pulp and using the formula:

$$R = \frac{\Delta\,(\delta \cdot V - M)}{\delta\,(M - \Delta \cdot V)}, \qquad (4.3)$$

where:

R – dilution of pulp (units);
V – volume of pulp sample (ml);
M – mass of pulp sample (g);
Δ – density of liquid phase of pulp (g/cm^3);
δ – density of solid phase of pulp (g/cm^3).

In bacterial leaching the pulp density may also be characterized by the amount of solid phase per unit volume of pulp. To this end the mass of solids contained in a given sample of pulp is determined; the solids concentration in the pulp is calculated by the equation:

$$C_{sp} = \frac{1000 \cdot m}{V} \text{ (g/l)} \qquad (4.4)$$

where:

C_{sp} – solids concentration in the pulp (g/l);
V – volume of pulp (ml);
m – mass of solids in pulp sample (g).

The solids concentration in the pulp and their dilution are related by:

$$R = \frac{1000 \cdot \delta \cdot \Delta - \Delta \cdot C_{sp}}{\delta \cdot C_{sp}}; \qquad (4.5)$$

$$C_{sp} = \frac{1000 \cdot \delta \cdot \Delta}{R \cdot \delta + \Delta} \qquad (4.6)$$

In biotechnological practice pulp dilution R ranges from 4 to 10 (from 9 to 20 % solids). Plant capacity in terms of solids throughput, as well as heavy metals concentration in the liquid phase of the pulp increases with increasing pulp density. It should be noted however that at high pulp densities ($R<4$), the solubility of oxygen in the pulp and the metal value recoveries decrease, while leaching time increases. Therefore when dealing with thick pulps equipment such as fermentors, reactors, conditioning tanks, etc. should be used, i.e. devices that provide an active mass transfer between the solid, liquid and gaseous phases of the pulp.

4.3. PREPARATION OF BACTERIAL CULTURES

In bacterial tank leaching the heavy metal ions concentration in the pulp attains extremely high values and the pH drops considerably [8, 17, 20]. For example, the Zn^{2+} content of the liquid phase of the pulp may be as high as 20–40 g/l; Cu^{2+} – 5–10 g/l; Fe^{3+} – 20–50 g/l; AsO_4^{3-} – 12–20 g/l [8, 17, 18, 19]. In addition, bacterial tank leaching is characterized by long residence times amounting presently up to 72 h [17]. Therefore the bacterial strains used in bacterial tank leaching should be adapted to the leaching conditions existing at high dilution rates approaching the critical values.

In preparing cultures for leaching it is not advisable to adapt bacteria to single minerals or chemical elements present in a given concentrate [9, 19]. Bacteria which have been adapted to arsenic added to the culture medium (for example 9K) may not be adapted to all the compounds occurring in the medium during arsenopyrite concentrate leaching [11].

The process can be carried out either by using bacterial cultures isolated de novo from natural microbial communities of the sulfide ore deposit, whose products are to be leached (see Chapter 2), or bacteria previously isolated from other deposits and cultured in different media. In the latter case bacteria can be grown both in a culture medium, for example 9K (see 1 in Fig. 4.3) and in media containing various sulfide minerals (see 2 in Fig. 4.3).

It may take as long as 6–8 weeks (Fig. 4.2, Section 1.) for bacteria grown in synthetic culture media (for example 9K) to adapt to the sulfide concentrate to be leached, depending on the composition of the latter. This is due to the fact that when cultured on synthetic media the bacterial strains lose their adaptative properties to the leaching medium containing the sulfide concentrate. For this reason, early stages of adaptation should be performed in the enrichment culture (under batch conditions) at pulp densities corresponding to 2–3 % solids.

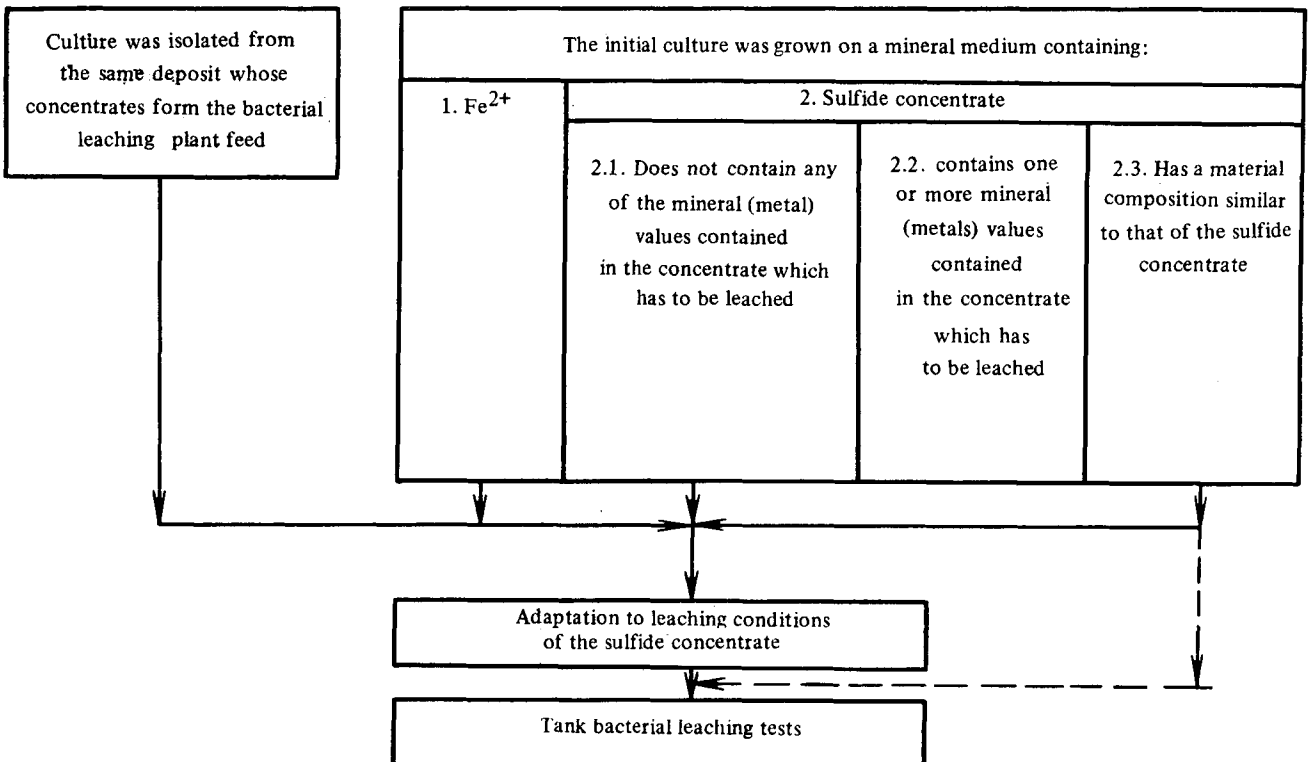

Fig. 4.3. Scheme for preparation of bacterial cultures

Adaptation is achieved by introducing into 3–5 flasks from 50 to 250 ml of medium 9K (pH 1.8–2.2), 20–100 ml of inoculum (late log phase culture, for instance, on 9K medium, and usually from 1 to 3 % (of the total solution mass) sulfide concentrate. Growth and oxidizing activity are checked once or twice a day by measuring the changes in pH, Eh, Fe^{2+}/Fe^{3+}, the amount of biomass and required metals in the liquid phase of the pulp. High metal ions (Fe, As, Cu, etc.) concentration in the liquid phase and low pH of the pulp are indicative of the effectiveness of bacterial leaching.

The flasks with the highest solubilization of the required metals are selected for further adaptation. Cultures from such flasks are transferred into fresh pulp with a similar or higher solid content, for example from 5 to 7 %. The solid content of the pulp is gradually increased in subsequent transfers to the ratio required for leaching. A culture is considered to be adapted to the sulfide concentrate and ready for use in tank leaching tests when several consecutive transfers on pulp with the required density constantly yield satisfactory metal solubilization.

Culture preparation may be carried out in shake flasks (with frequency of at least 270 rpm, and pulp density not exceeding 10 % solids), fermenters, aerated separating funnels (pulp density is not limited) or other vessels that provide for stirring, heating and aeration of the pulp.

Sulfide concentrates subjected to leaching may be classified into the following groups in relation to the concentrate on which bacteria are grown:

a) The sulfide concentrates to be leached (see Fig. 4.3, Section 2.1) do not contain any of the minerals (metals) on which the initial bacterial culture was grown. An example of this case is represented by the utilization of bacteria grown on arsenopyrite-pyrite concentrate for leaching a copper-zinc concentrate. Adaptation is achieved by several consecutive transfers, gradually increasing pulp density.

Adaptation of bacteria may also be carried out at pulp density used for culturing the initial bacteria, for example at 15–20 % solids. In this case consecutive transfers of the initial culture are performed on pulp of the initial produce which is gradually replaced with the sulfide material to be leached. In this way, the whole solid phase of the pulp is gradually replaced. The rate of replacement and frequency of transfers are determined empirically depending on the activity of the culture obtained.

b) The sulfide concentrates (see Fig. 4.3, Section 2.2) to be leached contain one or more metals (minerals) present in the sulfide concentrate on which the initial culture of bacteria was obtained. An example is the adaptation of bacteria grown on an arsenopyrite-pyrite concentrate containing from 5 to 8 % arsenic to an arsenopyrite-chalcopyrite concentrate under batch or continuous conditions. In this case adaptation is carried out as above (a). The adaptation time is reduced.

c) The sulfide concentrate (see Fig. 4.3, Section 2.3) to be leached differs in metal content from the concentrate to which the starting culture was adapted. An example of this is the adaptation of a culture grown on arsenopyrite-pyrite concentrate containing from 5 to 8 % of arsenic to the concentrate containing from 15 to 20 % of arsenic which is obtained from the

same deposit. Bacteria are adapted during several consecutive transfers at the same pulp density used for metal leaching.

d) The sulfide concentrate on which the culture is grown corresponds to the concentrate to be leached. In this case the culture may be used without prior adaptation (dashed line, Fig. 4.3).

It should be pointed out that while an adapted culture can effectively leach sulfide concentrates, the actual oxidizing activity depends on the specific leaching conditions as a whole (aeration, particle size of sulfide concentrate, mass transfer, ion composition of pulp, etc.).

4.4. BACTERIAL TANK LEACHING TESTS UNDER DIFFERENT CONDITIONS OF BACTERIAL GROWTH

The determination of the main parameters characterizing the final stage of the leaching process (degree of metal solubilization, leaching time, residual metal content of residues, etc.) at each stage of testing depends on a variety of factors: active mass transfer between solid, liquid and gaseous phases of the pulp, particle size of ground feed (sulfide concentrate), conditions of bacterial growth (leaching), etc.

Depending on the objectives, bacterial tank leaching may be carried out as a batch, semi-continuous or continuous bacterial culture. When leaching is performed in batch, the metabolism and leaching products are not removed and no fresh pulp is added during the process. Under these conditions testing aims, for instance, at assessing the amenability of the concentrate to bacterial leaching and the adaptation of bacteria to leaching conditions. In the case of continuous bacterial cultures, part of the metabolites and leach products is continuously removed from the tank and fresh pulp constantly supplied.

Semi-continuous (fed batch) and continuous bacterial tank leaching is both suitable for bacterial tank leaching tests on laboratory, pilot and semi-commercial scale.

During semi-continuous and continuous tests bacterial strains adapt to the series of factors typical of each apparatus; once the steady state is reached,

Table 4.3

Suggested Steps for Different Bacterial Tank Leaching Testing Steps

Leaching procedure	Testing steps		
	Bench scale	Pilot	Semicommercial
Batch	+		
Semi-continuous (repeated fed batch)	+	+	
Continuous	+	+	+

several parameters can be measured: kinetics of solubilization and recovery of the metal values in the pregnant liquor (liquid phase of pulp); kinetics of change in ionic composition of pulp; maximum flow rate. In addition, models which simulate the actual technological process and flowsheet optimization – including regeneration of leach liquors and further processing of solid residues for commercial purposes – can be developed. Suggested procedures for the bacterial leaching of sulfide concentrates suitable for the different testing steps are summarized in Table 4.3.

4.4.1. Bacterial Leaching in Batch Operation

During bacterial leaching in batch operation the products of bacterial metabolism and of sulfide concentrate leaching accumulate in the pulp, resulting in bacterial inhibition, a drop in sulfide oxidation rate and, eventually, a decrease in metal values solubilization. Moreover, when complex sulfides are leached, only some minerals are oxidized.

The size of the sample can range from 1 to 3 kg, depending on the number of tests, equipment capacity and pulp density.

Bacterial leaching in batch operation may be carried out in Erlenmeyer flasks, separating funnels, Pachuca tanks and in 250–1500 ml fermenters. Aeration of the pulp in the flasks is achieved by agitation on a shaker at a frequency of at least 270 rpm in order to prevent the solids from settling out of the pulp. If separating funnels are used for leaching, pulp aeration and agitation are ensured by airlifts to which air is supplied by a mainline or by thermostated microcompressors for aquaria.

Bacterial tank leaching tests in batch should be performed with an initial pulp density ranging from 5 to 10 % solids. Only after the main process parameters (pH, Eh, Fe^{2+}, Fe^{3+}, residual metal values content, etc.) are stabilized, can the thickness of the pulp be gradually increased up to 15–20 % solids. The ratio of volume of adapted inoculum to that of fresh pulp should be at least 1:3.

The size of batches used in tests aimed at assessing optimum leaching conditions should be such that sufficient residues remain for chemical assay.

The following example can be taken as a guideline for bacterial tank leaching batch tests.

The feed – an arsenopyrite-pyrite concentrate assaying 15 % of As – is ground to –0.074 mm. A late logarithm phase bacterial culture previously adapted to this concentrate is used as inoculum. 450 ml of medium, 90 g of concentrate and 150 ml of inoculum are placed in a fermenter or a 1000 ml aerated separating funnel. After inoculation the pH of the pulp is measured and, if necessary, adjusted to 1.8–2.2 with 1N H_2SO_4 or KOH solution. The control consists of the same pulp to which the same inoculum and a thymol are added to inhibit bacterial growth. Leach vessels are thermostated and aerated continuously.

The pH and Eh of the pulp as well as the Fe^{2+}, Fe^{3+} and As concentration in both the liquid and solid phases of the pulp are checked at time intervals fixed according to the process kinetics.

The information provided by these periodic measurements allows to evaluate the progress of the leaching process. The faster the Eh and the Fe^{3+} and As concentrations increase and pH decreases, the higher the leaching effectiveness.

A drop in the rate of change of these parameters indicates the end of the leaching process. Pulp samples taken during the test are specially treated to determine separately the metal values in the solid and liquid phases of the pulp (see Section 4.7).

. On the basis of these assays, the recovery of metal values (iron, arsenic, etc.) and of sulfur (see Section 4, 7) is calculated and the evolution in metal values concentration evaluated.

On completion of the test, the influence of the concentration of elements and of metal ions (e.g. sodium, magnesium, etc.) expected to be present in the full-scale process as well as the optimum values of other leaching parameters, such as pH of the pulp, are determined.

4.4.2. Bacterial Leaching of Sulfide Concentrates in the Repeated Fed Batch Process

The repeated fed batch process of bacterial culture is one of the simplest concentrate leaching processes as far as equipment is concerned. It ensures the periodic replacement of part of the pulp thus allowing the removal of the metabolism and metal oxidation products and the supply of fresh substrate.

Bacterial leaching of sulfide concentrates may be carried out in vessels arranged in series (fermenters, Pachuca tanks, separating funnels, etc.) with active mass transfer and forced periodical transport of pulp. In laboratory tests 2–6 vessels arranged in series are usually employed. The number of vessels required for the desired recovery by leaching depends on vessel capacity, dilution rate at each process stage and chemical and mineralogical composition of the sulfide concentrate.

In the repeated fed batch process the influence of the partial periodic replacement of the pulp on metal value solubilization is evaluated.

The main drawback of the repeated fed batch process is represented by the pulsed (non-chemostatic) pulp feeding which involves the periodic change of all the process parameters. Thus the repeated fed batch process is intermediate between the batch and the continuous processes. The shorter the time intervals between consecutive pulp replacements, the closer the performance of this process to that of the continuous (chemostatic) process.

Bacterial tank leaching via the repeated fed batch consists of two stages:

1) process set-up;

2) process monitoring during steady state operation.

During the set-up stage, the time intervals between successive pulp replacements are gradually decreased and the bacteria continuously adapt to the changing flow characteristics.

The steady state condition is reached when the main microbiological, chemical and physico-chemical process parameters are stabilized. The steady state condition is characterized by well defined leaching times and metal value extrac-

tion. The metal value leaching time corresponds to the residence time of the pulp in the series of vessels. Leaching time depends on metal values extraction, chemical and mineral composition, particle size of the feed, equipment used, intensity of mass transfer, etc.

All other conditions being equal, the shorter the residence time the lower the extraction. In tank leaching it is crucial that residence time be shortened to the greatest possible extent without affecting metal values extraction.

An example of a repeated fed batch process is described below.

A preliminary step consists in preparing the sulfide concentrate and the adapted bacterial culture (see Section 4.2). Then the process is started. In the first vessel, bacteria are grown on sulfide concentrate in a given pulp volume under batch conditions (see Section 4.3.). If the outcome is satisfactory, some of the pulp from the first vessel ($\Delta V = 0.1–0.3$ of the starting volume) is transferred to the second aerated vessel, and the same amount of fresh pulp is fed into the first vessel (Fig. 4.4). The pulp flow rate depends on the amount (volume) of pulp transferred and on the frequency of transfers and therefore these values should be kept constant during each testing stage. The time interval between successive pulp transfers during transition from batch to fed batch operation depends on the results of the assays and ranges on average between 36 and 48 h. The second vessel is filled by repeated transfers of equal volumes (ΔV) of pulp from the first vessel; the same amount of fresh pulp (ΔV) is fed into the first vessel after each pulp removal. Once the second vessel has been filled to the prescribed volume (V_2), the same volume of pulp (ΔV) is transferred from the second to the third vessel; then an equal amount of pulp is transferred from the first to the second vessel and ΔV ml of fresh pulp is fed to the first vessel. Once all the vessels of the series are full, a volume ΔV of pulp is removed from the last vessel (n); this loss is offset by the volume ΔV_{n-1} containing a mass Δq_{n-1}

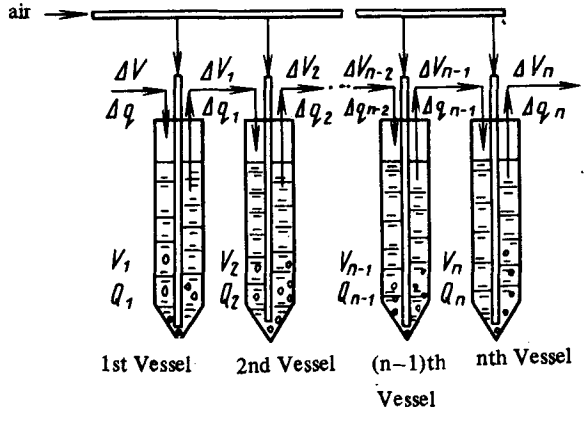

Fig. 4.4. Scheme of pulp movement for repeated fed batch bacterial leaching: $V_1, V_2, \ldots, V_n$ – pulp volume at each stage; $Q_1, Q_2, \ldots, Q_n$ – mass of the solid phase of pulp at each stage; $\Delta V_1 = \Delta V_2 = \ldots \Delta V_n$ – volume of transferred pulp; $\Delta q_1, \Delta q_2, \ldots, \Delta q_n$ – mass of the solid phase in transferred pulp

of sulfide concentrate (Fig. 4.4). Thus the volume ΔV of pulp moves through the series of vessels.

The residence time in the n-th vessel of the series is given by:

$$t_n = \frac{V_n \cdot \tau}{\Delta V} \text{ h} \tag{4.7}$$

where:

V_n – volume of pulp in the n-th vessel (ml);
τ – time interval between pulp transfers (h);
ΔV – volume of transferred pulp (ml).

The reciprocal of t_n is the rate of dilution D of the pulp for the n-th vessel.

$$D = \frac{1}{t_n} (h^{-1}) \tag{4.8}$$

The total leaching time T, in the whole series of vessels is given by the sum of the residence time of the pulp in all the vessels:

$$T = \sum_{i=1}^{n} t_i \text{ (h)} \tag{4.9}$$

If vessels with equal volumes of pulp are used for leaching, i.e. $V_1 = V_2 = ... = V$, the total time is given by:

$$T = \frac{V \cdot n \cdot \tau}{\Delta V} \text{ (h)} \tag{4.10}$$

where:

V – pulp volume in each vessel (ml);
n – number of vessels in series;
ΔV – volume of transferred pulp (ml).

The pulp transfer rate can be changed according to process requirements, but it is recommended that the ratio of volume transferred to the volume of the corresponding vessel does not exceed 0.5, because at higher values the leaching system becomes unstable.

Throughout the duration of the experiment, samples are taken for chemical analysis to determine the metal content of the solid and liquid phases of the pulp. From the results of these analyses, the output of residual solids and metal (arsenic) and sulfur extractions in the solid and liquid phases of the pulp of each vessel and in the whole series of vessels (see Section 4.6) are calculated.

4.4.3. Continuous Bacterial Leaching Tests

Continuous multi-stage bacterial tank leaching is carried out in appropriate laboratory facilities where the fresh pulp is continuously fed at a fixed flow-rate to the first vessel and the leached residue is continuously removed from the last vessel of the series.

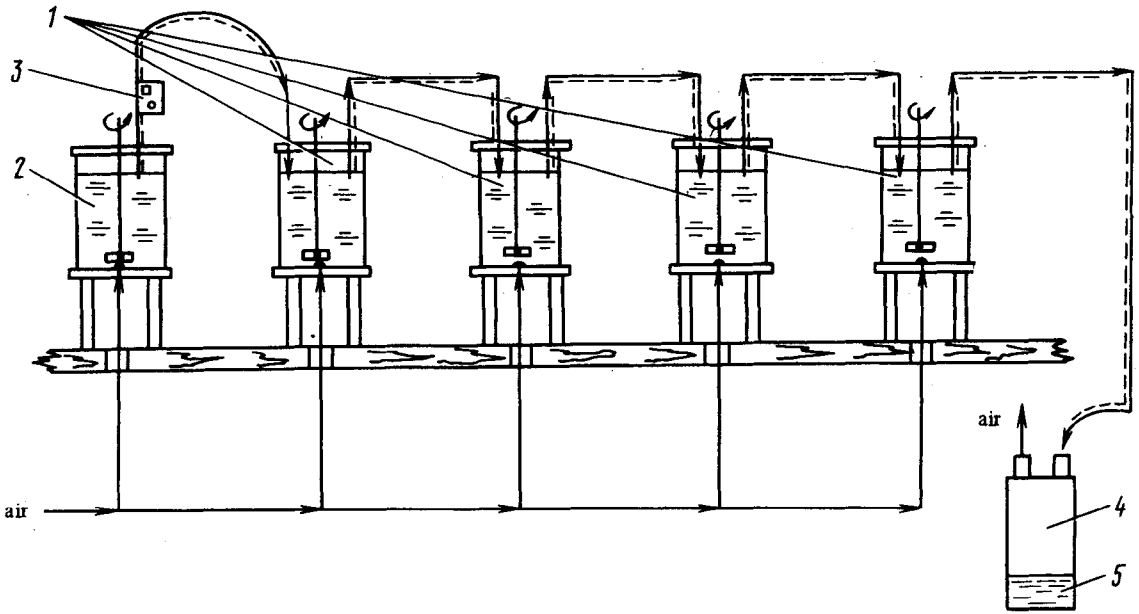

Fig. 4.5. The BILAF-1 plant of the Institute of Microbiology of the USSR Academy of Sciences:

1 – leaching reactors; 2 – conditioning reactor; 3 – pulp feeder; 4 – pulp receiver; 5 – pulp

→ – flow path of pulp

The set-up consists of a head tank for pulp preparation and a series of 2.5 l Pachuca tanks (from 5 to 10 according to needs). The pulp is fed from the head tank to the first Pachuca tank by means of an airlift, and, by gravity flow, to the subsequent tanks. Mixing and aeration in each Pachuca tank are ensured by specially designed airlifts [17, 21].

Another somewhat more sophisticated laboratory facility of this type called Bilaf-1. With this plant appropriate models of the process can be developed and scale-up carried out for commercial plant design. The installation consists of a series of 3–5 airtight 2–2.5 l reactors with pneumatic-mechanical pulp stirring.

The first reactor is used for pulp preparation; aeration and pulp transport are achieved by blowing air through it which produces a slight overpressure in the vessel. This reactor is also fitted with a device designed for adjusting the vertical position of the pulp intake pipe (Fig. 4.5). The pulp, in droplet form, is picked up by the air stream and transferred via the pulp intake pipe into the next reactor; the same air is used for pulp aeration in the reactors.

A smooth adjustment of the pulp flowrate to the subsequent reactors is achieved by varying the position of the pulp intake pipe. In this way, the selection of bacteria with high specific growth rates is favored, resulting in a decrease in leaching time and, at the same time, in an increase in metal solubilization.

Tank leaching in a continuous pilot plant allows to adjust the major factors affecting residence time – flowrate and CO_2 supply – in order to minimize its duration. In addition, it is possible to extend the tests to include the whole processing cycle: regeneration of leaching solutions, smelting or cyanidation of residues, evaluation of reagent consumption, etc.

A description of a bacterial leaching test carried out on a sulfide concentrate in the Bilaf-1 plant is given below.

In the first reactor a **T. ferrooxidans** batch culture is grown on a sulfide concentrate. The minimum volume of pulp in the reactor for effective mixing and aeration is about 0.5 l.

By the time leaching can be considered satisfactory, the pulp flow rate from the head reactor is 10 ml/h. From that moment on, the pulp is allowed to flow into and fill the other reactors. After stabilization of the main process parameters (pH, Eh, biomass amount and activity, heavy metals concentration in solution), the pulp flowrate can be increased by 15–20 % and can only be further increased after stable results are attained.

The first reactor is inoculated with a 3–5 day old culture, previously adapted to the sulfide concentrate and grown according to the batch – or repeated fed batch – technique, with the same pulp density adopted in the tests.

From 1 to 2 l of a 16.7 % solids pulp, inoculated with an adapted culture (500 ml) having the same density, are placed in the conditioning (head) reactor. Fresh pulp is periodically added to this reactor to make up for consumption, taking care that its volume never falls below the minimum required for acceptable mixing.

The residence or leaching time (t_n) of the pulp in the n-th reactor is given by:

$$t_n = \frac{V_n}{d}, \qquad (4.11)$$

where:

V_n – pulp volume in n-th reactor (ml);

d – flow rate (ml/h).

The rate of pulp dilution in the n-th reactor is given by:

$$D = \frac{1}{t_n} = \frac{d}{V_n} \ (h^{-1}) \tag{4.12}$$

The total leaching time T in the whole series of reactors is expressed by:

$$T = \sum_{i=1}^{n} t_n = \frac{\Sigma V}{d}, \tag{4.13}$$

where:

ΣV – total volume of pulp in the n reactors;

n – number of reactors.

For monitoring and control of the leaching process, pulp samples are taken from each reactor. The distribution of components in the solid and liquid phases is calculated from the results of chemical analysis.

4.5. BACTERIAL LEACHING FLOWSHEETS FOR SULFIDE CONCENTRATES

As shown in the foregoing, laboratory bacterial tank leaching tests – which have the objective of developing scale-up procedures – allow to identify and define the parameters governing the sulfide concentrate leaching flowsheets (Table 4.2). For each type of sulfide concentrate these flowsheets depend on the aim of the process, feed contents, specification of the products and specific hydrometallurgical processes for non-ferrous and rare metals [8, 17]. Bacterial leaching represents the main stage of flowsheets combining benefication and hydrometallurgy and consisting of several interrelated cycles, the most important of which are described in this paragraph (Fig. 4.6).

Preliminary operations include grinding and/or fine grinding of the feed to the desired particle size, removal of impurities and/or concentration of the desired metals or minerals by various benefication and hydrometallurgical methods.

For example, pyrite is removed from a complex sulfide ore by flotation which yields a copper-zinc bulk concentrate and a pyrite concentrate, with 92.1 % copper and 90.8 % zinc recovery (Fig. 4.7, Table 4.4).

Ores or concentrates containing large amounts of carbonate minerals should be preliminarily treated with sulfuric acid. This procedure may be adopted, for instance, for removing soluble copper compounds prior to bacterial leaching [17].

Bacterial leaching may consist of one or more stages, for instance three stages for chalcopyrite concentrate processing (Fig. 4.8) [17], and two for gold-arsenic concentrate which involves separating the leach residues into a coarse and a fine size fraction after the first stage of bacterial leaching and further leaching

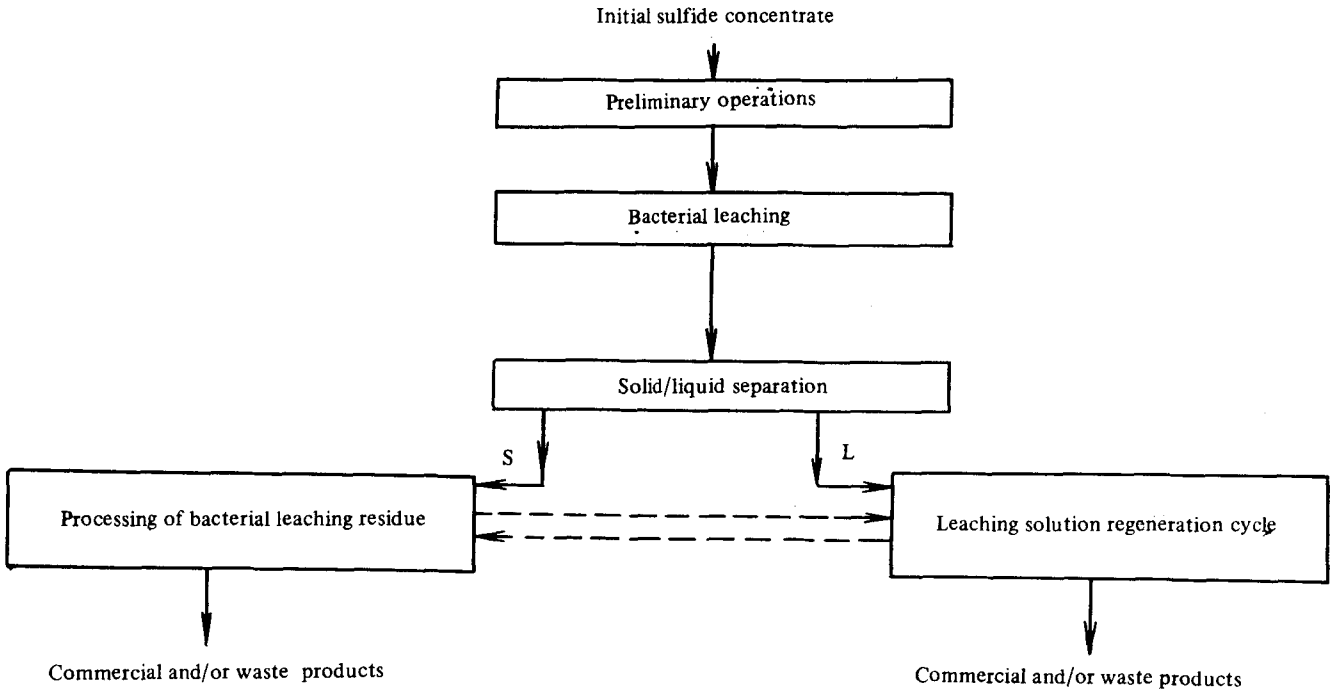

Fig. 4.6. Bacterial leaching flowsheet for a sulfide concentrate

S – solid phase, L – liquid phase

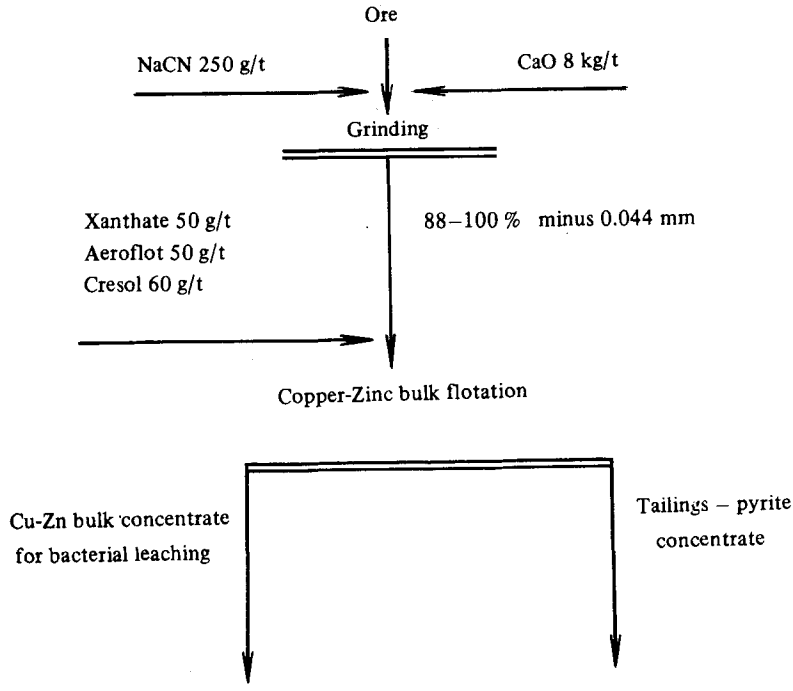

Fig. 4.7. Preliminary operations for bacterial leaching of copper-zinc sulfide ores (17)

of the coarse fraction in the second stage (Fig. 4.9). Elemental sulfur production is one way of diversifying the use of sulfide raw material during bacterial leaching with 75 % recovery of the initial amount of sulfide sulfur [3].

Processing of bacterial leaching residues, depending on the specification of the desired products, may comprise flotation, chemical leaching, cyanidation, smelting, etc. (Fig. 4.10, Table 4.5). For example, in the case of an arsenic concentrate containing gold, the pH of the bacterial leaching residue can be increased with CaO to values in the range from 9 to 12 with subsequent cyanidation producing a gold-containing solution and a residue [17]. The bacterial leaching residue is smelted when it contains large percentages of gold. Prior to smelting the residue should undergo chemical leaching in order to remove arsenium oxide compounds (Fig. 4.9) [17].

Regeneration of bacterial leaching solutions is carried out to obtain commercial and/or waste products and recycle solutions. Many different flowsheets may be designed for this purpose, each including several unit operations: precipitation, filtration, washing, etc. (Fig. 4.12) [17].

In the case of the above-mentioned arsenopyrite concentrates, the biomass can be recycled together with the liquid phase of the pulp as the arsenic and

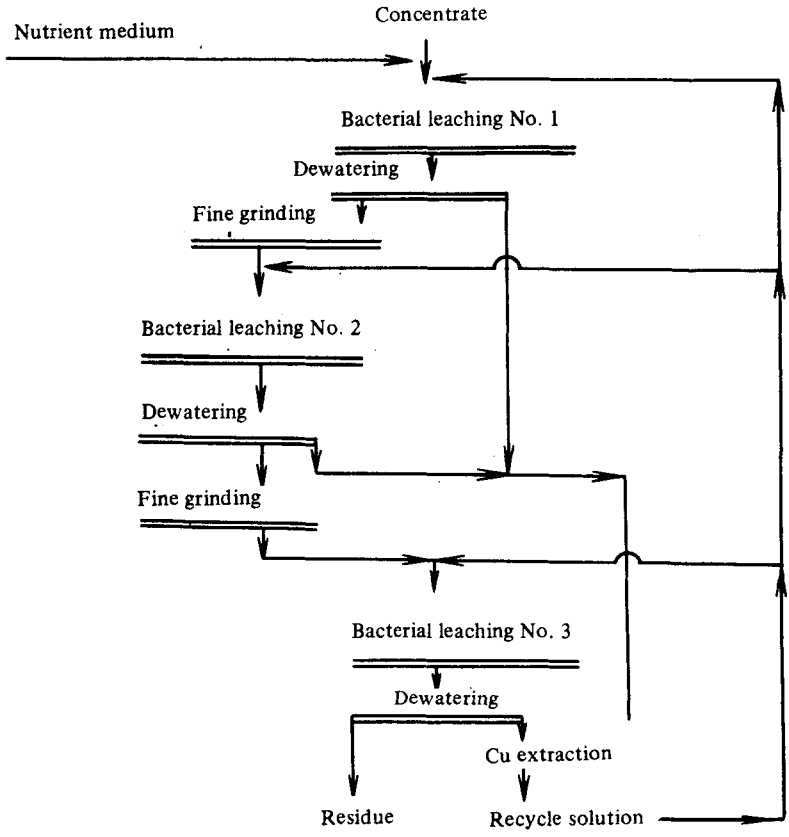

Fig. 4.8. Flowsheet of a three-stage chalcopyrite concentrate bacterial leaching

Table 4.4

Results of a Copper-Zinc Ore Flotation Operation

Product	Yield, %	Assay, %			Recovery, %		
		Cu	Zn	S	Cu	Zn	S
Cu-Zn bulk concentrate	32.73	8.15	8.6	37.1	92.1	90.8	31.0
Pyrite concentrate	67.27	0.36	0.44	42.1	7.9	9.2	69.0
Heads	100.00	2.80	3.10	39.17	100.0	100.0	100.0

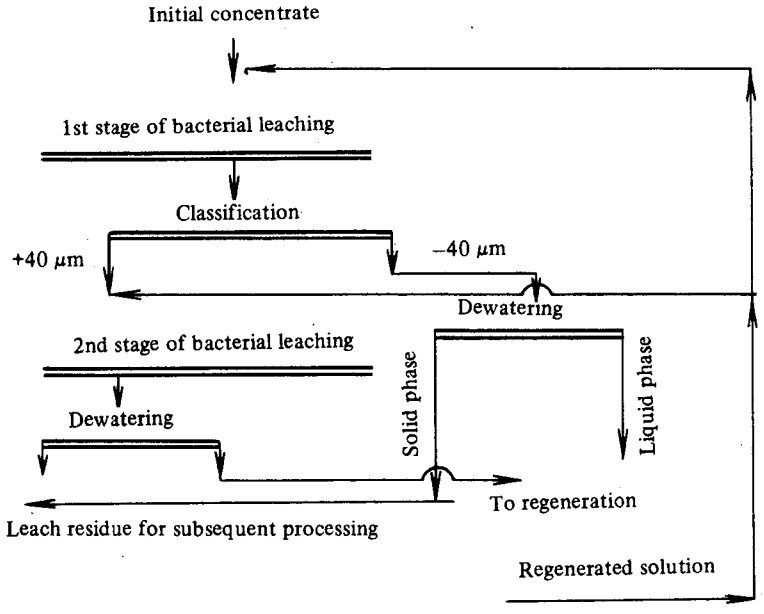

Fig. 4.9. Flowsheet of two-stage bacterial leaching of a pyrite concentrate containing gold and arsenopyrite with interstage classification of leach residue

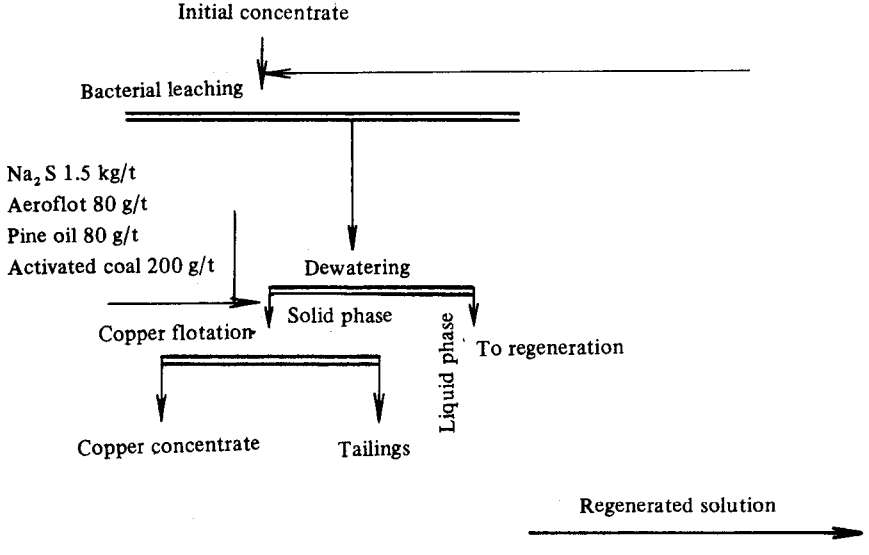

Fig. 4.10. Part of the Cu-Zn sulfide ore beneficiation flow-sheet

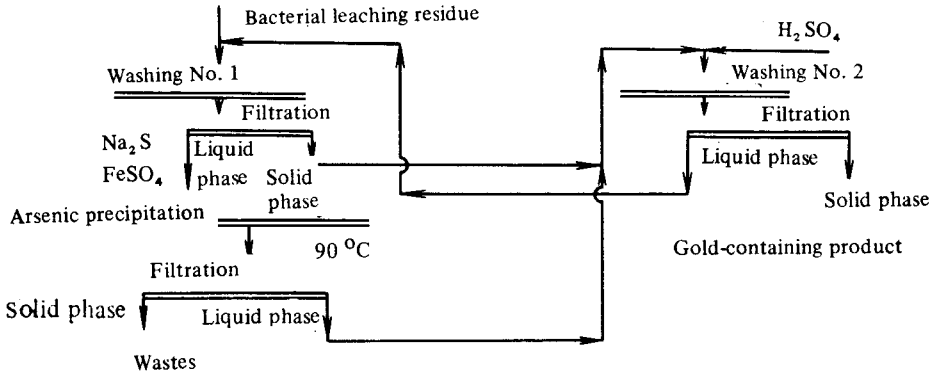

Fig. 4.11. Schematic diagram of two-stage counter-flow washing of the residue from the bacterial leaching of a pyrite concentrate containing arsenopyrite

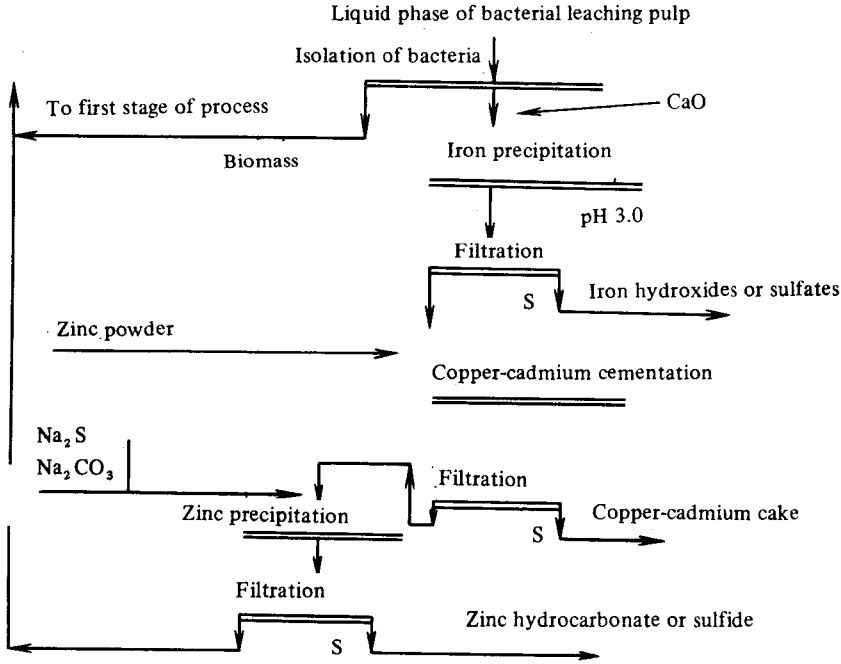

Fig. 4.12. Partial flowsheet of bacterial leaching of a Cu-Zn concentrate. Regeneration of recycle solutions

iron concentrations in solution only depend on the pH of the pulp [17]. It should be noted that the flowsheets for the treatment of bacterial leaching residues and those for the regeneration of solutions may be combined.

For instance, the spent solutions may be first used to wash the leach residues and subsequently sent to the regeneration circuit where the metals are separated by precipitation.

Table 4.5

Results of a Copper Flotation from the Residue of Bacterial Leaching of a Cu-Zn Concentrate

Product	Yield, %	Assay, %		Recovery, %	
		Cu	Zn	Cu	Zn
Cu concentrate	41.5	19.4	3.22	89.5	74.3
Tailings	58.5	1.64	0.79	10.5	25.7
Bacterial leaching residue (flotation feed)	100.0	9.0	1.8	100.0	100.0

4.6. SEMI-INDUSTRIAL BACTERIAL TANK LEACHING TESTS

Semi-industrial bacterial leaching tests can be carried out as continuous operations whenever favourable conditions exist for the growth of microorganisms. The continuous flow of the pulp is achieved by arranging the leaching vessels for series flow. The design of Pachuca-type devices is shown in Fig. 4.13 [17, 21].

The initial stage, i.e. that preceding the attainment of steady state conditions, is an important part of semi-industrial tests. First of all, the required amount of bacterial culture is prepared, i.e., bacteria are grown in advance on the sulfide concentrate and are adapted to leaching conditions. Cultivation of bacteria begins in the first unit of the series where sulfide concentrate (S:l=1:20–1:15) and nutrient medium are fed (see 4.4.3.). The intensity of the oxidizing process in the leaching vessel is evaluated from the amount of ferric iron present and from the pH and Eh values. The number of bacterial cells and their activity are also constantly monitored. As soon as the results can be considered satisfactory, the pulp is fed to the second unit of the series at a very low flowrate D, for example, 0.05 h^{-1}. The entire series of vessels is filled at this flowrate while the basic process parameters are continually monitored.

The start-up of the process is considered completed when the basic process parameters (pH, Eh, pulp density, ion composition of the liquid phase, activity and number of cells) are stabilized.

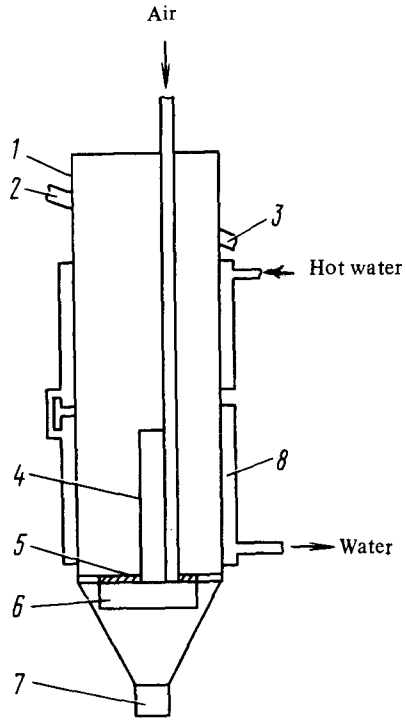

Fig. 4.13. Pachuca tank for bacterial leaching:
1 – Pachuca tank body; 2 – feed pipe; 3 – overflow pipe; 4, 6 – airlift; 5 – spider for airlift fastening; 7 – Pachuca tank discharge pipe; 8 – pulp heating jacket

In continuous operation, leaching may be performed using either the direct- or counter-flow technique. With the former method, the sulfide concentrate and the liquid phase are simultaneously fed into the first unit, pass through all the vessels and are discharged from the last one. In the counter-flow technique, the sulfide concentrate and the liquid phase move toward each other. During direct-flow leaching the build-up of inhibiting ions in the vessels is gradual and the bacteria have time to adapt to their increasing concentration. Therefore, bacterial activity in the pulp is not affected. In the counter-flow technique, bacteria are affected by the unfavourable conditions of the medium, which has a high inhibitor concentration, and it requires some time to adapt to these conditions, resulting in a significant decrease in their activity from the very beginning. Moreover, the counter-flow technique entails the separation of the solid phase from the liquid one on discharge from each vessel, and this considerably complicates the process as a whole.

The main vessels in the bacterial leaching plant (Fig. 4.14) were Pachuca tanks of 0.2 m^3 capacity, arranged in two parallel rows, five tanks to a row (3

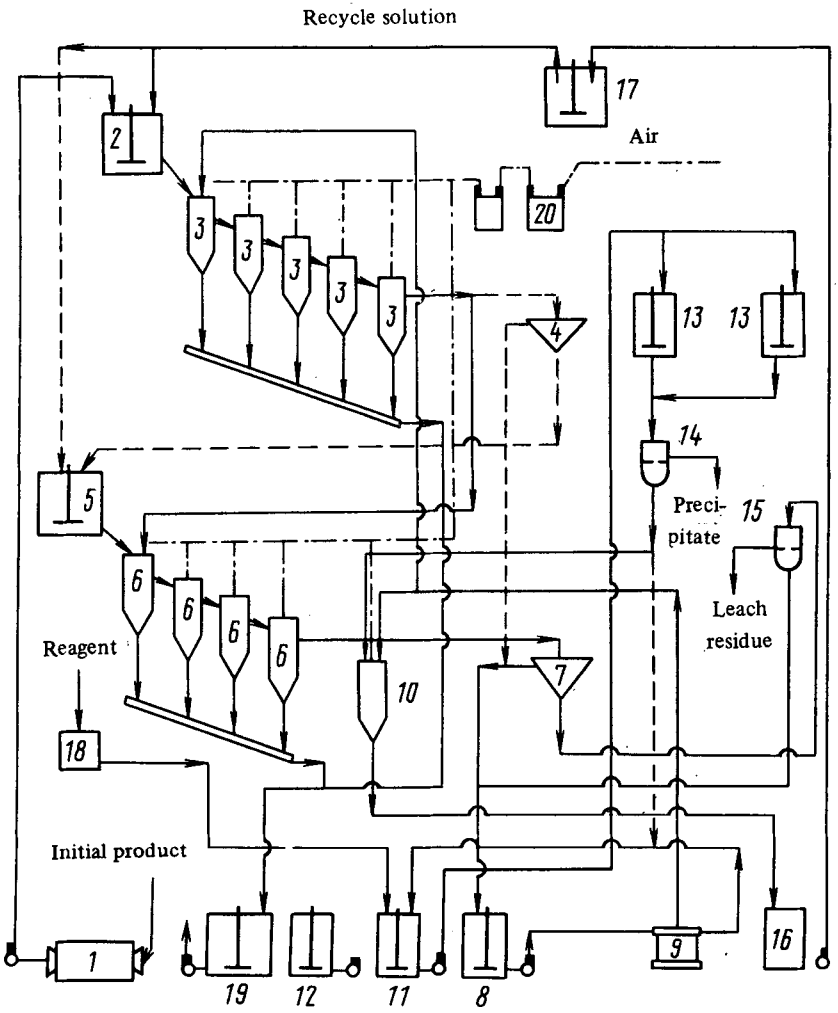

Fig. 4.14. Flowsheet of a semi-industrial bacterial leaching plant

and 5). Depending on the leaching time, which is determined by the nature of the concentrate to be processed, either both rows (leaching time 120–140 h) or one row (leaching time 60–70 h) are used, plant capacity being 60–75 kg of solids per day (300–400 l of pulp per day). The last Pachuca tank in one row (10) is not connected to the other tanks and is used to produce and regenerate the biomass fed to the head of the process via tank 2. The ground concentrate mixed with the solution at a given pH and temperature is also fed into this tank.

The Pachuca tanks are thermostated at 28–32 °C by means of circulating hot water in their jackets. The pulp flows by gravity from one Pachuca tank to

the next through overflow pipes and is stirred and aerated by air delivered to the airlifts through an air blower at pressures between $1.1 \cdot 10^5$ and $1.2 \cdot 10^5$ Pa.

Prior to leaching, the concentrate is ground, when necessary, in mill 1, and is then pumped, together with the solution recycled from storage tank 10, into conditioning tank 2.

The pH in this tank is adjusted to 2.5 or lower with sulfuric acid. To ensure continuous and uniform filling of the Pachuca tanks, a 1.5–2-day reserve of pulp is kept in the conditioning tank. From this tank, with 0.9 m^3 capacity, the pulp is continuously pumped by airlift to the first Pachuca tank and then flows by gravity to all the other tanks in the row.

If the leaching time is sufficient, the pulp from the last Pachuca tank is fed to a thickening cone; the thickened pulp is then pumped to the filter section, while the clear liquor, after joining the filtrate, is sent to the metal precipitation tanks and onto the bacterial regeneration unit. It should be pointed out that this solution may also be recycled without regeneration.

If longer leaching time is required, the pulp from Pachuca tank 5 is airlifted to the first tank of the second row. The pulp from the last tank in this row is then transferred for thickening to dewatering cone 4 or 7 and the thickened pulp then pumped to vacuum filter 15. The filter cake is a final leach residue and may undergo further processing. The filtrate joins the clear liquor and is fed to tank

Table 4.6

Semi-Commercial Bacterial Leaching Plant: List of Equipment

Description	Capacity, m^3	Quantity
1. Ball mill	–	1
2. Conditioning tank	0.9	1
3. Pachuca tank for leaching	0.2	5
4. Dewatering cone	0.3	1
5. Conditioning tank	0.4	1
6. Pachuca tank for leaching	0.2	4
7. Dewatering cone	0.3	1
8. Leaching solution tank	0.5	1
9. Biomass separator	–	1
10. Pachuca tank for solutions with biomass	0.2	1
11. Solution purification tank	0.5	1
12. Stand-by tank	0.5	1
13. Settling tank	0.5	2
14. Vacuum filter	0.06	1
15. Ditto	0.04	1
16. Recycle solution tank	0.5	1
17. Ditto	0.9	1
18. Pachuca discharge tank	0.9	1
19. Stirrer	0.03	1
20. Liquid-ring vacuum pump	–	2

8 and onto tank 11 where metals and, partly, sulfate ions are removed from the solution by addition of a lime suspension (up to pH 3–3.1) which is prepared in stirred tank 18. The pulp containing the precipitate is pumped to settling tank 13 where the solution is decanted and the precipitate passes through vacuum filter 14. The solution and filtrate are fed to Pachuca tank 10 for regeneration. From tank 10, the solution is fed to recycle solution tanks 16 and 17 and then returns to tank 2. In separator 9 the biomass is precipitated from the solutions. The resulting biomass may be sent either directly to tank 2 for pulp preparation or to tank 10 for regeneration. After separation of the biomass from the solution, solubilized metals are precipitated in tank 11. Should the plant need to be emptied for any reason, the pulp from the Pachuca tanks is fed to tank 19 for neutralization.

The plant may also be used for a two-stage process with intermediate replacement of the solution (dashed line). In this case the pulp from tank 5 is fed to cone 4. The clear liquor from the cone is fed to the leaching solution tank 8, while the thickened pulp is pumped to conditioning tank 5 where it is mixed with the solution. From the conditioning tank the pulp thus prepared is fed to the second leaching stage.

The plant is provided with automatic pulp temperature control and a pH meter.

A list of the main equipment used in the semi-industrial bacterial leaching plant is given in Table 4.6. The parts subjected to corrosive action may be made of X18H10T stainless steel or titanium.

4.6.1. Phase Separation after Leaching

After bacterial leaching the pulp is a suspension containing solid particles ranging in size from 0.5 to 100 μm. It also contains finer particles (from 0.1 to 0.5 μm) as well as colloid particles of ferric hydroxides, arsenates, etc., smaller than 0.1 μm and, rarely, 1 μm. The liquid phase of the pulp has a complex chemical composition and contains bacterial cells and organic metabolites besides various chemical compounds (as much as several tens of grams per litre). A large number of cells is also attached to the surface of solid particles. These pulp components considerably affect the solid/liquid separation.

In laboratory tests involving small amounts of pulp, the phases are separated by vacuum filtration of the suspension on filter paper. Normally, Büchner funnels are used for this purpose. The final dewatering stage for the leaching residue is drying.

If bacterial leaching is carried out continuously, solid/liquid separation must also be continuous. This usually consists of thickening and filtration or centrifuging.

Thickening of the pulp is performed in settling tanks, dewatering cones or thickeners. Batch settling tanks are vessels or basins without stirrers. They are fitted with a bottom outlet for discharge of the thickened pulp as well as overflow weirs for the clarified solution.

Settling tanks may only be used for batch bacterial leaching. In all other cases continuous thickeners, in which sedimentation is achieved by gravity, are

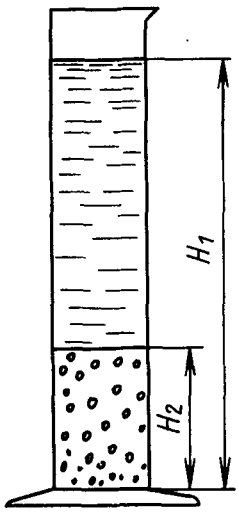

Fig. 4.15. Cylinder for the calculation of mineral particles settling rate

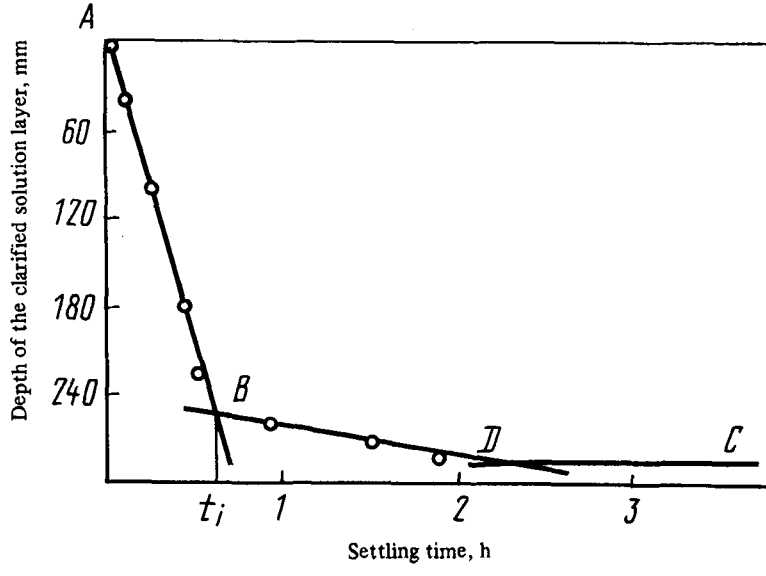

Fig. 4.16. Depth of the clarified solution layer versus time

employed. The latter include tapered settling boxes, thickening or dewatering cones and mechanical thickeners.

To design the area of the tank first of all the solid particle settling rate is found. A given volume of pulp sample is poured into a 2-l graduated cylinder

with a narrow strip of graph paper glued on the outside. The pulp is mixed by repeatedly turning the cylinder upside down. The latter is then placed on a table and a stopwatch is started. When a distinct boundary between the settling solids and clarified water is detected, the level of the settling solid mass is marked on the graph paper and time is recorded. The downward motion of the mud surface is recorded after fixed intervals and marked on the graph paper. Initially, time is recorded every few minutes but as the settling rate slows down, this interval is increased to several hours. The experiment should last at least 24 h. Observation data are used to plot (Figs. 4.15, 4.16) the depth of the clarified solution layer (Y-axis) against settling time in hours (X-axis). From the points plotted three straight lines are constructed which correspond to the three thickening zones in the thickener. The AB line characterizes the free-settling or clarification rate, the BD line describes the thickening zone and the DC line corresponds to the pulp compression zone. Transition from the clarified to the compression zone is represented by point B denoting pulp volume V_1 after a given settling time, at liquid-to-solid ratio R_1 (w/w). Point D indicates the volume V_2 of settled pulp at liquid-to-solid ratio R_2. Volumes V_1 and V_2 are read directly from the graph paper on the cylinder to calculate:

$$R_1 = \frac{V_1 \cdot \delta - P}{P \cdot \delta} \cdot \delta_1 \tag{4.14}$$

$$R_2 = \frac{V_2 \cdot \delta - P}{P \cdot \delta} \cdot \delta_1 \tag{4.15}$$

where:

δ – density of the solid phase (g/cm^3);

δ_1 – density of the solution (g/cm^3).

If the depth of clear water ($H_1 - H_2$) at point B is expressed in meters per t_1 (24 h), the settling rate is:

$$v = \frac{24\,(H_1 - H_2)}{t_1} \tag{4.16}$$

Hence, the required thickening area is:

$$S = \frac{R_1 - R_2}{\delta_1 \cdot v \cdot K_1},\ (m^2\,day)/t; \tag{4.17}$$

where:

K_1 is a safety factor of 0.7–0.8 allowing for irregular operation of the unit.

The required thickening area for both tapered settling tanks and radial thickeners may be calculated using equation (4.17).

A cone thickener is a conical tank with a 60° taper angle whose upper edge is surrounded by a circular overflow launder for discharging the supernatant clear liquid. The feed enters through a pipe located in the centre of the upper

part of the tank, which is provided with a goose-neck spigot discharge for the thickened pulp.

The diameter of the thickening funnel is selected such that the pulp upstream flowrate in the circular space does not exceed the settling rate of the finest particles. The latter is usually calculated using Stokes' formula for particles smaller than 0.1 mm:

$$V_s = \frac{54.5 \cdot d^2 (\rho - 1)}{\mu}, \text{cm/s} \qquad (4.18)$$

where:

d – particle diameter (cm);
ρ – particle density (g/cm^3);
μ – water viscosity (0.01 poise).

The drain capacity of the funnel (cone) is:

$$V_c = \frac{\pi \cdot (D^2 - d^2)}{4K} V_s, \text{m}^3/\text{h} \qquad (4.19)$$

The resulting funnel diameter is:

$$D = \sqrt{\frac{4K \cdot V_c}{\pi \cdot V_s} + d^2}, \text{m}; \qquad (4.20)$$

where:

D – funnel diameter (m);
d – diameter of the central pipe (m);
V_s – particle settling rate (m/h);
K – coefficient of 0.5–0.6.

Cylindrical thickeners are the most efficient and convenient devices for the first dewatering stage. They should be made of acid-resistant material. The density of the thickened pulp is usually 50–60 % solids.

Leach residues with such a density can be filtered. In small-capacity plants, vacuum filters or leaf press filters are normally used for filtration. Both types are intermittent. Vacuum filters are simple in design and easy to maintain and are provided with devices for precipitate flushing but cake removal must be done by hand.

Leaf press filters are also fitted with devices for precipitate flushing. They are particularly suitable for filtering suspensions with low content of finely dispersed particles (slimes, hydrate sediments, etc.).

Drum or disk continuous vacuum filters should be used in high-capacity plants. Vacuum for filtration is normally 500–650 mm Hg. The cake is blown by a 0.5–1 kg/cm^2 overpressure.

4.6.2. Metal Recovery from Solutions

Solutions recycling is of major importance in tank leaching since it provides a means of improving the process by ensuring the use of an adapted bacterial

strain. This step consists in removing solubilized elements from the solution and their transformation into finished products. The treatment of solutions after separation of the solid phase should therefore include the extraction of metals from the solutions as well as the recovery and recycling of active biomass.

The chemical composition of bacterial leach liquors may be very complex. For example, the pregnant solution produced by bacterial leaching of a sulfide concentrate containing arsenopyrite and gold may contain as much as 7–10 g/l of arsenic and 15–29 g/l of iron. Likewise, that produced by a bulk copper-and-zinc sulfide concentrate may contain from 2 to 4 g/l of copper and from 20 to 25 g/l of zinc.

The metals in solution should be selectively recovered from the pregnant liquors (see Section 4.11), for instance, as low-solubility compounds before recycling in order to obtain commercial products without affecting the microbial activity.

4.6.3. Equipment for Tank Bacterial Leaching

Equipment which is normally used for bacterial leaching is such as to ensure good contact between the bacterial solution and solid substrate over a required period of time under optimum leaching conditions. Such conditions include efficient stirring, adequate aeration, possibility to maintain the necessary temperature and high production capacity. Besides, this equipment should be reasonably simple and cheap, as well as reliable and provided with instruments for automatic process monitoring and control.

In determining the production capacity of the batch leaching equipment, the required degree of metal leaching or metal content of the solid residue should be taken into account. The leaching time is determined using the kinetic curve representing the target metal content of the solid phase versus time. Knowing the effective capacity of the unit (V) and leaching time (τ) the pulp productivity of the unit can be determined:

$$W = \frac{V}{\tau} \tag{4.21}$$

where:

V – the effective capacity of the unit (ml);

τ – leaching time.

If the effective capacity of the unit and the required pulp productivity are known, the mean residence time of pulp in the leaching unit can be determined according to the formula:

$$\tau_{mean} = \frac{V}{W} \tag{4.22}$$

In continuous reactors the incoming pulp is sufficiently rapidly stirred resulting in uniform composition of pulp throughout the entire volume of the unit and, therefore, some of the incoming particles are immediately removed with the ongoing pulp. To minimize the amount of solid particles with insufficient residence time, reactors are arranged in a direct flow series.

A variety of reactors and tanks can be used for bacterial leaching with mechanical or pneumatic stirring. The Pachuca tank (Fig. 4.13) with airlift stirring is the simplest device in this class. It consists of a cylindrical tank or column with conical bottom and a central vertical pipe with diameter 1/5:1/10 that of the tank. Compressed air is pumped through this pipe forming an emulsion with the pulp and causing it to move upwards. The pulp is thus mixed and aerated. However, pulp is not aerated to a sufficient degree in Pachuca tanks because large air bubbles rapidly pass through the pulp column, impeding saturation with oxygen. Saturation is of major importance when leaching metals from sulfide concentrates at high pulp density and in the presence of a large biomass.

Devices equipped with mechanical stirrers and compressed air supply are more efficient in this respect. They include tanks and mechanical stirrers of different designs, such as impellers, propellers and turbines. Impellers are, as a rule, low-speed devices which create laminar flow of the pulp. They ensure good mixing of pulps containing finely ground minerals but aeration is poor and it is therefore advisable to use them for pulp preparation prior to leaching. Propellers operate at much higher speeds (over 500 rpm) and are capable of stirring pulps containing coarser solids. Still higher speeds are attained with turbines which produce turbulent motion of the pulp. They can be very efficient for coarse granular solids at high pulp densities.

However, mechanical stirring does not produce a sufficient degree of aeration and for this reason an additional air supply must be incorporated. The air is dispersed by the stirrer and uniformly distributed throughout the pulp. Air is fed through special devices called diffusors, installed on the bottom, or through pipes placed on the reactor bottom.

Conventional acid-resistant chemical reactors with additional air supply may also be used for metal leaching.

All units should be provided with pulp heating devices, either water jackets, as shown in Fig. 4.13, or other heat exchangers fitted inside the working volume. Pulp temperature should be controlled automatically.

4.6.4. Monitoring the Bacterial Leaching Process

To ensure adequate bacterial leaching, all basic process, chemical and microbiological parameters should be continually monitored.

Process monitoring involves determination of size distribution of the feed, pulp density, flowrate, temperature and acidity, and air consumption.

The feed size distribution may be determined by screen analysis and pulp density may be measured by weighing samples of a given volume.

Temperature and acidity of the pulp and recycle solutions are monitored automatically. The redox potential of the liquid phase in the leaching vessels and conditioning tanks can be measured once or twice per shift. Rotameters are used to measure the air flowrate to the leaching vessels.

Chemical monitoring of the bacterial leaching process provides information on the chemical composition of the feed and of the solid residues. Leaching efficiency is periodically assessed through chemical analysis of metal values in

the pulp, e.g. copper or zinc in the conditioning tank and the leaching vessels. The metal content is determined, as a rule, both in the solid and liquid phases. The bi- and trivalent iron content of the leach liquors should also be determined. In addition, the bivalent iron content in the liquid phase is an indirect measure of intensity of bacterial oxidizing activity: the absence of bivalent iron in a leach solution indicates high bacterial oxidation efficiency. As far as iron and arsenic are concerned, it should be pointed out that, depending on the acidity of the medium, they may be present not only in the solution but also in the precipitate as oxides. Therefore chemical analyses should be carried out after acidification of the solution which results in solubilization of these precipitates.

Microbiological monitoring involves measuring the amount of biomass (number of cells per unit of volume) and activity of bacteria in the leach liquors). Besides the serial end-point dilution technique, biomass may also be determined using the methods described in Chapter 3. The amount of biomass and its activity should be measured not only in the conditioning and leaching tanks but also in recycle solutions.

Table 4.8

Fundamental Bacterial Leaching Factors for which Monitoring is Recommended

Factor	Point of monitoring	Periodicity
Particle size distribution of concentrate	Classifier overflow	Continuous or once or twice per day
Plant throughput	Inlet of Pachuca tank 1	Ditto
Pulp density	Conditioning tanks, Pachuca tanks	Ditto
Acidity	Ditto	Ditto
Temperature	Conditioning tanks, Pachuca tanks	Ditto
Biomass	Conditioning tanks, Pachuca tanks, recycle solution	Once per shift
Activity of solution	Conditioning tanks, Pachuca tanks, recycle solution	Ditto
Main components of feed	Inlet of Pachuca tank 1	Once per day
Main components of solutions	Conditioning tanks, Pachuca tanks, recycle solutions	Ditto
Ferro-ferric iron content	Conditioning tanks, Pachuca tanks, recycle solutions	Once per shift
Main components of end products	Filter cakes, recycle solutions	Once per day

Written records of all the measured values are recommended.

Table 4.8 shows the basic bacterial leaching parameters to be monitored, points of monitoring and suggested measuring frequency.

4.6.5. Costs of Bacterial Leaching Process

Owing to the relatively long pulp residence times (tens of hours) typical of present day bacterial tank leaching processes, the equipment capacity required may entail considerable investment and operating costs. Despite the obvious advantages of this method over other methods, e.g. pyrometallurgy, its industrial scale application must be carefully analysed in terms of cost-effectiveness, through comparison with other commercial techniques.

The main items of cost analysis are investment and operating costs; the former include the cost of process equipment, buildings, auxiliary structures, piping and electric connections; the latter comprise the cost of manpower (including management), fuel, electricity and water, reagents and plant maintenance as a whole.

On the basis of this analysis a feasibility study can be carried out and the bacterial leaching unit cost, i.e. the cost of processing one ton of concentrate, can be calculated.

The economic comparison between a bacterial leaching and a sulphatizing roasting and leaching operation treating 500,000 t per year of complex copper-zinc sulfide bulk flotation concentrate is given in the sequel.

Bacterial leaching is assumed to operate continuously for 330 working days per year, on a 3-shifts-per-day basis.

Permanent investment costs

The equipment costs are shown in Table 4.9.

Table 4.9

Equipment Costs

Item	Quantity	Unit cost, thous. roubles	Total cost, thous. roubles
Conditioning tank K4-2	1	20.4	20.4
Bacterial leaching Pachuca tank ($V = 500\ m^3$)	30	35	1050.0
Acid-resistant thickener П-18К (without tank)	7	13.7	96.0
Pumps	6	1.2	7.2
Filter	2	28.4	56.8
Compressor with motor	4	79.9	319.6
Auxiliary equipment	–	–	150.0
Total:			1700

Table 4.9, continued

Item	Quantity	Unit cost, thous. roubles	Total cost, thous. roubles
Pipelines, 10 %			170
Steel structures, 10 %			170
Erection work, 10 %			170
Rubberizing			100
Transportation and overheads of agents, 10 %			170
Total:			2480
Miscellaneous equipment and contingencies, 30 %			720
Grand Total			3200

Process equipment usually accounts for 60 % of total equipment costs. Consequently, the overall cost of equipment can be estimated as 5400 thousand roubles and with additional off-site expenditure, 5400 x 1.15 = 6200 thousand roubles.

Table 4.10

Cost of buildings and structures

Item	Volume, m^3	Cost per 1 m^3, roubles	Total cost, thous. roubles
Bacterial leaching plant	11000	15.0	150.0
Compressor house	4300	16.0	70.0
Filtration plant, pump house, stores	–	–	200.0
Tanks for thickeners	–	–	240.0
Other structures, contingencies	–	–	60.0
Total:			720.0

The overall cost of construction of buildings and structures with additional off-site expenditure can be estimated as: 720 x 1.3 = 940 thousand roubles (Table 4.10).

Overall capital investments in the construction of a bacterial leaching plant and process and auxiliary equipment can be estimated as: 6200 + 940 = 7140 thous. roubles.

Operating Costs. Reagent consumption and costs are shown in Table 4.11.

Table 4.11

Annual Reagent Consumption and Costs

Item	Unit	Annual consumption	Unit cost	Total cost, thous. roubles
Sulfuric acid (process)	ton	2000	29.6	59.2
Slaked lime (60 % act)	ton	50	15.0	0.75
Miscellaneous and contingencies, 10 %				6.0
Transportation and costs of agencies, 15 %				9.0
Total:				75.0

Annual operating costs of the copper-zinc sulfide concentrate bacterial leaching plant are shown in Table 4.12.

Table 4.12

Annual Operating Costs of the Bacterial Leaching Plant

Item	Unit	Unit cost	Quantity	Total, thous. roubles
A. Wages	persons	2000	40.28	80.0
B. Salaries	persons	2800	6.4	16.8
Total:				96.8
Social security	%	12	84.8	11.2
Total wages:				108.0
Materials	–	–	–	75.0
Power	thous. kW·h	20.0	35000	350.0
Fresh water	thous. m^3	190.0	448	85.0
Depreciation of buildings and structures	%	2.8	940	67.0
Depreciation of equipment	%	15.0	6200	930.0
Total depreciation:				997.0
Maintenance	%	50.0	940	470.0
Miscellaneous and contingencies	%	10.0	–	215.0
Grand total:				2300.0

Annual operating costs of the bacterial leaching plant are 2300.0 thousand roubles or 4.6 roubles per ton of concentrate.

Table 4.13

Economics of Concentrate Processing

Description	Unit	Bacterial leaching, cementation, electrolysis	Sulfatizing roasting – leaching, electrolysis
Annual capacity	thous. t	500.0	500.0
Permanent investments	thous. roubles	15700	18400
Operating costs	thous. roubles	7650	8700
Reduced costs	thous. roubles	10000	11460
Cost of processing per ton of concentrate	roub.	15.3	17.3

The above analysis shows that the investment, reduced and unit costs of the bacterial hydrometallurgical process are lower than those of the roasting–leaching process; 15.3 against 17.3 roubles per ton of concentrate (Table 4.13).

The total cost of this process should be about 1.5 million roubles per year less than that of the sulphatizing–roasting–leaching process.

4.7. PREPARATION OF THE PRODUCTS OF BACTERIAL LEACHING FOR CHEMICAL ANALYSIS. CALCULATION OF METAL BALANCE

Monitoring and control of the bacterial leaching process involves sampling pulps and solutions at different points of the flowsheet. The results of chemical analyses are used to calculate the material balance of the operation, and their reliability depends on sampling and accuracy in sample preparation and analysis. Samples should be representative, in ratio and composition, of the phases making up the pulp and the basic condition for achieving this uniformity of distribution of the solid phase in the pulp. In turn, uniformity is strongly affected by the following factors: method and intensity of stirring, equipment design, particle size distribution of the solid phase and pulp dilution. When leaching in Pachuca tanks is carried out using pulp not thoroughly mixed, the solid phase may be unevenly distributed in the tank: coarser and heavier pulp fractions may segregate at the bottom of the tank while smaller and lighter fractions remain at the top. Therefore, the pulp should be sampled after mixing; partial samples constituting the resulting average sample should be taken from the upper, middle and bottom sections of the tank. When the grain size of the solid component of the pulp is finer than 0.074 mm, the average sample should contain not less than 15 g of solids.

The method of handling samples depends on their composition and leaching conditions. During leaching of some minerals, e.g. arsenopyrite, pyrite, etc.

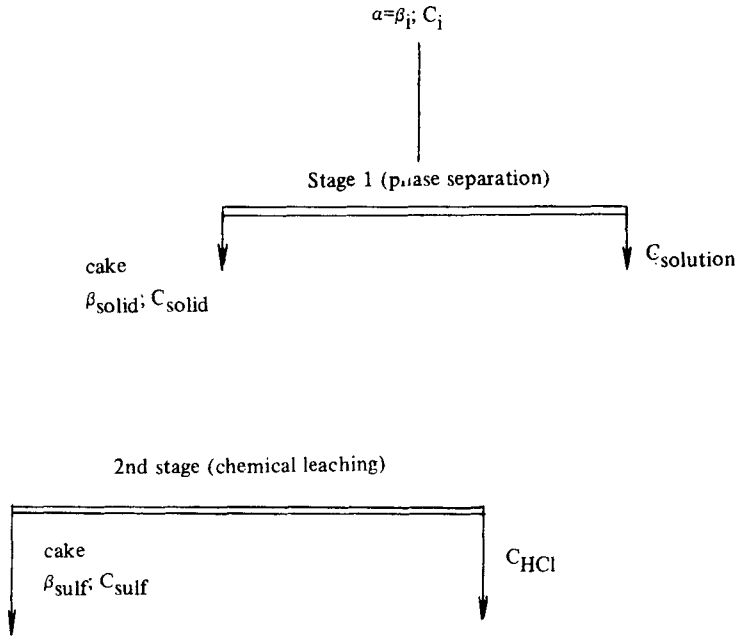

Fig. 4.17. Sampling flowsheet for chemical analysis

Symbols:

$\alpha=\beta_{initial}$; $C_{initial}$ – metal value contents in the initial product (%), (g/l);
β_{sulf}; C_{sulf} – metal value contents in sulfide component of the pulp (%), (g/l);
$\beta_{solid\ phase}$; $C_{solid\ phase}$ – metal value contents in the solid phase of pulp (%), (g/l);
$C_{solution}$ – concentration of the metal value in the liquid phase of pulp (g/l);
C_{HCl} – concentration of the metal value in the solution of chemical leaching (g/l)

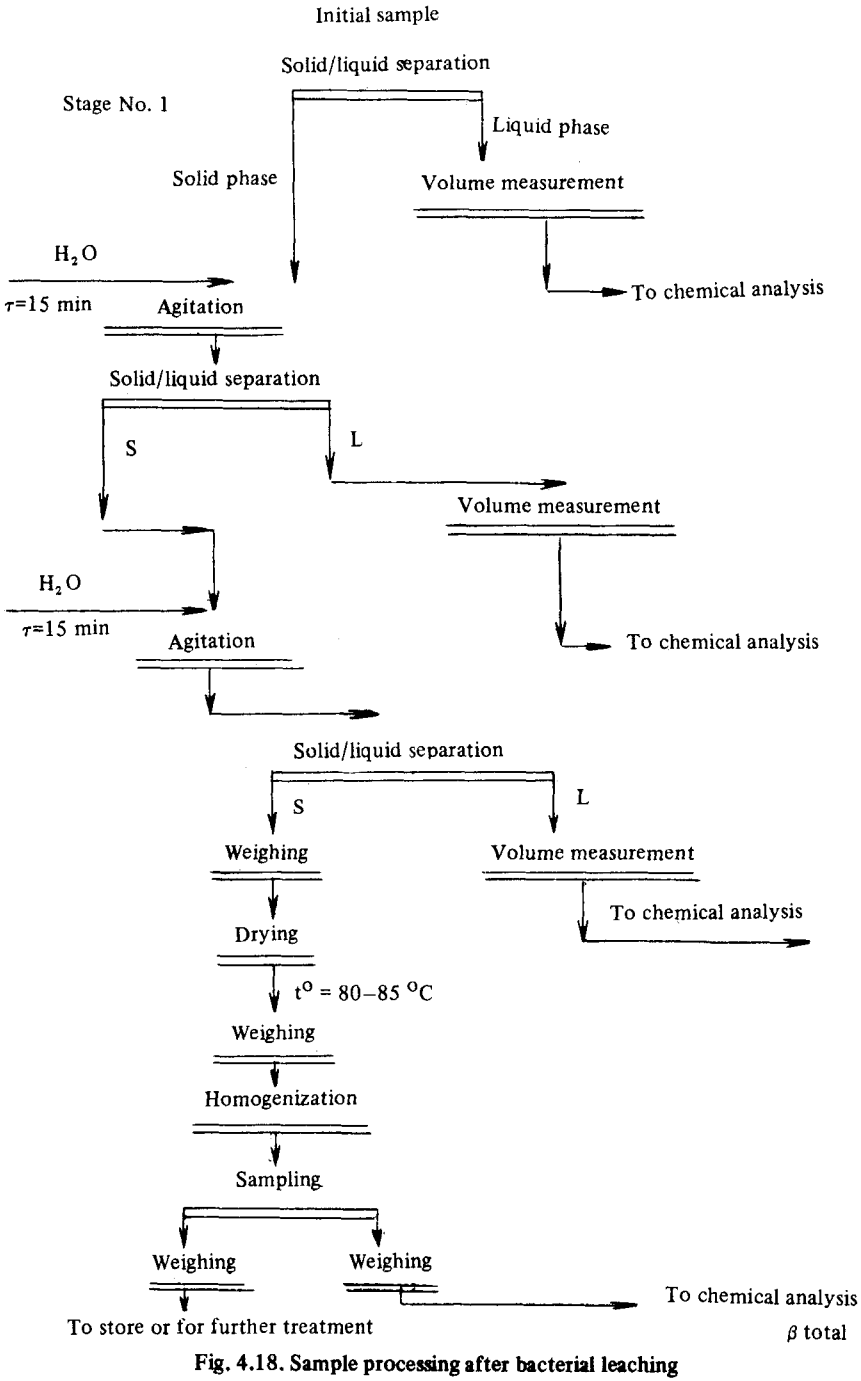

Fig. 4.18. Sample processing after bacterial leaching

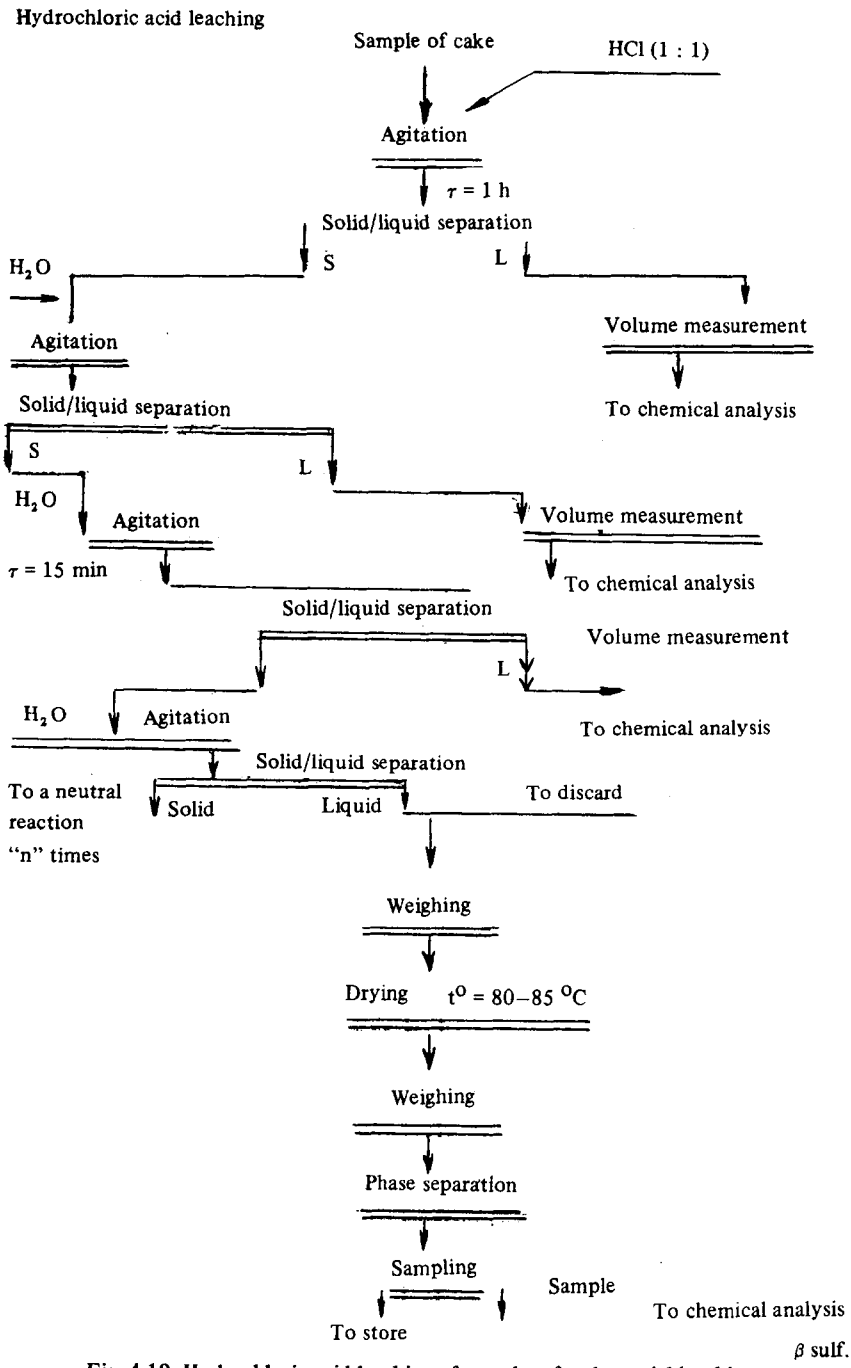

Fig. 4.19. Hydrochloric acid leaching of samples after bacterial leaching

practically insoluble iron and arsenic compounds, which constitute the new solid phase of the pulp, may form. Sulfide concentrates amenable to bacterial leaching may be classed into two groups: sediment-forming and non-sediment-forming. The latter group includes some copper-zinc sulfide ores and concentrates. The ability of a sulfide concentrate to form sediments is empirically determined in each case. For the purpose, several flowsheets are proposed which enable to treat concentrates capable of forming sediments (Figs. 4.17–4.19). Chemical analysis provides information on the ability of the sulfide concentrate to form sediments.

The preparation of sulfide concentrate for chemical analysis involves a solid/liquid separation step (Fig. 4.15.). Samples of sediment-forming concentrates are further treated with acid to dissolve the sediments produced during bacterial leaching (Fig. 4.19).

Solid/liquid separation is achieved either by centrifuging or by filtration. To thoroughly remove the liquid phase containing dissolved metals, the cake is washed at least twice. The volume of wash water added to mixing operations should be equal to or a multiple of the volume of the liquid phase in the pulp sample. After washing the cake is weighed, dried at 80–85 °C and then weighed again. The moisture content of the cake is then calculated using the equation:

$$W = (1 - \frac{m_2}{m_1}) \cdot 100 \quad , \tag{4.23}$$

where:

m_2 – mass of dried cake (g);
m_1 – mass of moist cake (g);
W – moisture content of cake (%).

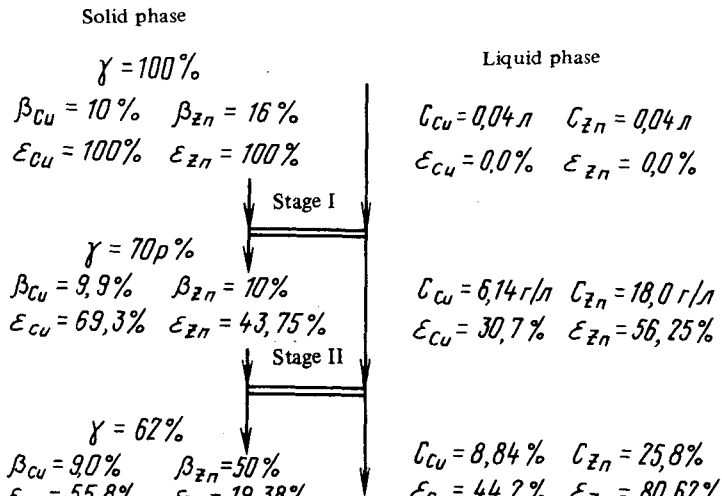

Fig. 4.20. Two-stage leaching of copper-zinc concentrate:
10 % Cu; 16 % Zn; R = 5; d = 1.0 g/cm³

The cake is then homogenized [12] and an average sample is taken for determination of the metal value in the solid phase of the pulp. When the concentrate can form sediments, the total metal values content of both sulfide and newly formed solid phase is determined. If no sediments are formed only the sulfide is assayed for metal contents.

The solid phase thus obtained is leached with hydrochloric acid in order to dissolve the solid precipitate formed during bacterial leaching. This step should be carried out by adding hydrochloric acid in amounts small enough as not to modify the dilution of the newly formed pulp with respect to the initial pulp. The volume of the added hydrochloric acid is determined by:

$$V_6 = \frac{m_4}{m_2}\left(V_1 + \frac{m_1 \cdot W}{100}\right), \tag{4.24}$$

where:

m_4 – mass of sample for chemical leaching (g);
V_6 – hydrochloric acid volume (m) (Fig. 4.19).

Hydrochloric acid leaching is performed in vigorously shaken Erlenmeyer flasks for 1 h (Fig. 4.19). The pulp then undergoes liquid/solid separation. The metal values content of the liquid phase is assayed, while the solid phase is repeatedly washed to effluent neutrality. At least the first two wash waters are assayed for metal values content. The washed sample is dried at 80–85 °C, homogenized and sampled to determine the sulfide metal content.

The distribution of the metal (metals) values between the liquid, solid and newly formed solid phases of the pulp is calculated from the chemical data. Metal balance is calculated under the assumption that the volume of the liquid phase of the pulp remains unchanged. Therefore, the metal concentration in the initial pulp is equal to the sum of its concentrations (expressed in the same units) in the liquid and solid phases of the pulp and in the precipitate formed during leaching.

$$C_i = C_{sol} + C_{HCl} + C_{sulf} \tag{4.25}$$

The notation is explained in Fig. 4.17.

To convert metal content (%) of the solid phase to concentration (g/l) the following equation is used:

$$C = \frac{\gamma \cdot \beta \cdot d}{10 \cdot R}, \tag{4.26}$$

where:

γ – sulfide concentrate feed (solid) (%);
β – metal content of feed (%);
d – density of liquid phase in initial pulp (g/cm^3);
C – metal content of pulp (g/l);
R – dilution of initial pulp (units).

Substituting in Eq. (4.25) C_i and C_{sulf} with β_i and β_{sulf} from Eq. (4.26) we get:

$$\frac{100 \cdot a \cdot d}{10 \cdot R} = C_{sol} + C_{HCl} + \frac{\gamma_{sulf} \cdot \beta_{sulf} \cdot d}{10 \cdot R} \tag{4.27}$$

$a = \beta_i$ = metal content in the initial pulp.

By solving Eq. (4.27) for γ_{sulf} we get Eq. (4.28) with which the yield of sulfide concentrate can be calculated:

$$\gamma_{sulf} = \frac{100 \cdot a \cdot d - C_{sol} \cdot 10 \cdot R - C_{HCl} \cdot 10 \cdot R}{\beta_{sulf} \cdot d} \tag{4.28}$$

The metal value extraction in the sulfide concentrate is given by:

$$\epsilon_{sulf} = \frac{\gamma_{sulf} \cdot \beta_{sulf}}{a} ; \tag{4.29}$$

the metal value extraction in the liquid phase is $100 - \epsilon_{sulf} = \epsilon_{sol}$,

where:

ϵ_{sulf} – metal extraction in sulfide concentrate (%);

ϵ_{sol} – metal extraction in liquid phase (%).

When no precipitates are formed, $C_{HCl} = 0$ and hence Eq. (4.28) may be rewritten:

$$\gamma_{sulf} = \frac{100 \cdot a \cdot d - C_{sol} \cdot 10 \cdot R}{\beta_{sulf} \cdot d} \tag{4.30}$$

If a non-leachable component, e.g. gold, is present in the sulfide concentrate its yield may be determined as the ratio of its content in the feed to that in the residue:

$$\gamma = \frac{100 \cdot a_{Au}}{\beta_{Au}}, \tag{4.31}$$

where:

a_{Au} – gold content of feed (g/t);

β_{Au} – gold content of leach residue (g/t).

The yield of sulfide concentrate may also be determined by the ratio of sulfide mass in the residue to that in the feed:

$$\gamma = \frac{100 \cdot m_{prod}}{m_{initial}}, \tag{4.32}$$

where:

$m_{initial}$ – mass of product before leaching (g), (kg);

m_{prod} – mass of product after leaching (g), (kg).

If polymetallic products are leached the metal balance can be calculated for all metals in a similar way. The calculated data are recorded in table form or graphically (Fig. 4.20, Table 4.14).

It is recommended that experimental conditions, chemical analyses of daily samples and metal balance calculations are summarized in table form. An example of such a table (4.15) for the bacterial leaching of a pyrite concentrate containing arsenopyrite and gold is given overleaf.

Table 4.14

Example of data arrangement for metal balance calculation

Operation	γ, %	βZn, %	C_{Zn}, g/l	βCu, %	C_{Cu}, g/l	ϵZn, % s.ph.	ϵZn, % l.ph.	ϵCu, % s.ph.	ϵCu, % l.ph.
Feed to 1st stage Initial pulp	100.0	16.0	0.0	10.0	0.00	100.00	0.00	100.00	0.00
Reject from 1st stage Leached pulp	70.0	10.0	18.0	9.9	6.14	43.75	56.25	69.35	30.70
Feed to 2nd stage Leached pulp of 2nd stage	70.0	10.0	18.0	9.9	6.14	43.75	56.25	69.35	30.70
Reject from 2nd stage Leached pulp of 2nd stage	62.0	5.0	25.8	9.0	8.84	19.38	80.62	55.80	44.20

s.ph. – solid phase

l.ph. – liquid phase

Table 4.15

Suggested form for recording bacterial tank leaching parameters. The example refers to a pyrite concentrate containing arsenopyrite and gold

Date and time	Unit No	Liquid phase											
		pH	Eh	Fe, g/l			As, g/l			SO_4^{2-}, g/l	m*, g/l	A**, g/l·h	Fe, %
				3+	2+	Σ	5+	3+	Σ				
1	2	3	4	5	6	7	8	9	10	11	12	13	14

Solid phase

As, %	S^0, %	Yield, %	Fe, %	As, %	Au, g/t	S^{2-}, %	m*, g/g	A**, g/l·h	As, %	Fe, %	Au, %
15	16	17	18	19	20	21	22	23	24	25	26

Table 4.15, continued

Newly formed solid phase

Yield	As, %			Fe, %			S^o, %	As, %	Fe, %	S^o, %
	5+	3+	Σ	3+	2+	Σ				
27	28	29	30	31	32	33	34	35	36	37

Recycle solutions

Litres for 24 h	As, g/l			Fe, g/l			SO_4^{-2}, g/l	m*, g/l	A**, g/l·h
	5+	3+	Σ	3+	2+	Σ			
38	39	40	41	42	43	44	45	46	47

Experimental conditions

Solids in pulp, %	Flow, l/h	t, °C	Air consumption, l/min	Stirrer, rpm	Pulp in unit, l	Pulp processed, l	Cake, kg	Cake humidity, %	Titrant H_2SO_4/KOH, l	Added fresh water, l
48	49	50	51	52	53	54	55	56	57	58

* Bacterial concentration in solution (on solid phase of pulp): g/l (g/g)
** Oxidative activity of bacteria (g/l·h)

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Chapter 5
IN-SITU, DUMP AND HEAP MICROBIAL LEACHING

5.1. GENERAL

Microbial in-situ (in-place) leaching can be considered as a specialized underground extraction system consisting of the microbiologically enhanced dissolution of metal values from run-of-mine ores with grades ranging from above the cut-off grade (cut-off grade is defined as the average grade of the run-of-mine ore below which mining is no longer profitable) [36] to the so-called submarginal or submilling grades. A typical example of the latter is the rock forming the remnants of underground mined-out stopes, which are generally mixed with caved country rock, or the leaner parts of orebodies mined by open-cast methods.

Leach solutions are injected into – and percolate through – the rock mass and, after dissolution of the desired metal values, are collected and pumped to the metal recovery plant. Microbial dump leaching can be defined as a "metal scavenging" method, employed for recovering metal values from lean ores (sometimes incorrectly called "waste rock"), usually the submarginal-grade overburden of open-pit mining operations, i.e. that part of the orebody containing rock with grade below the cut-off and which must be removed in order to enable access to the richer parts of the mineralization.

The blasted rock is accumulated in dumps located in the vicinity of the open pit. The top of the dump is irrigated with leach solutions containing microorganisms. These solutions percolate through the broken rock mass, solubilizing the metal values contained therein and becoming loaded with the latter. The pregnant solutions flow out of the bottom of the dump and are finally collected in ponds and then pumped to the metal recovery plants.

Microbial heap leaching is an ore solubilization method whereby the run-of-mine ore, fragmented (and sometimes suitably sized) is piled up in regular layers called "lifts" on appropriately prepared areas, so as to form heaps. The top of these heaps is irrigated with solutions containing microorganisms in the same way and with the same objective as dump leaching. Metal solutions are collected and their metal values recovered in basically the same way as dump leaching.

Base-metal sulfide- or pyrite-containing uranium ores – which on account of either (a) their low metal values content, (b) the very finely intergrown mineral components, (c) the nature of their gangue minerals, or (d) the small amount of exploitable reserves cannot be profitably mined and beneficiated using con-

ventional ore processing methods or combined flotation and tank bioleaching processes -- can be advantageously processed with one (or more) of these three methods.

Microbial in-place, dump and heap leaching differ from abiotic leaching methods in that a microbial flora, capable of enhancing the leaching process, is present in the leach solutions and within the broken rock mass. Despite the fact that they are applied to quite different types of mineralized rock and therefore present numerous differences, which distinguish them from one another, from the conceptual point of view these three leaching methods are basically similar in many aspects and have some important technological features in common which are discussed below.

Leaching of fragmented rock masses has long been practiced empirically but only in this century have scientists endeavoured to optimize the process. Interest in this field was heightened in the 1950's, following the discovery of the biocatalytic role played by microorganisms in the dissolution of minerals and rocks. The next logical step was the development of appropriate models for scaling up the results obtained with small-scale laboratory or pilot plant equipment designed to simulate commercial operations. As in other fields of mineral and chemical engineering, the early models were semiempirical and quite simple. Over the years, however, these have been replaced by increasingly complex and sophisticated tools. The models are still being perfected even though present knowledge is adequate for making fairly accurate predictions of new project efficiency. The most important models applied to bioleaching kinetics of ore particles still have, however, mostly a theoretical importance and are more suited to reactor-leaching scale-up studies. A thorough understanding of the factors affecting the bioleaching of fragmented rock masses is however a necessary prerequisite for the correct evaluation and application of such models.

5.2. BIOLEACHING EFFICIENCY

A better understanding of the problems posed by the bioleaching of fragmented rock masses and of the means of solving them is afforded by the concept of leaching efficiency, which is also essential for the quantitative assessment of the results of these operations.

The more effective the bioleaching process, the greater the metal value dissolved from the rock and the higher its rate of dissolution. The percent ratio of the metal value dissolved to that present in the rock is called "metal recovery" (R_m); the percent ratio of the rate of metal value dissolution obtained by a given commercial process to the maximum rate attainable in optimum conditions (achieved, for instance in a laboratory or pilot plant), is called "kinetic efficiency" (E_k); bioleaching efficiency (E_B) is defined as the product of metal recovery multiplied by kinetic efficiency:

$$E_B = R_m \cdot E_k \tag{5.1}$$

The variation of R_m and E_k ranges from 0 to 1; likewise E_B also varies from 0 to 1.

Metal recovery, R_m, depends on a variety of conditions amongst which the following three are fundamental for in-place, dump or heap leaching:

a) contact of the leach solutions containing microorganisms with the highest proportion of exposed surface area of the mineral to be leached;

b) maximization of surface are of the mineral to be leached accessible to leach solutions;

c) access of gases required for microorganisms' metabolism (O_2 and CO_2), and essential for the oxidation process (O_2) to the highest proportion of wetted surfaces of the mineral to be leached.

The first condition can be quantitatively expressed as the maximization of the "exposure ratio" (R_W) and can be written:

$$R_W = \frac{\Sigma A_a}{\Sigma A}, \tag{5.2}$$

where:

ΣA_a – surface area of metal-values containing mineral accessible to leach solutions at a given particle-size distribution π_i of the leaching feed (e.g. the run-of-mine ore in the case of in-place bioleaching);

ΣA – exposed surface area of metal-values containing mineral at particle-size distribution π of the leaching feed which corresponds to complete liberation from the gangue or from other minerals of the mineral to be leached.

The second condition can be quantitatively expressed as the maximization of the "percolation ratio" (R_p), by equation:

$$R_p = \frac{\Sigma A_W}{\Sigma A_a}, \tag{5.3}$$

where:

ΣA_W – surface area of mineral to be leached contacted by the leach liquors;

ΣA_a – has the same meaning as in Eq. (5.3).

Finally, a quantitative expression of the third condition can be obtained by maximizing the "oxygenation ratio" (R_O):

$$R_O = \frac{\Sigma A_O}{\Sigma A_W}, \tag{5.4}$$

where:

ΣA_O – surface area of mineral reached by the volume of oxygen required to oxidate the metal sulfide and

ΣA_W – has the same meaning as in Eq. (5.3).

We can write:

$$R_m = k\,(x, y, z, \ldots)\, R_W R_p R_O, \tag{5.5}$$

where k (x, y, z) is a coefficient which accounts for the effect of other influencing factors such as temperature, redox potential, nature of gangue, etc, which are not explicitly mentioned in the present analysis.

Substituting the R_i in Eq. (5.5) with their expressions (5.2), (5.3) and (5.4) we obtain:

$$R_m = k\,(x, y, z, \ldots)\,\frac{\Sigma A_O}{\Sigma A} \tag{5.6}$$

Access of oxygen to the mineral particles is therefore essential for the success of a bioleaching operation.

5.3. BIOLEACHING OF FRAGMENTED ROCK MASSES

The fragmented rock mass, whether it remains in place or is removed from the stopes or pits to form dumps or heaps, must satisfy certain conditions in order to achieve optimum bioleaching efficiency, i.e.:

1) the establishment of the most favourable conditions for microbial growth;

2) total contact of leach solutions, as well as gases and microorganisms, with the crystal lattices of the minerals to be solubilized; i.e. maximization of exposure, percolation and oxygenation ratios;

3) total and ready removal of dissolved metal ions by leach liquors;

4) recovery of pregnant solutions, without losses of dissolved metal values, from the fragmented rock at the same flow-rate at which they are introduced and their collection in appropriately designed and located ponds.

The above conditions are discussed in detail in the sequel.

5.3.1. Establishment of Most Favourable Conditions for Microbial Growth

The commercial applications of microbial leaching seem, up until now, to have concerned the action of sulfur and/or iron oxidizing microorganisms on iron- or base-metal sulfides. As illustrated in Chapter 1, microbial growth is affected by physical and chemical factors.

5.3.1.1. Temperature

Temperature is by far the most important physical factor, though little can be done, in actual commercial operations, to produce variations in its naturally established values. However, it is important, at the feasibility study stage, to evaluate the predictable temperature range existing within the fragmented rock mass.

First of all a distinction must be made between the temperature of the environment where the operation takes place, with its daily and, in particular, seasonal variations, and that inside the fragmented rock mass. As repeatedly demonstrated by the findings of Malouf and Prater [115], Bruynesteyn and Duncan [28] and Bryner et al. [29] the biological oxydation of pyrite and chal-

copyrite by **T. ferrooxidans** attains its optimum at about 35 °C, whereas at 20 °C in certain instances it has been found to be negligible and exhibits a minimum at 65 °C for pyrite and 55 °C for chalcopyrite [135]. Thus it appears that environmental conditions have a marked effect on the biocatalytic activity of the genus **Thiobacillus**, even though published data are still somewhat scanty.

Two situations which are likely to frequently occur in commercial operations, are worthy of careful examination at the feasibility study stage. The first concerns mine sites where winter may last several months, with outside temperatures constantly far below 0 °C. It has been ascertained that even under such conditions in-place and dump bioleaching can be implemented [34, 138, 156]. Stirkov et al [156] report that despite the fact that the ambient winter temperature in a test dump falls to as low as −5 °C and that of the leach solutions distributed in the dump may drop to as little as 2 °C, the temperature of the effluent pregnant liquor is about 11 °C throughout the year. At San Valentino di Predoi, the mine site is characterized by heavy snowfall from October to May, with temperatures often falling to 25 °C below zero in winter and seldom exceeding 15 °C in summer. It has been ascertained that the air temperature in the mine is 8 °C throughout the year, whereas that of the water ranges from 6 to 7 °C [138]. At Denison Mine (Ontario, Canada), it has been found that temperature has a marked effect on iron oxidation rates from pyrite which is reflected in bioleaching efficiency during winter months. However, in this period the temperature never falls below 10–12 °C in the underground stopes [34]. It is reasonable to believe that underground mines are naturally thermostated at temperatures far higher than outside; consequently, in-place leaching can be profitably carried out by recirculating leach liquors which may even warm up due to the heat generated by metal sulfides oxidation. Stirkov et al. [156] attribute the constancy of effluent temperature to the thermostatic action of the ore mass, but the importance of the heat produced by exothermic sulfide oxidation reactions must be given due consideration [156]. The findings of Beck [10] and Brierley [20] in this regard are very significant. Beck [10] measured, in certain points of the dumps at Bingham Canyon, temperatures as high as 60–80 °C and peaks of up to 48 °C in some effluents where the number of **T.ferrooxidans** cells per cubic centimeter of solution was negligible. Brierley [20] reports temperatures of 63 °C in some parts inside the Chino Mine dumps. Jennings et al [86] observed that in their laboratory leaching tests on Copper Mountain copper ore containing some pyrite, bacterial activity appeared extremely temperature-dependent. Consequently, in designing the dumps of a pilot plant, provision was made for heating the leach solutions to at least 21 °C by steam-injection spargers in the pump sumps. When winter temperatures dropped to 4.5–10 °C, the results of the laboratory tests were replicated by a pilot dump, where bacterial activity failed to commence. Solution heating practiced at another test dump throughout leaching, raised the temperature in the pyrite zone to between 18 and 24 °C and successfully initiated bacterial activity. Further, microbial activity did not diminish even during the coldest months, when the temperature inside the dump dropped to an average of about 15.5 °C. Jennings et al [86] calculated that considering pyritic heating sources, a dump 60 m high would be self-sufficient. Although the calculations are not shown, this con-

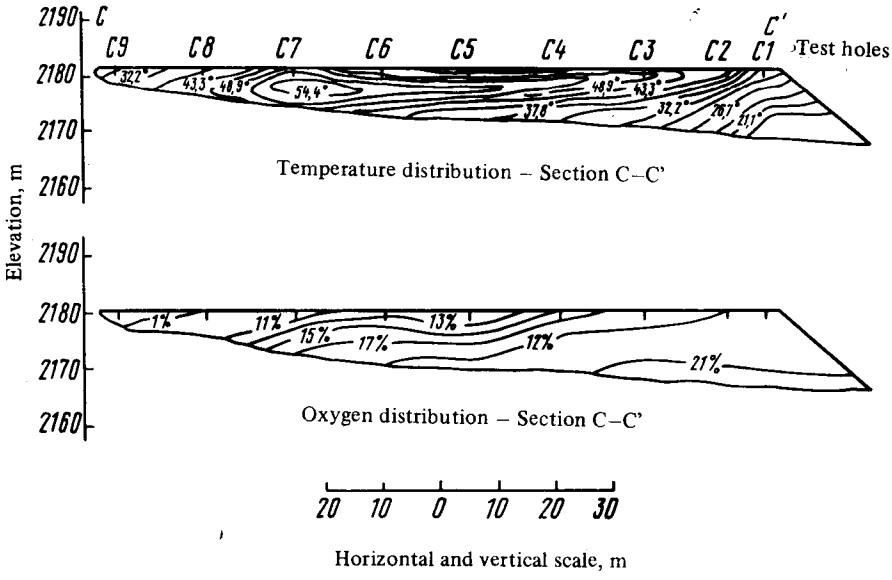


Fig. 5.1. Cross-section through Midas Test Dump, operated by Kennecot's Utah Copper Division. Temperature (^{o}C) and oxygen profiles were measured through concentrically nested casing pipes in holes (C1–C9) drilled into dump

clusion is of paramount importance since it proves that the height of the fragmented rock mass is a critical geometric parameter for estimating self-heating capability.

In conclusion, at the given physical and chemical properties of a fragmented rock mass, a critical height does exist, below which self-heating probably cannot take place.

Cathles and Apps [38] conducted a major investigation on temperature evolution within a commercial test dump (see Fig. 5.1 for an example of temperature profiles). Assuming only vertical air convection, they developed a finite difference one-dimensional model of the leaching history inside the dump, based on the shrinking core model mixed kinetic which, after suitable calibration, matched fairly well the leaching history of the test dump. The authors confirmed, on rational grounds, earlier fragmented data and observations, by arranging them in an orderly and organic framework and it emerged that:

1) air convection plays an important role in the fragmented rock mass leaching process;

2) fragmented rock mass zones where exothermic oxidation reactions take place become heat sources; thus, over time, the dumps heat and any leaching model must account for the temperature dependence of the leaching process;

3) fragmented rock mass height and permeability and lixiviant application rate decisively affect the metal value leaching rate.

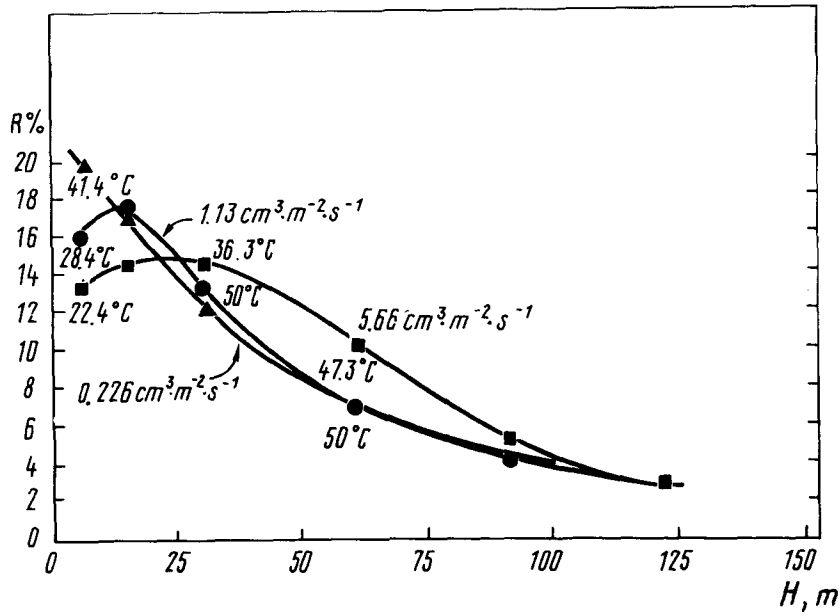


Fig. 5.2. Leach rate (R) as a function of dump depth (H) for various irrigation rates. Optimum dump height is 12 to 21 m for conditions at most operations. Higher irrigation rates, although detrimental for leaching of small dumps, can be beneficial for dumps about 23 m high

A comprehensive review of the finite difference model, which includes air convection, heat balance, temperature-dependent mixed-oxidation kinetics and bacterial catalysis, along with the testing process, is given by Cathles [37]. In a subsequent paper, Cathles and Schlitt [40] developed a more sophisticated model that allows for horizontal as well as vertical air flows. Based on these findings, the authors deduced an optimum height for the fragmented rock mass, shown in Fig. 5.2, ranging from 15 to 30 m, thus supporting Jennings et al's claim [86]. The model also led to the formulation of some practical recommendations for dumps over 60 m high; for example, permeability should be maximum and irrigation more intensive than in lower dumps and possibly increased in the hot zones.

5.3.1.2. Microorganisms

Bryner et al. [29], Beck [10] and other authors [82, 89, 90, 91, 161] observed that copper leaching resumed at temperatures above 50 °C. This is to be attributed, in their opinion, to the fact that chemical leaching is favored at higher temperatures and thus prevails over biological leaching. The discovery of thermophilic iron-oxidizing bacteria capable of oxidizing metal sulfides above 50 °C, observed by several researchers, in high temperature zones of dumps

[21, 24, 71], thermal springs [22, 23, 66] and complex sulfide orebodies [22, 66, 90], however, called in question this explanation.

With a view to elucidating this situation, large-column tests were conducted in order to simulate bioleaching processes in elements or unit dumps. Large columns were equipped for continuous temperature monitoring to enable temperature profiles of several zones inside the fragmented rock mass to be plotted. From these tests it emerged that temperature steadily rose up to 55 ^{o}C, accompanied by a decline in the **T.ferrooxidans** population, a decrease in pH and in increase in oxygen consumption, copper content of the pregnant liquor and Fe^{2+} to Fe^{3+} conversion [39, 123, 126]. According to the authors these findings may well prove, indirectly, the existence within the fragmented rock mass, of a thermophilic microorganism responsible for heat production, oxidation and copper solubilization. In addition to catalyzing the exothermic ore-oxidation reactions, it may also control the reaction rate by adjusting the temperature to optimize catalysis efficiency, as a result of metabolic optimization. Such a "feedback mechanism" may explain the observed constancy of temperature. However, we cannot conclude that thermophilic microorganisms alone are favored within the dump. As Ehrlich and Fox [57], Groudev et al. [71] and Goodman et al. [68] point out, the microflora of a commercial operation is characterized by considerable variety, as can be seen from Table 5.1, referring to the dumps of the Vlaikov Vrah Mine in Bulgaria [71] and Tables 5.2 and 5.3 referring to the San Valentino di Predoi mine in Italy [138]. Most likely, the existence of temperature gradients inside the fragmented rock masses allows the formation of multiple ecological niches. The latter permit the simultaneous subsistence and integration and hence cooperation in the bioleaching process of the different species.

The presence of sulfate-reducing microorganisms in the dumps, reported by Groudev et al. [71], should be taken into account in the design of new bioleaching operations of fragmented rock masses. These microorganisms indicate the existence, within the broken rock mass, of a reducing anoxic environment, which hinders the evolution of the bioleaching process. Under these circumstances, chemical precipitation of ions can take place and sulfate-reducing bacteria, such as **D. desulfuricans**, can accelerate the conversion of metal sulfate ions, including copper sulfate, to sulfides. Cooper and Ayala [42] observed this reprecipitation phenomenon on trench walls in the dumps at Cananea.

Widely differentiated microorganisms can either significantly enhance or hinder bioleaching. The studies carried out by Tsuchiya et al [164] and Trivedi and Tsuchiya [162] on microbial mutualism in nickel and copper sulfide bioleaching are of particular interest in this sense. These authors suggest that if microorganisms of the genera **Thiobacillus** and **Beijerinckia** are grown together, the former may fixate carbon dioxide and the latter nitrogen, which are both required even where carbon and nitrogen are absent from the culture media. In short, carbon and nitrogen compounds should be exchanged between the two microorganisms. As proof of this mutualism, the growth of **T. ferrooxidans** is five times greater in the mixed culture than in the isolated one and copper recovery in the mixed culture is more than threefold that of the pure culture. In addition, the reaction kinetics are faster in the mixed culture.

Table 5.1

Examples of microflora in a copper waste dump [71]

Groups of microorganisms	Taxonomic position
Thiobacilli	T. ferrooxidans T. thiooxidans T. thioparus T. denitrificans
Sulfur and phototrophic bacteria	Colourless Green Red
Iron bacteria	Gallionella Leptothrix
Sulfate-reducing bacteria	Desulfovibrio and others
Heterotrophic bacteria	Bacillus Aerobacter Pseudomonas Caulobacter
Molds and yeasts	Cladosporium Penicillium Trichosporon Rhodotorula
Microscopic algae and Protozoa	Ulotrix Euglena Amoeba Eutepia

Table 5.2

Viable counts of T. ferrooxidans and T. thiooxidans in solid and liquid samples collected from different sites in the San Valentino di Predoi mine (Italy)

Bacteria and acidity	Cells per litre in six different samples					
	A	B	C	D	E	F
T. ferrooxidans	10^3	2×10^3	2×10^3	8×10^2	5×10^4	5×10^4
T. thiooxidans	10	10	20	8	10^2	10^2
pH	2.8	3.8	3.6	3.8	n.d.	n.d

n.d. – no data

Viable Counts of Heterotrophic Bacteria in Liquid Samples Collected from Different Parts of the San Valentino di Predoi Mine (Italy)

Microorganism	Colony-forming units per liter in sample			
	A	B	C	D
Pseudomonas spp (maltophila, putida, stutzeri, vesicularis, etc.)	3×10^3	10^6	10^6	10^4
Enterobacter sp.	$< 10^2$	9×10^3	5×10^4	6×10^2
Achromobacter sp.	$< 10^2$	2×10^2	2×10^2	5×10^2
Caulobacter sp.	$< 10^2$	2×10^2	3×10^2	$< 10^2$
Alcaligenes sp.	$< 10^2$	$< 10^2$	$< 10^2$	2×10^2
Bacillus sp.	$< 10^2$	2×10^3	5×10^3	9×10^2
Unidentified gram-negative motile bacteria	5×10^2	7×10^3	7×10^3	10^2
Unidentified gram-positive motile cocci	$<10^2$	$< 10^2$	$< 10^2$	$<10^2$

Fungi were present in all liquid samples and their concentration varied from 70 to 600 CFU/L according to the sample. Anaerobic bacteria were undetectable in all samples.

However, contrasting results are reported by Groudev et al. [71], who observed at least three detrimental effects attributable to the mixed microflora:

1. Competition for available oxygen and other useful ions, with precipitation of the latter on the surface of the ore minerals.

2. Utilization of living cells of **T. ferrooxidans** as nutrients by other species: in some sections of the Vlaikov Vrah (Bulgaria) dumps the authors found a particularly high number of amoebas (as much 500 per cm^3) and protozoa that also feed on **T. ferrooxidans.**

3. Inhibition of **T. ferrooxidans** by organic compounds secreted into the ground by other microorganisms, such as **Caulobacter.**

A knowledge of the microflora present in a fragmented rock mass undergoing bioleaching may therefore be important in determining bioleaching efficiency. Prediction and manipulation of the microflora composition of a dump are somewhat difficult where experimental data are lacking. However, as Groudev et al. [71] point out, such modifications can be accomplished during industrial operation in a number of ways, for instance by pH or Eh control of the lixiviants, modification of lixiviant composition, or breeding of microbial strains in the laboratory using genetic bioengineering techniques. These genetic modifications should improve both the bioleaching capabilities of the microorganisms and their adaptation to the environment, resulting in the gradual replacement of "wild" **T. ferrooxidans** strains.

Finally, it can happen that some microorganisms subtract part or all of the metal values from the pregnant liquors: this phenomenon has been observed by Campbell et al. [34] in a stope of the Denison Mine, where fungi were adsorbing uranium resulting in its disappearance from the pregnant solutions.

5.3.1.3. Nutrients and Toxic Elements

It is very well known from general microbiology that microorganisms need several chemical elements for their growth. The majority of these have been identified for **T. ferrooxidans** too and are comprised in the composition of the now classical media currently employed in bacterial leaching tests, such as, for instance, Leathen's medium [103] and Silverman and Lundgren 9K medium [151]. Despite being commonly used by researchers all over the world, the composition of such media does not always appear to correspond to optimum growth conditions [117, 153]. Furthermore, as Silverman and Lundgren have already ascertained [151] the basal salts of the culture media may become toxic when they exceed a certain concentration, which in certain cases may even be the same as that of the well-known 9K medium.

Sometimes the concentration of certain salts in the leach solutions is strongly affected by chemicals used in mining: a typical instance is the high concentration of nitrogen compounds found at Denison [34] resulting from the use of ammonium nitrate-based explosives in stope development.

An exhaustive investigation of the waters to be used in preparing leach liquors and the constant monitoring of the latter, in parallel with laboratory testing of the microbial strains used in the mine, is therefore strongly recommended and should enable the preparation of the most suitable nutrient content. Checks of this kind should also allow to detect the presence of toxic compounds or elements.

5.3.1.4. Aeration

A necessary prerequisite for the success of bioleaching is efficient aeration, by diffusion, of the fragmented rock masses.

Furthermore, as already mentioned, at Cananea and Gaspe it was observed that a predominantly reducing environment may flourish readily inside the fragmented rock masses. Copper is precipitated from the pregnant solutions, a drawback that can be obviated if all the zones, even the remotest, are thoroughly aerated. The alternation of wet and dry periods is advisable (as will be discussed in more detail in the sequel).

The access of oxygen to spots in the fragmented rock mass where oxidation reactions take place is guaranteed via two transport mechanisms: either by diffusion through the interconnected intergranular voids (which, as Harris [75] points out, can be considered as the pores of a giant pseudo-particle) or by dissolution in the leach liquor.

Now, the maximum solubility of O_2 in water at 35 ^{o}C is 8 $g \cdot m^{-3}$, corresponding to 0.25 $mole \cdot m^{-3}$; the moles of Cu liberated from chalcopyrite per mole of O_2 are less than 2:8.5 = 0.23523, corresponding to 14.95 g of Cu. Thus

the weight of Cu liberated by 8 mg of O_2 is lower than 0.0037 g [75]. Hence, if all dissolved O_2 in the leach liquor were utilized for chalcopyrite oxidation, then the concentration of copper in solution in one pass should not exceed 0.0037 $kg \cdot m^{-3}$.

Since experience has shown that the concentration of copper in solutions naturally flowing out of leach dumps is at least two orders of magnitude higher than the figure given above, obviously a greater amount of oxygen than that dissolved in the leach solutions must reach the oxidation spots and this can only happen by diffusion throughout the fragmented rock mass. Diffusion is therefore the major mechanism of oxygen transport inside the fragmented rock mass. Auck and Wadsworth [4] recognized the practical importance of the drainage rate for the satisfactory diffusion of oxygen throughout the coarse ore mass, and advanced the concept of Limiting Drainage Rate (LDR), i.e., the drainage rate of the leach solution from the fragmented rock mass, where conditions of the rock fragments ensure that oxygen up-take per unit surface is the same for every particle-size class. This concept is significant because the same amount of time is required for both LDR and the above condition to be reached. Its understanding is therefore vital if an adequate distribution over time of the various phases of coarse rock mass wetting and drainage is to be ensured, since under dry conditions aeration is improved, even if somewhat inconsistently. The importance of adequate aeration is visible in the design of some dumps. For example, at Butte, Montana (U.S.A.), in the so-called "finger dumps", the air at 0.283 $m^3 \cdot s^{-1}$ compressed at 828 kPa was forced [137] through five PVC 100 mm injection lines with 1.6 mm holes at 750 mm centers. Brimhall and Wadsworth [25] found, in the absence of microorganisms, that the amount of copper liberated during leaching of low-grade porphyry ores is directly proportional to the amount of oxygen reacting with the ore. In addition, their findings confirm the importance of wetting and rest cycles, which had long been suspected [79]. The major role played by adequate dump aeration in bioleaching efficiency has also been stressed by several other researchers, including Mrost and Lloyd [122], Stirkov et al. [156], Bhappu et al. [12], Ritchie and Harries [74], Ritchie [73, 136] and Karavaiko et al. [102].

5.3.2 Contact of Fluids and Microorganisms with Mineral Surfaces

In Section 5.2 it was shown that bioleaching efficiency is proportional to exposure, percolation and oxygenation ratios. Therefore, in a properly designed bioleaching operation of a fragmented rock mass, conditions must be such that these three parameters are maximized.

5.3.2.1. Factors Affecting Exposure Ratio

Ore minerals usually occur in the host rock (gangue) in the form of veinlets, clusters and dispersions. In primary ores the latter form is by far the most common. In secondary sulfides (enrichment-zone sulfides) besides dispersions, veinlet-like formations are often found, which stem from the redeposition of

sulfides in natural planar cracks, termed "fissures", of the host rock from downward percolating solutions. Clusters are only frequent in the richest zones of virgin orebodies.

All the dispersed ore particles are accessible to leach solutions if at least one of the following conditions is satisfied:

– either the rock contains so many interconnected cavities and natural fissures that all the ore particles at least partially emerge on the surfaces of the cavities, or

– the leach solutions react with the rock barriers separating adjacent ore particles, dissolving them.

Porosity. A wealth of valuable information on the natural exposure ratio of rock can be gained from the knowledge of its porosity.

Rock pores are interstitial openings between grains capable of absorbing water. Porosity of a rock, denoted with the dimensionless quantity n, is a fraction expressing the proportion of the aggregate volume of pore space to total volume of the rock [136]:

$$n = \frac{V_p}{V_t}, \tag{5.7}$$

where:

V_p – volume of pores in V_t,

V_t – total volume of rock particle.

In rock, porosity varies over a very wide range. It can be almost zero in hard rocks such as fresh granite, diabase, gabbro and marble, rising to 40 % or more in certain limestones and tuffs and as much as 52.94 % , the maximum [8] in loose granular soils, whereas for an average sandstone 15 % is a typical value [59, 67]. Volcanic rocks can also exhibit very high porosity, due to the preservation of the sites of volcanic gas bubbles; in volcanic rocks, the system of pores is not always well connected [67].

The porosity of fresh igneous rocks seldom exceeds 1–2 %, but as weathering progresses, porosity tends to increase to 20 % or more.

However, only microscopic and mineralogical analyses of significant rock samples can provide the information needed to evaluate the natural exposure ratio and the possibility of increasing it (resorting to artificial weathering by means of acid leach solutions) and to determine the grain-size distribution required of the comminuted rock to achieve the desired exposure ratio.

5.3.2.2. Permeability and Degree of Natural Fissuring

Obviously, except for certain uraniferrous sandstones, the exposure ratio of a rock in place is usually too low to yield economically acceptable leaching efficiencies. For this reason in the majority of cases, the rock must be fragmented at least by blasting with explosives. However, the attainment of a satisfactory exposure ratio is a necessary, but not sufficient condition for the correct performance of a bioleaching process. As shown in Section 5.3.1.4, it is crucial that air and leach liquors reach even the remotest anfractuosities in the frag-

mented rock mass. There are two ways of quantitatively characterizing fragmented rock masses under this aspect: permeability measurements and quantification of the degree of natural fissuring of the rock.

Permeability can be defined as "the ability of rocks to transmit fluids" [141]. Permeability of a rock exists by virtue of its porosity, but a porous rock may not necessarily be permeable. It is not directly proportional to porosity but is dependent on pore size, total amount of pore space and in particular their interconnection. A knowledge of permeability is necessary to gain a knowledge of the degree of interconnection between the pores or fissures. A fragmented rock mass can be considered, according to Harris [75] – who was one of the first to develop a model for this type of bioleaching – as either an aggregate of incoherent, more or less porous particles having their own size distribution (corresponding to the "particulate condition") or a single giant "pseudo-particle" having its own complex network of pores. Therefore two porosities have to be taken into account: the porosity of the individual particles and that of the giant "pseudo-particle". Similarly, permeability will be different depending on whether an individual particle or the whole fragmented rock mass is considered.

The rate of discharge flow of a fluid through the pore passages is given by Darcy's law, which most rocks and, it is reasonable to assume, fragmented rock masses, obey. Two analytical expressions of this law have been proposed. The first, applicable in the case where the fluid involved is water at constant temperature (about 20 ^{o}C) has the form:

$$q_x = K \frac{dh}{dx} A, \tag{5.8}$$

where:

q_x – flow rate ($L^3 \cdot T^{-1}$) in the x direction
h – hydraulic head with dimension (L)
A – cross-section area normal to x (dimension L^2)
K – permeability coefficient having dimensions of velocity (e.g. metres or centimetres per second).

When the temperature varies considerably from 20 ^{o}C, or when other fluids are to be considered, Darcy's law can be written:

$$q_x = \frac{K}{\mu} \frac{dp}{dx} A, \tag{5.9}$$

in which p is the fluid pressure (equal to $g \cdot \rho \cdot h$) with dimensions of $F \cdot L^{-2} (kg \cdot m^{-2})$, and μ and ρ are viscosity (with dimensions $F \cdot L^{-2} \cdot T$) ($kg \cdot m^{-2} \cdot h$) and density of the permeant respectively and

g – the acceleration of gravity.

For water at 20 ^{o}C, $\mu = 1.005 \cdot 10^{-3}$ N·s·m^{-2}, $\rho = 1$ kg·m^{-3}, $g = 9.81$ m·s^{-2}.

When Darcy's law takes this form, the permeability coefficient K is independent of the fluid properties. Its dimensions are those of area (e.g. square meters or square centimeters). Before the introduction of the International System of Units (SI) [60, 165] permeability was commonly expressed by the Darcy unit:

1 Darcy equals $9.87 \cdot 10^{-9}$ cm^2. Table 5.4, taken from [136], gives typical values of permeabilities calculated for water at 20 °C; 1 Darcy corresponds approximately to 10^{-3} $cm \cdot s^{-1}$.

From Eqs. (5.8) and (5.9) we get:

$$k = \frac{\rho \cdot g}{\mu} K \tag{5.10}$$

In mining, k is called the coefficient of percolation and is used more frequently than K. For water at 20 °C we can write:

$$k_{20\,^oC} = \frac{1.00 \cdot 9.81}{1.005 \cdot 10^{-3}} K_{20\,^oC} = 9761.2 \cdot K_{20\,^oC} \tag{5.11}$$

Rocks can be classified according to their coefficient of percolation into impervious ($k < 1.16 \cdot 10^{-6}$ $m \cdot s^{-1}$), weakly permeable ($1.16 \cdot 10^{-6} < k < 116 \cdot 10^{-6}$ $m \cdot s^{-1}$), moderately permeable ($116 \cdot 10^{-6} < k < 5.8 \cdot 10^{-3}$ $m \cdot s^{-1}$) and highly permeable ($k > 11.6 \cdot 10^{-3}$ $m \cdot s^{-1}$) [59]

Table 5.4

Permeabilities of Typical Rocks (from [136])

Rocks	K ($cm \cdot s^{-1}$) for rocks with water (20 °C) as permeant	
	Laboratory	Field
Sandstone	3.10^{-3} to 8.10^{-8}	1.10^{-3} to 3.10^{-8}
Shale	10^{-9} to 5.10^{-13}	10^{-8} to 10^{-11}
Limestone	10^{-5} to 10^{-13}	10^{-3} to 10^{-7}
Basalt	10^{-12}	10^{-2} to 10^{-7}
Granite	10^{-7} to 10^{-11}	10^{-4} to 10^{-9}
Schist	10^{-8}	2.10^{-7}
Fissured schist	1.10^{-4} to 3.10^{-4}	

According to Chamberlain [41], the lower coefficient of percolation limit for acceptable leaching is from $2 \cdot 10^{-6}$ to $5 \cdot 10^{-6}$ $m \cdot s^{-1}$.

Permeability tests play a major part in assessing in-situ leaching feasibility of an orebody and its knowledge allows to predict whether leaching fluids and gases will flow naturally through a formation or whether resort must be had to blasting in order to produce a sufficient degree of permeability for successful leaching. Furthermore, the effectiveness of blasts detonated to improve fluid flow in impervious ("tight") formations can be easily judged from permeability data. Permeability may be measured either by means of laboratory tests on specimens of rock, necessarily limited in size, or via field tests. The most suitable field tests fall into three categories: constant head, variable head, and pumping tests [41]. For more details on this important subject the reader should

consult [41, 128]. However one major discrepancy is to be found between permeability values derived from laboratory tests and those measured in field tests. The reason for this discrepancy, some examples of which are shown in Table 5.4, lies usually in the regular sets of open joints and fractures throughout the rock mass. Because of this, laboratory and field determinations should be supplemented by the measurement of a parameter suitable for characterizing the degree of natural and/or induced (for instance by blasting) fragmentation.

5.3.2.3. Quantitative Characteristics of the State of Rock Fracturation

In-situ rock masses can be classified for engineering purposes, according to their state of fracturation. An understanding of the quality of a given rock mass is therefore a necessary prerequisite to the accurate design of bioleaching processes applied to rock masses. In this regard, considerable progress has been made worldwide in recent years in the field of rock mass classification on structural grounds. The classifications proposed by Barton et al [7], Louis [111], Wickham et al. [175], Lauffer [102], and Bieniawski [13, 14] can be conveniently adopted. Specifically, in the system proposed by Bieniawski, a numerical parameter is calculated called "Rock Mass Rating" (RMR), that estimates the rock quality using Deere's index "Rock Quality Designation" (RQD) [49, 85], based on the relative amount of fracturation and alteration observed in drill cores. Actually, the RMR seems more suitable for prediction of tunnel support requirements and since its derivation is more complex than that of Deere's index, only the latter will be dealt with in detail here.

The RQD index is most simply calculated as the percentage of the total length of a diamond drill core occurring in intact lengths greater than 0.1 m:

$$RQD = 100 \sum_{i=1}^{i=n} \frac{l_i}{L}, \qquad (5.12)$$

where:

l_i is the length of the i^{th} core length greater than 0.1 m, n is the number of intact lengths greater than 0.1 m and L is the length of borehole over which RQD is required.

Table 5.5

Joint Set Number (J_n)

A. Massive, no or few joints	0.5–1.0
B. One joint set	2
C. One joint set plus random	3
D. Two joint sets	4
E. Two joint sets plus random	6
F. Three joint sets	9
G. Three joint sets plus random	12
H. Four or more joint sets, random, heavily jointed, "sugar cube", etc.	15

If, as Barton et al [7] suggest, together with RQD the joint set number J_n is considered, and the latter is given a numerical rating according to Table 5.5, the ratio RQD/J_n represents the structure of the mass and is a crude measure of block or particle size.

5.3.2.4. Rock Breakage Indices

The natural permeability and exposure ratio of most igneous rocks to which copper sulfides mineralizations are often associated is usually too low to ensure satisfactory access of the leach solutions to the mineral particles. For in-place leaching the rock often needs further fragmentation using explosives, most of it being left in place, whereas for heap leaching fragmentation must be completed in crushing plants. A special case of in-place leaching is represented by bioleaching which is carried out in the remnants of certain stoping operations or even in new mining operations. When the rock is broken by means of blasting followed by crushing, both comminution processes must be carefully designed since their costs may be such as to decisively affect the final decision on project feasibility [83]. When resort is made to explosives the orebody is developed in the traditional way in order to enable access of drilling machines to the blasthole sites. An accurate assessment of such development costs is strongly recommended and they should be analyzed in depth in the feasibility study. As mentioned, rock breakage involving explosives may entail high costs: a detailed study of all the influencing parameters is therefore extremely useful. The two major cost items in rock blasting are borehole drilling and powder factor, i.e. the consumption of explosive per unit volume or mass of blasted rock. Drillability indexes have been drawn up for estimating the incidence of drilling costs. One such index, widely used is the drillability scale originally developed by Protodyakonov [132, 141] called the coefficient of rock strength. Semi-empirical methods of borehole geometry and round patterns are described in detail in the manuals cited in the bibliography [35, 64, 101, 130]. In the choice of explosive the acoustic impedances of the rock (product of density times velocity of sound) and of the explosive (product of density times detonation velocity), which should be as close as possible, must be taken into account. A relevant example of the correct blast design for preparation of an underground bioleaching operation is given in a report on the Agnew Lake Mine project. The desired grain size distribution of the fragmented rock mass was achieved through the combination of correct borehole geometry (spacing and diameter) and the use of the most suitable explosives [100]. Table 5.6 shows the blast design parameters at Agnew Lake. The discrepancy between measured and theoretical impedance values is to be attributed chiefly to the fact that conditions of the explosive in the blasthole differ considerably from theoretical ones.

The power factor has been satisfactorily used in a computer model developed to calculate fragmentation costs for in-situ leaching operations as a function of blast design and deposit parameters [143]. In the most general case, whereby the orebody is covered by an overburden of thickness x, and ore zone thickness y is known, it has been suggested [143] that the powder factor increases with the depth of the ore zone according to the equation:

Table 5.6

Characteristics of the Rock of Agnew Lake Mine and of the Explosives Used for their Blasting (after [100])

Explosive	Density, $kg \cdot m^{-3}$	Velocity of detonation, $m \cdot s^{-1}$	Explosive pressure, MPa	Pressure transmitted to the rock, MPa	Brizanyte energy utilized, %	Acoustic impedance, $kg \cdot m^{-2} \cdot s^{-1} \times 10$	
						calculated	measured
Nitrogel*	1000	4960	271	455	53	4.96	2.0
Hydromex M-210**	1520	6640	631	970	70	10.09	3.0
Powermex 500***	1200	4000	400	650	61	4.80	2.43
Powermex 300***	1200	4000	490	650	62	4.80	2.15
Rock	n.s.	–	–	–	–	–	10.60

* Nitrogel is an explosive containing TNT [63].

** Hydromex M-210 is a TNT slurry explosive [63].

*** Powermex 500 and 300 are two cap-sensitive slurry explosives [63, 100].

n.s. – not specified.

$$P = \alpha + \beta (x + y), \tag{5.13}$$

where:

α and β are user-specified parameters. It should be pointed out that all such parameters are empirical and can be determined from the factors appearing in their analytical expressions (like α and β in Eq. (5.13) by resorting to field tests – carried out according to standard procedures described in the bibliography – or by relying on previous experience with the same type of rock.

As a rule, in dump leaching fragmentation costs are nil since the rock composing the dumps is, by definition, the "waste" overburden of the orebody. This overburden has to be removed in order to make the ore accessible, and its blasting is therefore a mining cost. After all, bioleaching is, in this case, nothing else than a "scavenging" operation which does not entail any prerequisites as far as rock size is concerned.

A quite different situation arises in heap bioleaching. In this case, in fact, the bioleaching operation is a true metal extraction process carried out with biochemical means on the run-of-mine ore and which replaces conventional mineral dressing techniques. Therefore, the pursuit of the highest possible bioleaching efficiency and, hence, exposure ratio, is imperative. Usually, the fragmentation produced by blasting is not sufficient to achieve acceptable exposure ratios and the run-of-mine ore has to be further comminuted in crushing plants located in the vicinity of the mine site. It should also be pointed out that crushing usually generates fines (–3 mm size fraction, in the case of heap leaching), whose proportion in the crushed product may attain, depending on the rock characteristics, relatively high values. As will be discussed in more detail in Section 5.3.2.5, the presence of high proportions of fines can have a detrimental effect on percolation ratio and, possibly, metal extraction. Consequently, it may be advantageous to separate the finer sizes by screening and to extract the metal contained therein by other methods (for instance vat leaching or flotation followed by pyrometallurgy of the concentrates). Under such conditions, crushing and screening costs must be included in the cost analysis of the bioleaching operation as a whole. The experience gained in this field means that screening costs can be estimated with a sufficiently high degree of accuracy. The evaluation of the energy required to crush a given rock from one grain-size distribution to another is not as straightforward and no suitable theoretical procedure has yet been developed. However, a semi-empirical procedure developed in the early 1950's by the American technologist F.C. Bond [15] yields fairly accurate predictions of the energy required to crush a given rock. This procedure is based, practically, on the determination – by means of a purpose-designed laboratory ball or rod mill, operating according to prescribed guidelines – of the so-called "Work Index" (W_i), the energy in kW·h required to comminute 1 short ton (907 kg) from theoretically infinite size to 80 % by weight passing the screen with square openings of 100 μm.

The energy, expressed in kW·h per short ton of rock, required to comminute a feed 80 % of which passing the F-micrometers square openings of a screen to a product 80 % of which passing the f-micrometers square openings of another screen, is obtained from the equation:

$$E = 10\,W_i\left(\frac{1}{\sqrt{f}} - \frac{1}{\sqrt{F}}\right) \qquad (5.14)$$

5.3.2.5. Flow Path Distribution Inside a Fragmented Rock Mass and Some Influencing Factors

The discussion in the previous Sections highlights the marked effect rock fragmentation methods, grain size distribution within the fragmented rock mass (the fines, by filling the voids between the coarser particles, may shift the process towards pseudo-particulate leaching) and its evolution over time (for instance, weathering of the gangue results in production of slimes), can have on process efficiency. In particular, the channeling of leach solutions can impair bioleaching performance, since certain zones in the rock mass may be isolated from the liquors.

On the basis of the above considerations and the data reported by Howard [78], concerning the Kennecott's Chino Mine in New Mexico, Roman [139] suggested using the distribution of the distances between preferential solution flow paths to develop models of the leaching processes taking place within a fragmented rock mass. Howard's very interesting data were obtained with the so-called γ-γ-exploration method, in which γ-rays emitted by a source inside a well drilled through the dump, bombard the material forming its walls and are reflected by collisions with the atoms of this material. The number of reflected γ-rays is statistically related to the density of the atoms of the material surrounding the source. Given the emission intensity of the source, the distance between source and detector and the wall geometry, the number of γ-rays reaching the detector is proportional to the density of the material surrounding the well. Analysis of the data gathered using this method has revealed two density classes in the investigated dumps. If, as is very likely, most of the solution flow occurs in those zones of the dump having lower density, in preferential flow paths, the γ-γ-plot also gives the distribution of their centers. The ratio between the indices characterizing the center line distribution and particle-size distribution of the fragmented rock mass, which for the Chino dump was 30:1, was successfully used by Harris in his laboratory tests with leaching columns. This ratio is proposed as a design criterion for fragmented rock masses, especially dumps and heaps, where lower grades of rock to be leached necessitate a more efficient leaching process.

Similar investigations conducted by Daniel et al. [47] on the dumps at Rum Jungle, Australia, demonstrated that, due to the different meteoric water flow regimes present, density varies significantly, from 1150 to 2000 $kg \cdot m^{-3}$. It also emerged that liquids have a long retention time and that during the dry season between 0.1 and 1.5 m of the surface layers dry up.

The problem of non-homogeneous flow rates through the fragmented rock masses has been studied by Naismith and Kirchner [127] on a pilot plant scale. The flow regime generated within the model dump appeared to depend on the flow rate per unit surface. It can be stated that generally for low flow rates ($< 300 \cdot 10^{-6}$ $m \cdot s^{-1}$) flow distribution, a function of flow rate, is erratic. The

solution diverges into narrow rivulets, thus effectively wetting only a limited part of the fragmented rock mass. For higher flow rates ($>500 \cdot 10^{-6}$ m·s^{-1}) the flow distribution pattern is more stable and complete, and moreover, flow rate has a negligible effect on liquor distribution.

Given the relatively little research work conducted on this important topic, no detailed guidelines can be laid down governing rock mass design in terms of bioleaching process efficiency. However research to date does highlight the entity of damage that can result from bad designing. In fact, a heterogeneous flow may cause sizeable volumes of rock to be excluded from the dissolution processes, resulting in metal value recovery losses.

One reaction which has a decisive effect on the leaching process is the oxidation of Fe^{2+} to Fe^{3+}, an active oxidizing agent integral in the dissolution of several copper ores. During leaching, ferrous iron is introduced into or forms within the fragmented rock mass in three ways [176]:

1) recycling the spent solution from the metal extraction plant;
2) reduction of Fe^{3+} during oxidation of sulfides and certain other minerals;
3) dissolution of iron-containing ores.

The conversion of Fe^{2+} to Fe^{3+} is effected mainly by oxygen and bacteria and depends, in practice, on a large number of variables. Ferrous iron is oxidized by oxygen according to the following reaction:

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \qquad (5.15)$$

and, at room temperature, it is governed by the kinetic equation [74]:

$$-\frac{d\ln(Fe^{2+})}{dt} = k_b P_{O_2} + k_t (Fe^{2+}) P_{O_2} \qquad (5.16)$$

where:

k_b and k_t may be functions of the sulfate and hydrogen ion concentrations as well.

The reaction rate is second order with respect to ferrous iron concentration and first order with respect to oxygen concentration or partial pressure. While the rate is pH-dependent, it is not greatly affected by variations of pH below 1.5 [152]: however, above pH 2 in sulfatic solution the reaction rate increases quite rapidly with pH. This conclusion is true for the chemical oxidation process of Fe^{2+}. Ferric ion is, in turn, only slightly soluble in aqueous solutions, less so than ferrous iron. When its quantity is exceeded, due either to the oxidation of Fe^{2+} to additional Fe^{3+} or to a rise in pH, ferric iron hydrolizes according to equation (5.17) and a new equilibrium is reached.

$$Fe^{3+} + 3\,H_2O \leftrightarrows Fe(OH)_3 + 3H^+ \qquad (5.17)$$

In reality, the phenomena are much more numerous and complex and hydroxides or colloidal oxides may form, or more or less complex salts may precipitate. These ferric compounds, which can redissolve slowly, especially following "ageing", may encrust the copper ore surfaces, slowing down their dissolution. Alternatively, they partially or totally obstruct the interstices between the rock fragments and within the rock pores, altering the leach solution's flow regime.

An exhaustive review concerning the equilibria existing in aqueous solutions containing ferric, ferrous and sulfate ions, published by Bhappu et al. [12], emphasizes the importance of leach liquor pH in the formation of precipitates. Since the redox potential of leach solutions inside the fragmented rock masses depends to a great extent on the ferric: ferrous iron ratio ($[Fe^{3+}]/[Fe^{2+}]$), its measurement may be useful in data acquisition. To date, the continuous monitoring of leach solution redox potential has been practiced only in the semi-commercial plant of Vlaikov Vrah, in Bulgaria, as well as at a number of plants in the USSR [71, 89–91].

In a dump at Cananea [42] where, after five years of operation, a trench about 23 m deep was dug, inspection of the trench walls revealed thick layers of copper and iron sulfates and, in particular, of jarosite, goethite, hematite and chalcantite, whose precipitation is aided by impervious clay layers and the predominantly reducing environment in the deeper zones of the fragmented rock mass. Similar conditions were reported by Jennings et al. [86] during the already mentioned bioleaching test carried out in a pilot plant consisting of two 1200-t copper ore dumps.

Obviously, very little can be done to modify the conditions within the existing fragmented rock mass; the only possible remedial actions involve the removal of iron from the solution by precipitation in settling ponds, the partial re-

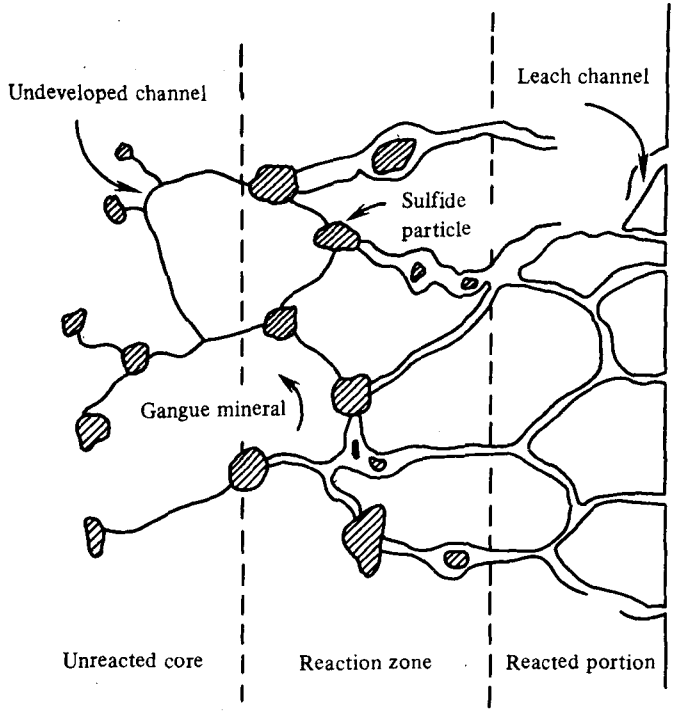


Fig. 5.3. Diagram for channels and reaction for a reacting ore particle

placement of iron-rich solutions with fresh water or, finally, the control of solution pH. The importance of accurate control over both iron content and pH of the leach liquors during commercial operations was pointed out by Auck and Wadsworth [4]. These authors, completing the work begun by Brimhall and Wadsworth [25], identified the physical and chemical parameters involved in the chemical leaching of fragmented rock. Their findings, referred to laboratory tests of two porphyry copper ores, may be considered valid in the general sense for other bioleaching processes. Auch and Wadsworth's investigation focused on extraction rates of copper, iron and gangue components as well as important factors such as acid consumption, penetration rate of leach solutions into the fragmented rock mass, and the influence of fragment particle-size distribution, drainage rate, pH and temperature on oxygen consumption. First they demonstrated substantial differences in extraction kinetics for copper, iron and gangue elements, (magnesium, calcium and aluminium). Copper solubilization should occur as leach solutions penetrate into small channels whose walls are encrusted with basic iron sulfates (Fig. 5.3), connecting the external particle surfaces with the mineral grains dispersed within the rock. The sulfide particles constitute the copper and/or iron sources, while they obstruct the inter-

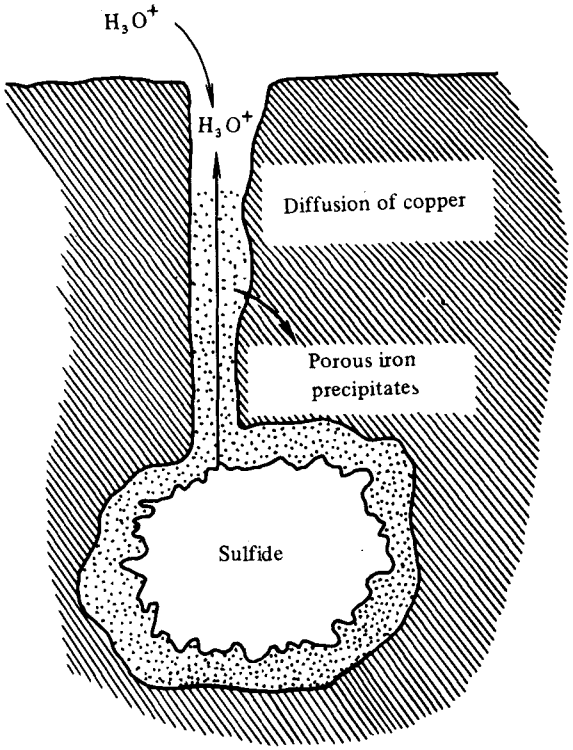


Fig. 5.4. Detail of sulfide particle leaching process

stices, blocking the flow of leach solutions and retarding channel development. In order for leaching to occur, leach solutions must contact the sulfide particle. This can only happen if the leach liquor is sufficiently acidic to solubilize the basic iron precipitate clogging the channel (Fig. 5.4). In this way, the leach liquor is enriched in ferric iron which is then available for the release of copper. The solubilized copper finally diffuses through the porous residual basic precipitate blocking the channel, into the leach liquor, which forms a film of water around the rock fragment containing the channel. However, as soon as the pH exceeds 2.5 or 2.6, the process slows down considerably: the removal of iron salts and their retarded formation at low pH values explain the importance of this parameter in the leaching cycle. The dissolution of the gangue elements seems to take place, by contrast, according to penetration paths of the leach liquors, related to the state of fracturation of the rock. The geometry of this process is entirely different from that of the distribution of the pores leading to the sulfide particles.

In chalcopyrite leaching, an initial decrease of leach liquor pH is observed, which tends to stabilize at around pH 1.9 [18]. This buffering effect, a major feature of the leaching system, produces equilibrium in steady state conditions of H_3O^+-dependent reactions involving both the metal sulfides and the gangue components. Braun et al. [18] stressed the importance of the following buffering reactions associated with chalcopyrite dissolution:

Acid-conserving reactions:

$$CuFeS_2 + \frac{5}{4}O_2 + 5H^+ \rightarrow Cu^{2+} + Fe^{3+} + 2S^o + \frac{5}{2}H_2O \qquad (5.18)$$

$$FeS_2 + \frac{3}{4}O_2 + 3H^+ \rightarrow Fe^{3+} + 2S^o + \frac{3}{2}H_2O \qquad (5.19)$$

$$CaCO_3 + 2H^+ + SO_4^{2-} \rightarrow CaSO_4 + CO_2 + H_2O \qquad (5.20)$$

Acid-producing reactions:

$$S^o + \frac{3}{2}H_2O \rightarrow 3H^+ + SO_4^{2-} \qquad (5.21)$$

$$Fe^{3+} + \frac{3}{2}H_2O \rightarrow 3H^+ + \frac{1}{2}Fe_2O_3 \qquad (5.22)$$

$$Fe^{3+} + \frac{7}{2}H_2O + \frac{2}{3}SO_4^{2-} \rightarrow \frac{5}{4}H^+ + \frac{1}{3}Fe_3(SO_4)_2(OH)_5 2H_2O \qquad (5.23)$$

From equations (5.22) and (5.23) it is clear that hematite and jarosite are formed, the latter much more abundantly, as has frequently been observed in the course of such commercial operations.

5.4. PRELIMINARY TESTS

Prior to designing the actual bioleaching process, a series of determinations must be made and tests carried out in order to assess its feasibility and to establish optimum technico-economical conditions for its implementation.

Some determinations are specific to the method considered, whereas the tests are common to all of them. These tests, called "amenability tests", which aim at evaluating the amenability of the ore to bioleaching and the process parameters (solubilization kinetics and metal values recovery as related to particle size distribution of the broken rock mass, irrigation flow rate and periodicity, optimum temperature, sulfuric acid consumption, nutrient composition) are carried out with the shake-flask, air-lift percolator, column and test-heap techniques, discussed in detail in sections 3.1.2.–3.1.5 of Chapter 3.

The determinations required for all three methods concern the mineralogical and chemical composition of the ore: they are discussed in Section 3.1.1. of Chapter 3. Other determinations, the relevancy of which is restricted to at the most two methods, will be discussed during the description of such methods.

5.5. IN-SITU (IN-PLACE) LEACHING

The depletion of the richest zones of known orebodies has compelled mining companies to search for new orebodies while developing at the same time new mining techniques for those parts of known orebodies containing valuable minerals below the cut-off grades specified by current market conditions.

Cut-off grade is defined as "the grade of material which produces a revenue equal to the production costs through to sales, together with depreciation and the minimum profit" and is used to distinguish ore from waste [76] or as "the determination of the lowest grade that can be extracted at which total profits are maximized" [170]. There are, however, some orebodies where low grades and/or scarcity of reserves do not justify the investments required for their exploitation with conventional technology, and this is where in-place leaching and bioleaching are applicable.

The process is, in principle, quite simple and, as Rouse points out [140], has been occurring naturally for millions of years. In fact, when sulfide ores located in the outcrops were exposed to atmospheric oxygen and water (and probably to microorganisms) acidic products were formed and solubilized. In-place leaching can be carried out in underground stopes, either in virgin orebodies (Fig. 5.5) or in the remnants of conventional stoping methods (Fig. 5.6) which, owing to mixing with caved wall rock and/or to the low metal value grade and/or to the intervening instability of the mine openings, can no longer be profitably and safely mined by conventional means. The two cases do not differ, in principle, in terms of the dissolution process and equipment involved, but the development operations are different. In-place leaching can also be carried out in outcropping orebodies, or parts thereof, as an alternative to conventional open-pit methods.

The following points should be stressed:

1) in-place bioleaching does not differ to a great extent, in principle, from straight chemical in-place leaching, since microorganisms only catalyze or accelerate the chemical process;

2) commercial experience has been limited to the exploitation of those copper and uranium orebodies for which conventional stoping methods were unprofitable;

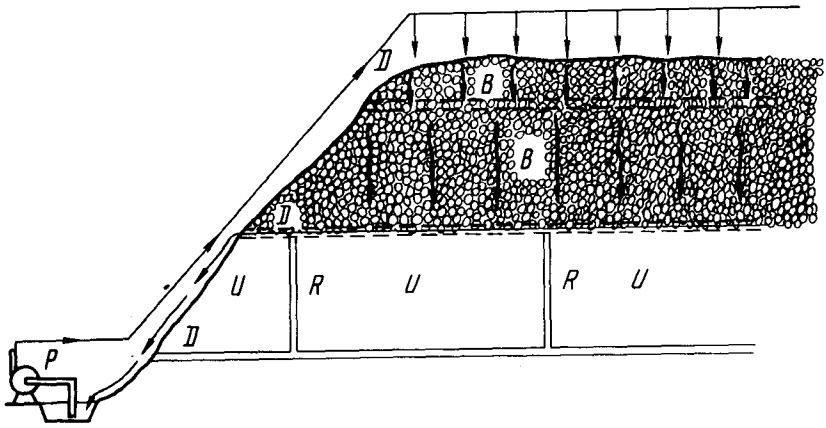


Fig. 5.5. Schematic of in-situ leaching of virgin, vein-shaped orebodies. Longitudinal section:

D – drifts; B – broken ore; U – unmined ore; R – raises; P – pump

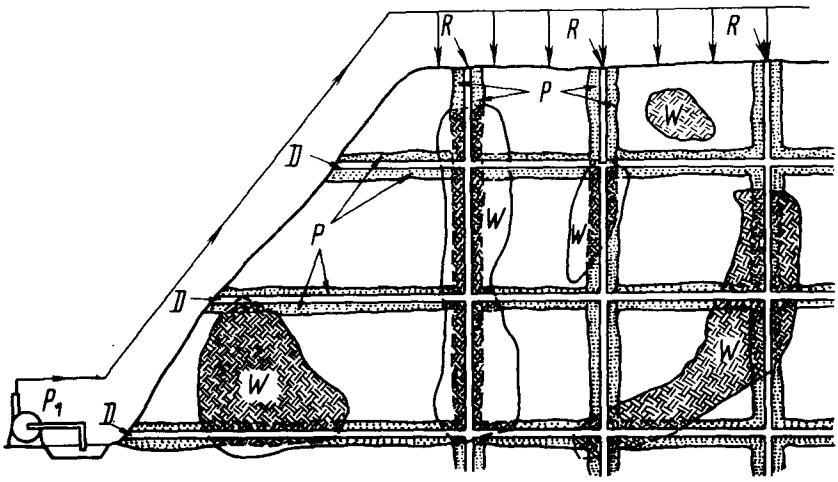


Fig. 5.6. Schematic of in-situ leaching of vein-shaped depleted orebodies. Longitudinal section:

D – drifts; R – raises; P – pillars; W – waste; P_1 – pumps

3) in-place leaching efficiency cannot be accurately predicted since no reliable methods exist for determining the metal-value content and proven ore reserves both before and after the operation.

Following the discovery of **T. ferrooxidans,** several bioleaching tests on copper and uranium were undertaken on an industrial scale in the 1950's.

Copper mines investigated include the Degtyarskii mine in the Soviet Union [155], the Miami mine in Arizona,U.S.A. [62], the Kimbley Pit, Nevada [154], and the Kosaka mine in Japan [158]. More recently two virgin orebodies, Old Reliable, Arizona and Big Mike, Nevada, were fractured by conventional blasting, to increase their permeability to leach solutions [43, 114]. In the early 1980's a successful commercial underground in-place leaching test was carried out at Gunpowder Mine, Queensland, Australia in 55,000 tons of blasted run-of-mine ore composed of chalcocite, chalcopyrite and pyrite with minor amounts of copper sulfides in a host rock of sandstones, arkoses and quartzites [33]. During the first six months of operation, copper recovery was approximately 33 %. Recently pilot tests have been carried on copper oxide orebodies [45], and three underground in-place leaching tests have been set up in European complex sulfide mines: one at Avoca in Ireland, with the financial support of the European Community [32, 167], another – in the depleted complex sulfides mines of Rammelsberg in West Germany [104], and the third – supported by a contribution from the Italian National Research Council, in a chalcopyrite mine in Northern Italy, where, due to the slump in the copper market, conventional mining, milling and flotation operations were suspended in the early 1970's [138]. In-situ bioleaching in two uranium mines, the Stanrock mine, Ontario, Canada [53, 118–120] and the Pitch mine in Gunnison National Forest, Colorado,U.S.A. [116], has yielded several hundred of tons of uranium. Only scanty information is available on the one recorded experiment on a virgin uranium mineral orebody, which was undertaken in the 1970's at Agnew Lake Mine, Canada [100, 143, 145, 168].

All of these tests have led to a better understanding of this technology.

The application of in-situ leaching and bioleaching appears to depend on certain variables related to the geological and topographical features of the orebody, the local meteorologic and hydrologic regimes and to some rock-mechanical properties of the ore and host rock. The geological and topographical features include:

1) location with reference to orography;

2) form and size;

3) lithology and mineralogical composition of the ore and gangue.

Topographical features may in themselves have a marked effect on the economy of the commercial leaching operation. In fact, in order to accede to the stopes of a deep-seated orebody, for instance, at least one shaft is required, properly equipped with hoisting facilities, entailing much higher costs in terms of both investment and operation, than access via adits to a similar orebody located above the topographic ground surface. The combination of topographic and hydrological features, amongst which water table (i.e. the geometric surface below which the ground is saturated – in its simplest form, when the ground is homogeneous and continuous, it is a plane), is a major factor to be taken into consideration in the feasibility study of the in-place leaching project.

The leach solutions are introduced into the orebody by:

1) Percolation "per descensum" or gravity percolation: the leach solutions flow around the particle surfaces without flooding them. This permits the fairly

efficient diffusion of the gaseous phase through the liquid film coating the particles.

2) Flooding the rock mass: the leach solutions may flow either "per descensum" or "per ascensum", but the required aeration may be disturbed in some way. As pointed out by Bruynesteyn [26], when the top of the broken ore mass is inaccessible, as can happen in small stopes filled with ore, this may be the only practicable method. Laboratory tests were conducted in air-lift percolators to evaluate the effect of four percolation regimes involving trickle and flood leaching [133]. It seems that continuous contact of the leach solution with the ore material, through circulation/flooding enhances metal dissolution in percolation. This finding appears to contradict the claim [26] that flooding prevents the access of the required amount of oxygen to the whole broken rock mass. It is quite likely however that this discrepancy is only apparent due to the fact that aeration of the leach liquor in air-lift percolators is different than in the actual broken rock mass in a stope.

3) Surface spraying of the exposed walls of the stopes using water spouts, sprinklers or atomizers.

Clearly, mode 1 may only be adopted in the case of a fragmented (rubblized or hydrofractured) rock mass. Any preexisting adits, shafts or more generally, cavities, should be isolated from the lixiviant flow. If sufficient amounts of ore remain on the exposed surfaces of these cavities, leaching may be done according to option 2. In any case, an accurate hydrologic survey of the lithospheric zone of the orebody will establish the position of the water table and permit an estimate of both ground water pollution potential and pregnant liquor losses.

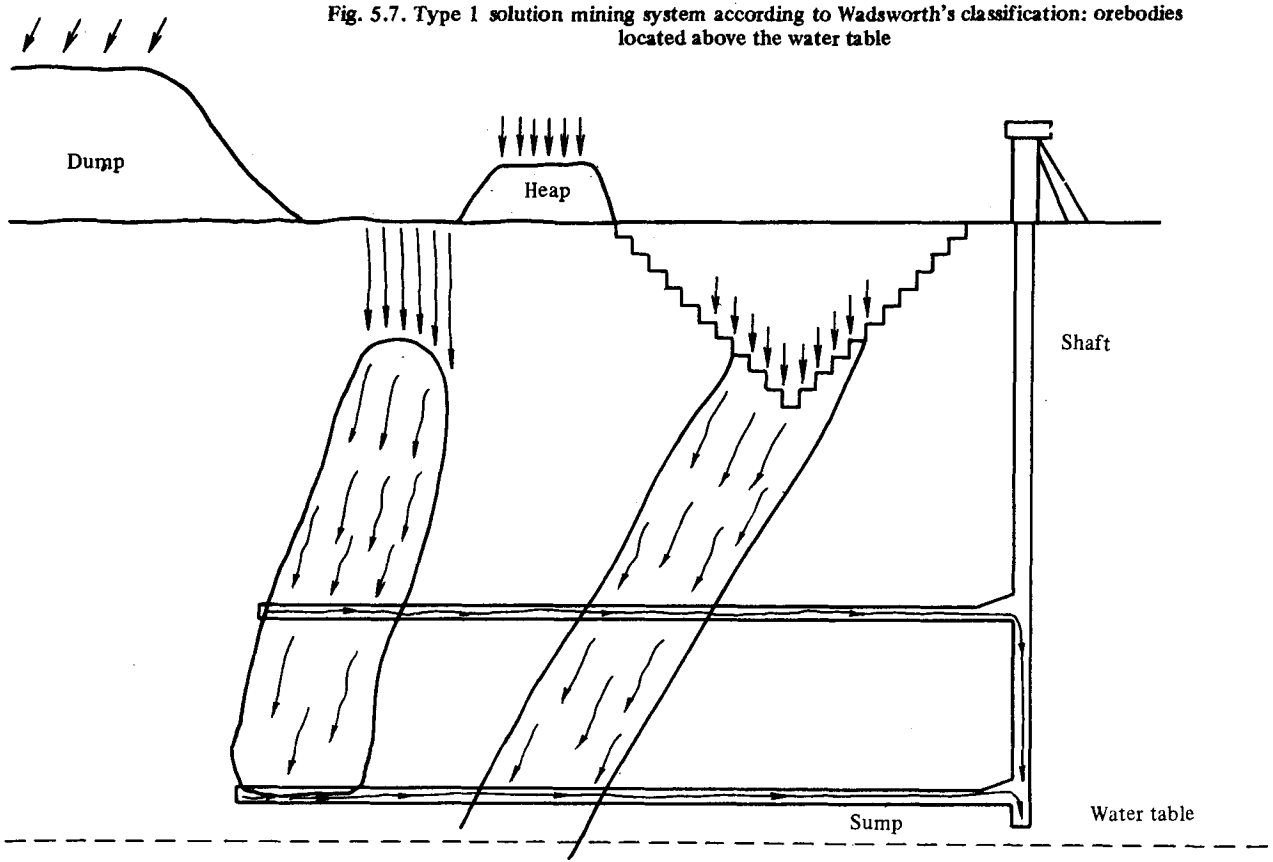
Wadsworth [171] proposes three general types of solution mining systems, based on position with respect to the water table. Orebody portions either outcropping or exposed in the bottom of open pits, as well as deposits within the lithosphere but above the water table, belong to Type 1 (Fig. 5.7). Type 2 (Fig. 5.8) includes those orebodies both partial and whole, located below the natural water table, but not so deep that conventional underground or borehole techniques are economically impracticable. Type 3 (Fig. 5.9) deposits are located so far below the natural water table that conventional mining methods are often uneconomic.

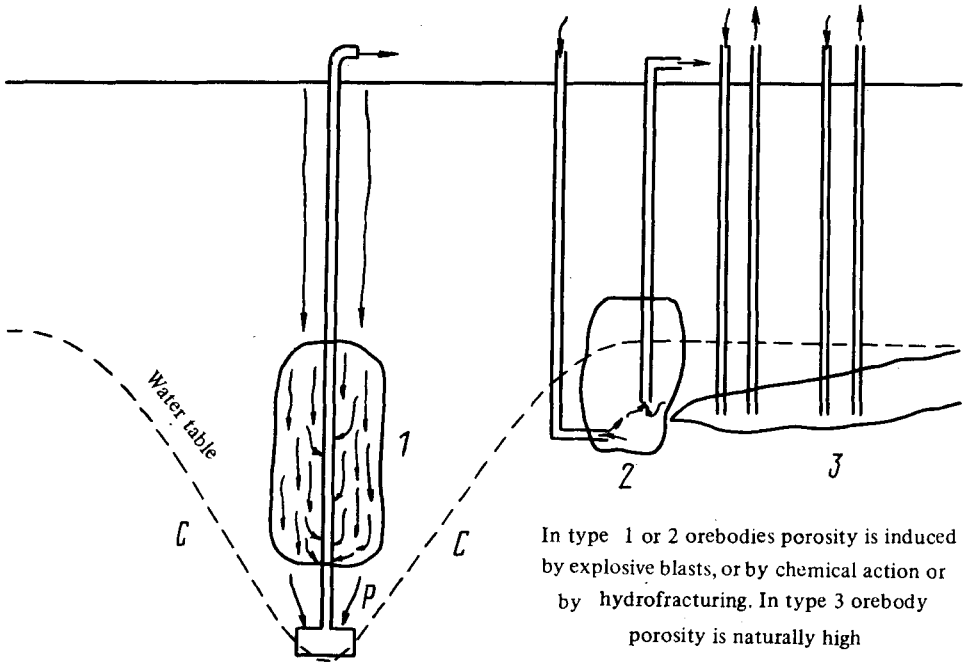
Type 1 deposits can be leached in one of the above ways, and in each case similar hydrological and chemical conditions prevail.

In-situ leaching of Type 2 deposits may require some preliminary development work, such as shaft sinkings or ramp drivage, to excavate adits and drifts. Through these openings, the rock mass is blasted by conventional explosives and thus fragmented to the size most suited to leaching. If miners are working underground, the water table must be artificially lowered by a conventional mining drainage system such as a drainage drift or pump. To prevent dilution, the water table must stay lower than the level of the sump of the pregnant solution reclaiming pump. Seepage of pregnant solutions into the natural water system must be avoided to prevent aquifer pollution and metal value losses.

Moreover, complications may arise when part or all of the water in the natural water table is saline (for instance due to proximity of the sea); the mixing

Fig. 5.7. Type 1 solution mining system according to Wadsworth's classification: orebodies located above the water table





In type 1 or 2 orebodies porosity is induced by explosive blasts, or by chemical action or by hydrofracturing. In type 3 orebody porosity is naturally high

Fig. 5.8. Type 2 solution mining system according to Wadsworth's classification: orebodies located below the water table at moderate depth

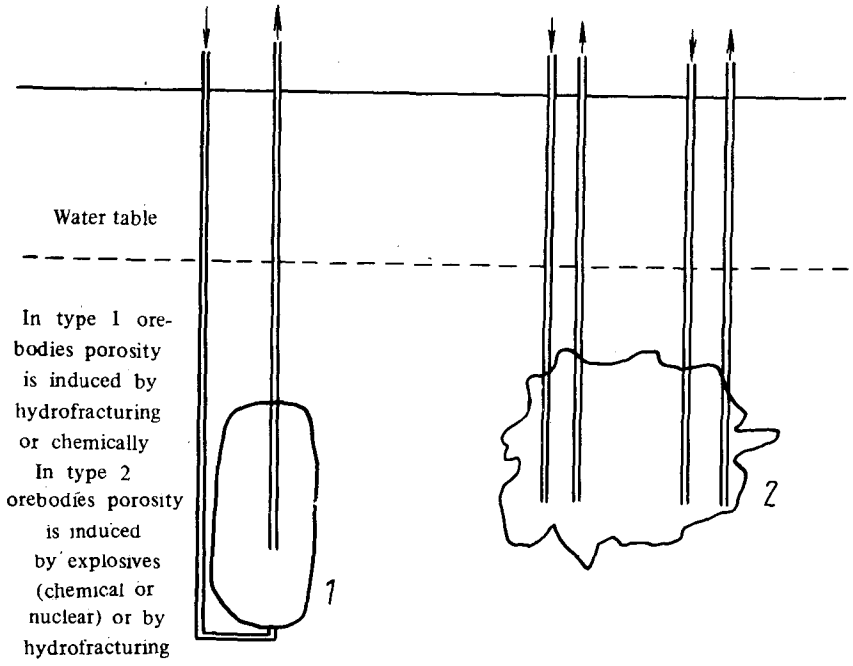


Fig. 5.9. Type 3 solution mining system according to Wadsworth's classification: orebodies located deep below the natural water table

of such water with leach solutions may create environmental conditions which inhibit the growth of the desired microbial flora.

Detailed below are problems encountered in the leaching of Type 3 deposits that have been investigated jointly by industrial as well as scientific organizations.

As far as meteorology is concerned, the temperature of the natural environment may affect both microbial activity and operating costs. There are mines, located in high mountains and/or at high latitudes, where outside temperatures may remain below 0 °C for several months of the year. Under these conditions, bioleaching in open pit mines may even cease during the coldest months, due to freezing of surface waters. In any case, provision should be made for adequate heating of all plants where metal recovery from pregnant solutions is carried out. The case of underground mines may be somewhat different and, in some ways, economically more advantageous. Underground mines are usually natural thermostats and the temperature in the stopes is constant all the year round. During the coldest periods the fresh waters –which enter the mine through its caved outcrops – freeze and their inflow ceases, but in the underground stopes, even a few tens of meters below the outcrops, solution circulation continues undisturbed. In certain instances, especially when there is plenty of room in mined-out stopes and tunnels excavated in strong rock and where ventila-

tion is very effective, this situation may be availed of by using these cavities for installing the metal recovery plant, such as copper cementation launders [138].

Orebody shape and size are also important; the more regular and compact the orebody, the more uniform the contact of the fluid streams with the broken ore mass. A relatively thin vein-shaped orebody, for instance, less than 5 m thick, extending in strike and depth for several hundred meters, will require the installation of more leach solution injection points than will a compact orebody.

The location relative to the orographic surface is of considerable practical importance, as the leach solutions usually circulate downwards. They collect in the deepest accessible part of the orebody and subsequently must be pumped to the surface for the recovery of dissolved metal values.

The deeper the orebody, the higher the hydraulic head the pump has to overcome to circulate the pregnant liquors and hence the higher the incidence of power per unit mass of metal value recovered. The brake power required to deliver Q $m^3 \cdot s^{-1}$ of a liquid with total dynamic head of H meters can be calculated using the equation [99]:

$$P = \frac{Q \cdot H \cdot \rho}{\eta}, \tag{5.24}$$

where:

ρ – density of liquid;

η – pump efficiency.

Total dynamic head consists of the sum of the dynamic suction lift, plus static discharge head, plus velocity head, plus the exit and velocity losses, plus the head necessary to overcome the friction losses in the discharge line.

A rough estimate can be made using for H, in (5.24), the total static head, i.e. the vertical distance from the surface of the pregnant-liquor in the collecting pool to the point of free discharge in discharge piping.

Hence, pumping costs increase with depth. The energy required to pump 1 m^3 of water against a geometric head of 100 m is, theoretically, 0.972 MJ, and practically, about 1.44 MJ [124]. Given that leach solutions outflowing from in-situ dissolution should contain about 3 $kg \cdot m^{-3}$ of copper, it is clear that depth may become an insurmountable economic limitation. At the El Mesquite in-situ uranium leaching operation, which is not bacterially mediated, the leach solutions are already pumped from a depth of 100 m with flow rates ranging from $38 \cdot 10^{-3}$ to $57 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$ [30].

The lithology and mineralogical composition of the ore, as well as the gangue and host rock, affect the success of the operation. The presence of metal sulfides, particularly pyrite, generally indicates environmental conditions favorable to the growth of bacteria **Thiobacillus**, whereas, the presence of molybdenum, which is highly toxic to these microorganisms [125], may inhibit their activity. Carbonate gangues may cause the leach solution pH to increase beyond the optimum point for growth of **Thiobacillus**, which is about 2.35 [112]; above pH 4.0, its growth virtually ceases. Such a detrimental pH can be avoided by acidifying the leach solutions; this procedure, however, may become prohibitively expensive.

Further undesirable consequences may result from the chemical effects of acid leach solutions on some gangue types. The debris in these cases often consists of very fine clays and silt, that may clog the passages between and within the rock fragments, preventing the leach solutions from thoroughly wetting the rock mass. Certain porphyry monzonites and altered granites often cause this problem. Dudas et al. [54] emphasize the serious detrimental effects resulting from the presence of phyllosilicates in the microcracks of the mineralized rock.

Another important factor, as pointed out by Wadsworth [171], is natural in-situ weathering that provides access paths for solution penetration. Weathering can either accelerate or retard dissolution, depending upon the particle sizes involved. Particle fragmentation and clayey slime formation can sometimes result from accelerated leaching.

Tectonics and the mechanical properties of rock also play an important role: since their origin, the orebodies have usually undergone the same tectonic evolution as the lithosphere in which they were formed. Thus, they have been subjected to all the stresses transmitted through the earth's crust. Structural geology teaches that, when a disturbance propagates through a region of the earth's crust, the mechanically weaker (crystal) areas will yield. These failures appear as more or less extended shear surfaces, or "faults", along which relative displacements, or "throws", of the rock masses can occur. These range from a few centimeters to thousands of meters. In addition, true fragmentations or "tectonizations" of the rock subjected to the stress, are observed. Discontinuities in the orebody may interfere with the leaching process: in the case of faults, the slip surfaces may actually become preferential flow paths of the leach solutions as Spedden et al. [154] showed with semi-industrial tests. The leach solutions can then totally bypass adjacent mineralized zones or even disappear in the faults, which then behave like sinkholes, causing irreversible loss of metal values. A similar problem may occur when more or less extended zones of the orebody have undergone strong local fracturing, producing an irregular network of cracks, into which the leach solutions penetrate. The presence of clays within the fractures further exacerbates the situation. On the other hand, as pointed out by Bhappu and Fletcher [11] the success of an in-situ leaching operation depends on the accessibility of the leach solutions to the whole mineralized mass. They cannot interact unless intimate contact is achieved, which requires exposition and accessibility. The latter can be gauged, taking into account several factors, including the degree of dissemination, the volume and the shape, and therefore the specific surface and grain size, mineral value concentrations within the rock matrix and the porosity and permeability of both the ore and country rock. Particularly unfavorable conditions could result from low ore and high country rock porosity. In this case, the leach solutions tend to escape through the country rock before being collected. In conclusion, intact rock is not very amenable to in-situ leaching; however, in considering fractured rock, an understanding of the fracturation geometry is required.

The application of the rock characterization methods discussed in Section 5.3.2.4 affords the most suitable means for achieving the desired bioleaching efficiency.

5.5.1. In-Situ Bioleaching of Depleted Orebodies

The first, and to date, the only commercial in-situ bioleaching attempts furnishing valid operational data, have involved, with the exception of the Gunpowder Mine, the exploitation of orebodies considered depleted by conventional mining standards. The previously-mentioned Dergtyarskii mine, Soviet Union [155], Kosaka and Tsuchihata mines, Japan [158], and Miami mine, Arizona [62], provide examples of the downward percolation technique applied to type I orebodies according to Wadsworth's classification [171]. At Dergtyarskii (Fig. 5.10) the leach solutions were prepared in bacterial regeneration tanks through which air was sparged. The solutions were then pumped, through polythene pipes, to the injection wells, diffusing through to the ore blocks overlying the mined-out stopes and the corresponding adits and drifts. The pregnant solutions were conveyed, by means of gully-drains, to a central sump (10) from which they were pumped to the surface. The pregnant solutions were subsequently conveyed through a limnographic station (11) where microbial concentration was determined, and to clarification tanks (12) where the residual suspended solids were settled, finally reaching the precipitation launders (13) where copper was recovered. The spent solutions were then recycled to the preparation tanks.

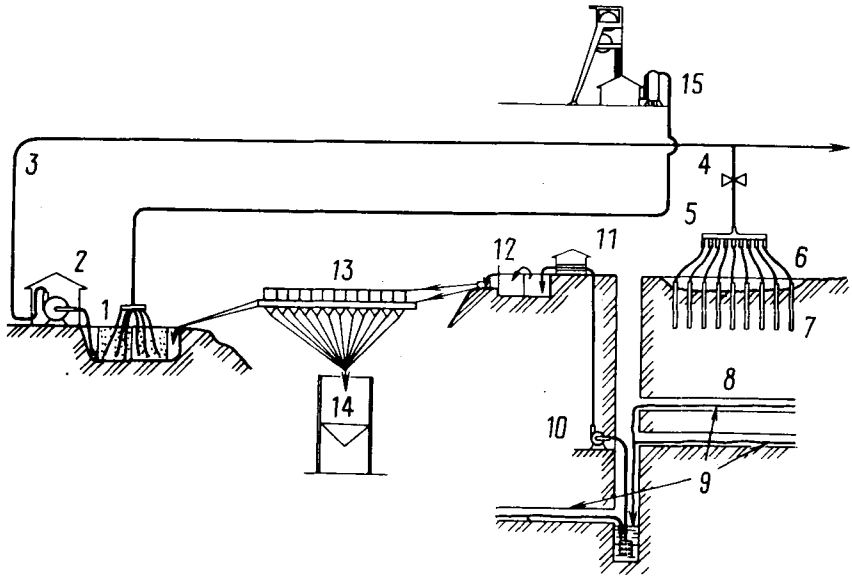


Fig. 5.10. Bioleaching at Degtyarskii mine:
1 – air sparging of recycled solution; 2 – pump station; 3 – solution distribution line; 4 – valve; 5 – solution distribution manifold; 6 – solution lines; 7 – injection wells; 8 – orebody; 9 – drainage gullies; 10 – pregnant solution pump; 11 – limnographic station; 12 – clarification tanks; 13 – precipitation launder; 14 – cement copper bins; 15 – compressor station

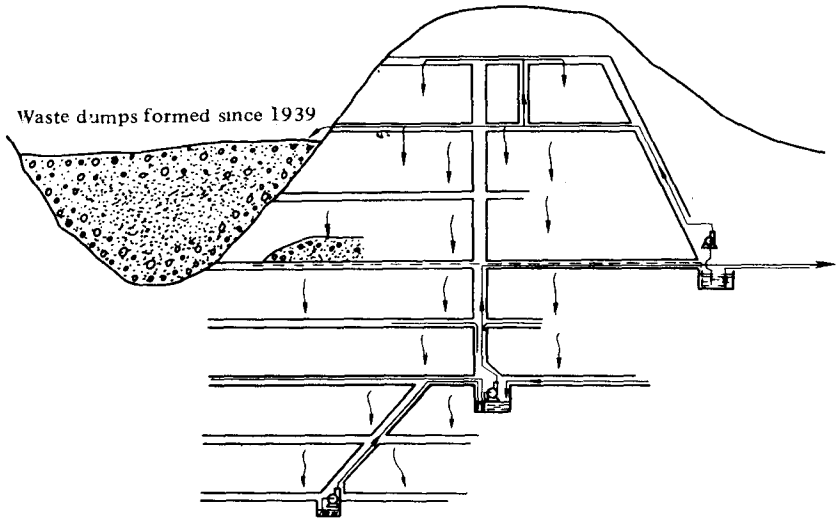


Fig. 5.11. Schematic diagram of bioleaching operations at Motoyama deposit, Kosaka mine

At Kosaka the areas to be leached were first blasted with conventional explosives. Subsequently the lixiviants were injected through boreholes drilled in the walls of the adits and drifts (Fig. 5.11). At Tsuchihata the copper minerals were dispersed as thin veinlets within a tufaceous Tertiary breccia and rhyolite. The rock was very likely porous and permeable, since the lixiviants were introduced into the caved zones at the surface and through the upper drifts. The mine extended about 400 m deep and pregnant liquors were collected in pump sumps located at various levels.

The bioleaching of block-caving remnants has been successfully conducted since 1941 in the Miami mine, in Arizona (USA), where stoping with conventional mining methods was practised between 1910 and 1959. "Block-caving" mining [5] entails isolating ore prisms from the surrounding orebody. Each prism constitutes a "block", and the ore is weakened at its edges by cut-off raises that define it and give a firm pillar. The sides of the blocks can exceed 50 m in length. On occasion, the cut-off raises may have to be interconnected by a shrinkage stope to facilitate breaking the ore at the edges. The base of the block is concurrently prepared by driving a network of drifts and crosscuts to create a support system of ore pillars. When the pillars are blasted, the ore of the block is thus undercut and breaks away under gravity loading. Ideally, the collapse of the ore block is accompanied by the development of cracks, fissures and fractures, producing large blocks of ore. As they move downward, these blocks crush each other, grinding the ore to a size able to pass through the drawpoints (Fig. 5.12). Drawing of the ore stops as soon as its average grade drops below the cut-off grade; however, at this point, the total metal-values content of the residual rock mass may still be considerable.

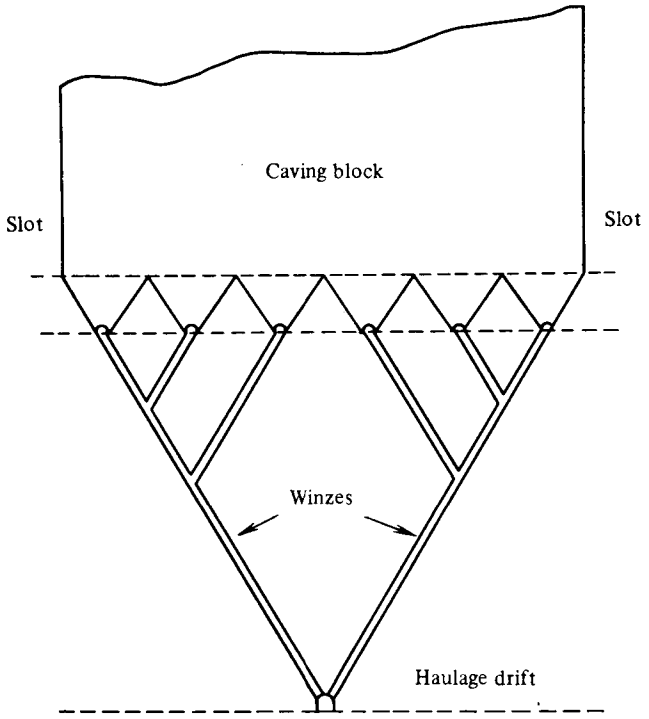


Fig. 5.12. Schematic diagram of block-caving stoping method (vertical section)

Because the rock is intensively fractured and may continue fracturing, the penetration of leach solutions is facilitated.

The copper in the Miami mine orebody, ranging from 0.2 % to 1.0 %, is mainly in the form of oxides, which are very difficult to beneficiate in the mine's concentrator. The leached rock consists of pillars of ore fractured during the block-caving operations, mined ore left between the original block-cave drawpoints, and portions of the orebody originally neglected because of their submarginal grade. Since the country rock is fairly impervious, pregnant liquor losses are very limited; leach solution recovery was 91 % in 1962 and 93 % in 1963 [134], with a copper content of 1.8 $kg \cdot m^{-3}$ and a circulating flow rate of $12.62 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$. The evaporation loss is now about 10 %, having gradually decreased due to increasing rock mass saturation, following the transient initial stages of the process.

In 1977, the leach solutions, either sprayed onto or injected through the surface of the collapsed pit bottom, trickled through 183 m of broken rock, the last 46 m a higher-grade mixture of copper ore and gangue. The leach solutions required three to four weeks to flow from the surface to collecting galleries 300 m deep. The pregnant liquor collected in a reservoir there and was then

pumped to the surface solvent-extraction plant. By January 1970 the Miami mine had produced about 130,000 tons of copper by in-situ leaching [62]. Overall recovery, when the project terminates, is expected to exceed 50 %.

No examples of depleted orebodies leached by flooding are known. Rabb [134] reports on Anaconda Copper Co.'s decision to apply this method, following the successful recovery of copper from the waters that flooded two of its deep mines during a five-month labor strike in 1935.

The Stanrock uranium mine [120], a type-I orebody, is a typical example of leaching by surface spraying of old stopes. This deposit is part of a large flat bed with an average dip of 15^{o}, favoring the slow migration of the leach solution towards the lower areas, where it flows into sumps and is pumped to the surface. Pairs of miners, using 50 mm rubber hoses fitted with tapered fire nozzles, completely washed two mined-out adjacent stopes per shift. The stopes varied from 45 to 75 m in length with an average floor area of 500 to 700 m^2. Stope washing was cyclic rather than continuous; that is, periods of washing were alternated with periods of three to four month "resting periods" during which the microorganisms produced more soluble iron. The mined-out stopes of the Milliken mine were leached in a similar way. Here the deposit is a bed whose dip ranges from 10^{o} to 30^{o}, consisting of a conglomerate of pebbles cemented by a matrix of uranium minerals and from 4 % to 6 % metallic sulfides, primarily iron. Conventional mining ceased in August 1964, when an industrial scale bioleaching experiment was undertaken in 100 mined-out stopes. In just one year 58 tons of uranium were recovered from 356,000 m^3 of leach liquor with an average uranium content of 0.135 $kg \cdot m^{-3}$. The pregnant solutions collected in the mine's lowest level and were pumped to the surface in three stages of about 330 m each, comprising a total geometric head of about 1,000 m

Table 5.7

Uranium recovery by bacterial leaching in the mines of the Elliot Lake (Ontario, Canada) area. Ore: quartz pebbles conglomerate with a pyritized (from 10 % to 15 % pyrite) sericitic matrix; uranium minerals: brannerite, uraninite, coffinite and thucholite.

Name of mine	Manpower engaged in underground leaching operations, N	U_3O_8 production, kg/month	Average underground temperature, ^{o}C	Method of washing of stope walls	Pregnant solution U_3O_8 content, $kg \cdot m^{-3}$	Optimum wetting cycle time, months
Stanrock	6	4 536	18	H.p.w.	0.14 to 0.45	3 to 4
Denison	4	n.s.	n.s.	H.p.w.	0.07	n.s.
Milliken	8	4 800	> 18* 14**	H.p.w.	0.06	3

n.s. – not specified; H.p.w. – high-pressure washing; * – summer time; ** – winter time.

[61]. In 1967 Stanrock Uranium Mines, Ltd., recovered between 6.8 and 7.3 tons of U_3O_8 per month from 1,200 stopes, having an average floor area of 557 m^2 [56]. Bioleaching was successful also in the Denison uranium mine, where the leach solutions were sprayed with lawn sprinklers and 12 stopes were monitored by just one operator [56]. The results obtained in these mines are summarized in Table 5.7.

5.5.2. In-Situ Leaching of Virgin Orebodies

For this type of deposit, the initial development work consists of rendering the ore mass permeable to lixiviants and gases. The necessary fracturing of the rock can be accomplished in several ways, depending on both the rock-mechanical properties of the orebody and the environmental constraints of the local area. The most conventional means entails the application of chemical explosives to well-known stoping methods, such as block-caving or sub-level stoping. In this case, the mine must be developed according to the criteria adopted for conventional mining, to give the drilling and blasting teams access to the whole mineable orebody. Long-hole drilling techniques, which utilize, for instance, down-the-hole (D.T.H.) drilling machines for blastholes with diameters up to 200 mm [98], may contribute decisively to the economic success of the operation.

Rock-mechanical investigations should precede the decision to use this stoping method. In the case of block-caving, for instance, it has become normal practice to estimate the RQD, as well as the "cumulative fracture frequency" curves of the rock, to determine its caving characteristics [77]. Fracture frequency curves are constructed by measuring the lengths of rock core, obtained by drilling, that have specified fracture spacing. The cumulative lengths are then calculated, expressed as a percentage of borehole length, and plotted. The curves, therefore, indicate any structural weakness inherent in the rock weaknesses relevant for caving, as expressed by the so-called "cavability factor".

Rabb [134] reports that the Anaconda Copper Co. developed such a system at one of its South American operations: however, probably only chemical leaching was involved. Half the original orebody, a low-grade water-soluble oxide copper ore, was mined with the conventional block-caving method and was utilized for heap-leaching on the surface. This partial mining initiated such intensive fracturing of the remaining rock that a leaching operation could then begin; in fact, for each ton drawn, an estimated 2.5 tons remained "completely cracked" and 6.5 tons were highly fractured. The pregnant liquors, with seepage losses into the country rock ranging from 10 % to 15 %, were efficiently drained through the network of raises forming the ore-spillage system of the block-caving method. Rabb estimates that 65 % extraction could be feasible within a few years.

In any case, fracturing by explosives for in-situ leaching must not only produce the desired grain size but must also induce maximum cracking in the rock fragments, to facilitate lixiviant penetration.

When the deposit is outcropping with high-grade upper parts, these portions can be mined first using conventional open pit mining methods, followed by the

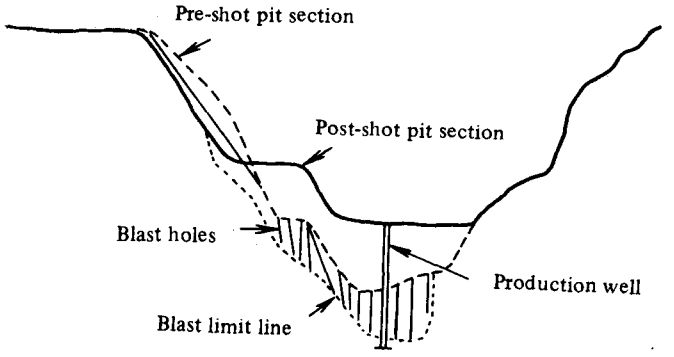


Fig. 5.13. Big Mike Mine: pit cross-section before and after blast

exploitation of submarginal ores by in-situ leaching. This sequence was followed at the Big Mike mine, about 30 miles south of Winnemucca (Fig. 5.13) [172]. After 350,000 tons of waste overburden had been stripped, 100,000 tons of 10 % Cu run-of-mine ore were mined by a conventional open-cast mining method forming a pit on whose slope some 600,000 tons of submarginal ore were left.

The in-situ leaching test was conducted on 500,000 tons of this residue, blasted by means of 6,300 m of 146, 230 and 250 mm blastholes charged with 200 tons of conventional explosives. On the average 330 g of explosives were used per ton of run-of-mine ore. The rock fragments, averaging 230 mm in size, formed a dump on the bottom of the pit. The lixiviants were sprayed over the whole area using sprinklers fed by a network of 50 mm diameter PVC pipes; the leach solutions percolated downward and collected in a centrally-located shaft. A pump, located 50 m deep at the bottom of the shaft, conveyed the pregnant solutions to the surface.

During the first year of operation the flow rate of the pregnant liquor was $12.6 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$ with a copper content of 2.0 $kg \cdot m^{-3}$.

A similar procedure was adopted to exploit the submarginal ores of the Old Reliable mine, Arizona [110, 144]. The deposit was an almost vertical "breccia pipe", containing about four million tons of a 0.8 % chalcocite, chalcopyrite, malachite, calcantite and chrysocolla run-of-mine ore. The country rock contained lavas of Cretaceous age, intruded and mineralized by the so-called "Copper Creek granodiorite". The orebody, which lies at the foot of a ridge, is 180 m long by 90 m wide, and 150 m deep below the outcrops. The deposit was fragmented by blasting 1,800 m of "coyote tunnels" charged with 2,000 tons of conventional ANFO explosive. The coyote tunnels had been prepared on three levels, one at the base of the orebody, the second 30 m higher and the third 50 m above the second. The explosive charges on all levels were detonated simultaneously, but the intermediate level was fired 100 ms after the first and the lowest level was delayed 150 ms beyond the intermediate.

The average ore fragment size ranged from 270 to 300 mm. The blasted area was terraced by crawler tractors, the benches were six meters high and varied from 4.5 to 45 m wide. Centrifugal pumps conveyed the leach solutions through a main 150 mm line from which 50 mm distributor lines branched out. Spray nozzles, attached to the distributor lines at 12 m centers, spread the leach solutions over a 2.3 ha with a flow rate of $69 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$, equivalent to $3.04 \cdot 10^{-6}$ $m^3 \cdot s^{-1} \cdot m^{-2}$. The flow regime was reached within about six months and the pregnant liquor contained 2 $kg \cdot m^3$ of copper. By December 1973 daily copper production was 10 tons.

The problem of in-situ leaching of a virgin orebody is addressed in three papers published by D'Andrea et al. [43, 44, 45] reporting details of preliminary investigations concerning ore characteristics, the project and the role of conventional explosive blasting, as well as an assessment of the fracturing produced.

The Emerald Isle mine, operated by El Paso Mining and Milling Co., near Kingman, Arizona, and the Sierrita mine, operated by Duval Corporation, Tucson, Arizona, where most of the copper is in the form of chrysocolla, were studied. Even though chrysocolla is not amenable to bioleaching, the approach to the problem and the data obtained are applicable to bioleaching projects using similar deposits where the copper is in sulfide form. For this reason, some relevant project details are mentioned.

The authors used a mathematical model to predict copper recovery, and attempted to develop a relationship between copper recovery and grain size distribution within the fragmented rock or even the technological parameters of the blasts. At the Emerald Isle mine this research was aimed at developing a leaching process for about $3 \cdot 10^6$ tons of ore beneath 60 m of overburden and 200,000 tons of ore exposed on a pit bottom, where mining had ceased due to prohibitive costs related to the break-even stripping ratio. The "stripping ratio" of a pit is the ratio of overburden actually removed to mineral mined [166]. The "break-even stripping ratio" is defined as follows:

$$\frac{\text{Recoverable value per ton ore} - \text{production cost per ton ore [129]}}{\text{Stripping cost per ton waste}}$$

The physical properties of the rock in place are reported in Table 5.8, while Table 5.9 presents the results of tests on the drill cores recovered before and after the blast.

At Sierrita, a block of outcropping orebody about 400 m^2 in area, 33 m deep and 13,000 m^3 in volume was fractured. Data on drill cores recovered before and after the blast are reported in Table 5.10.

The blast resulted in nonuniform permeability values. In Zone A before the blast, values ranged from 0 to $2 \cdot 10^{-3}$ $cm \cdot s^{-1}$, whereas after the blast they fell to between 10^{-4} and 0.8 $cm \cdot s^{-1}$. The in-situ leaching tests were restricted, however, to zone A, where two separate and distinct operations were conducted in two adjacent areas. Zone A_1 contained 13,000 tons of ore and Zone A_2, which was not blasted, included about 100,000 tons. This division permitted a comparison between leaching efficiency in the undisturbed rock and the rock fractured by the blast. The rock-mechanical data in Tables 5.9 and 5.10 clearly reveal a correlation between the RQD and average fragment size, indicating the

validity of this parameter in estimates of fracturation characteristics for leaching purposes. In Zone A_1, fractured by the blast, the leaching tests lasted 117 days while they lasted 190 days in the undisturbed Zone A_2. The results obtained for the two zones are shown in Table 5.11; the effect of the fracturation produced by the blast was sufficiently great to warrant blasting in Zone A_2 as well, if operations were to resume.

Table 5.8

Physical properties of rock at Emerald Isle mine

Property	Zone	
Name	A	B
Porosity, %	20.6	16.3
Density, $kg \cdot m^{-3}$	2290.0	2280.0
Permeability, Darcy*	n.s.	0.65
Longitudinal velocity, $m \cdot s^{-1}$	2430	2850
Torsional velocity, $m \cdot s^{-1}$	1260	1530
Compressive strength, MPa	17.924	n.s.
Tensile strength, MPa	0.496	n.s.
Young modulus, MPa	5860	n.s.

n.s. – not specified.

* The Darcy corresponds to a flow of 1 $cm^3 \cdot s^{-1} \cdot cm^{-2}$ for a pressure gradient of 10^5 $Pa \cdot cm^{-1}$.

Table 5.9

Rock-mechanical investigation of the drill cores at Emerald Isle mine

Property		Zone				
Name	Unit	A		B		
		Before blasting	After blasting	Before blasting	After first blasting	After second blasting
Core recovery	%	86	35	93	83	67
RQD*	%	65	11	70	40	31
Average size (50 % W)	10^{-3} m	163	< 25	163	78	38
Maximum size	10^{-3} m	660	229	838	500	406

* Rock Quality Designation. 50-mm diameter drill cores.

Table 5.10

Rock-mechanical investigation of the drill cores at Sierrita mine

Characteristics	Before blasting	After blasting		
Interval between holes, m	—	7.5	6.0	4.5
Core recovery, %	98	79	52	37
RQD*, %	37	24	10	10
Average size (50 % W), 10^{-3} m	66	48	23	15
Maximum size, 10^{-3} m	838	280	229	229

* RQD – Rock Quality Designation.

Table 5.11

The results of leaching tests at Emerald Isle mine

	Zone A_1	Zone A_2
Ore leached, Mg	13,605	90,700
Grade of ore – copper, %	1.0	1.0
Duration of leaching, days	117	190
Average running time, h/day	20.5	23.2
Leach influent:		
pH	1.18	1.10
Copper, $kg \cdot m^{-3}$	0.147	0.091
Iron, $kg \cdot m^{-3}$	8.5	–
Pregnant liquor:		
Flow rate, $m^3 \cdot s^{-1} \cdot 10^{-3}$	3.62	7.31
pH	1.3	–
Copper, $kg \cdot m^{-3}$	0.562	0.646
Iron, $kg \cdot m^{-3}$	6.2	–
Total copper production, kg	13 154	64 411
Overall copper recovery, %	9.67	7.10
Daily copper recovery, $\% \cdot 10^{-3}$/day	82.65	37.37

After 17 weeks in Zone A_1, recovery was 10 % while in Zone A_2, only 7 % recovery was obtained in 27 weeks. One of the two papers [44] describing this experiment reports an attempt to calculate copper recovery in terms of grain-size distribution, according to a computer program developed by Madsen et al. [113] for process kinetics.

A comparison of computed and experimental data, however, reveals the inaccuracy of this procedure: actual recovery was considerably lower than

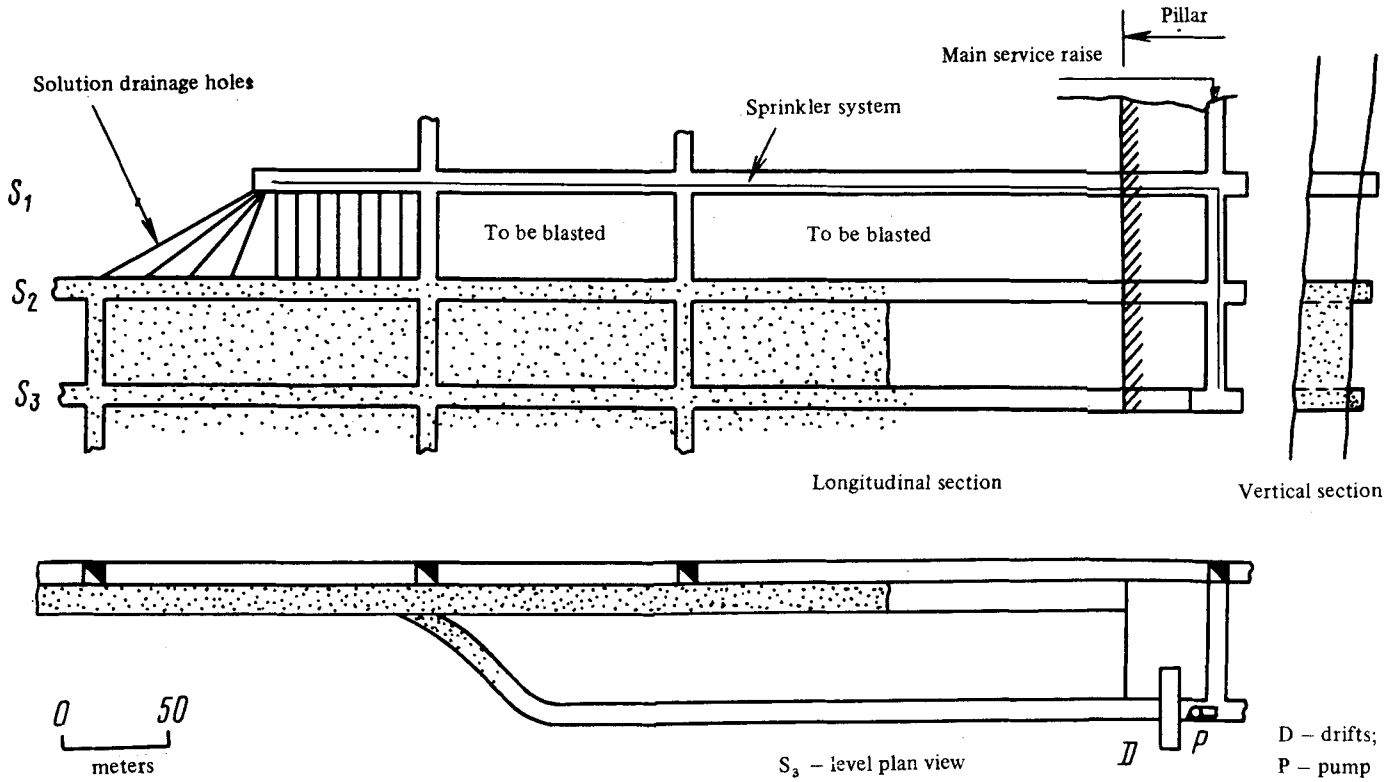


Fig. 5.14. Agnew Lake Mine: schematic diagram of preparation operations for in-situ leaching in an experimental stope

the calculations predicted, due to the model's limitations, and because the fracturation data derived from drill cores indicated relatively small grain size resulting from additional breaks during drilling. This attempt is of great interest, however, since the computational data represent an ideal limit.

One of the two known in-situ bioleaching projects using a virgin orebody was conducted at the Agnew Lake mine in 1975 [117]. This deposit is located southwest of the Sudbury basin – 60 km west of Sudbury, Ontario – and consists of a highly fractured oligomictic quartz pebble conglomerate containing urano-thorite in two zones stratigraphically separated by about 180 m [143]. These two zones strike W–E in surface and SW–NE in depth, while the dip is 85° close to the surface, decreasing to 45° about 1,000 m deep. A first experimental stope was developed in a zone where the deposit is 7.5 m thick and dips 65° to the south.

The project involved the excavation of a long-strike sublevel drifts 9 m wide, 1.5 m in the bedrock, to allow drilling of boreholes parallel to this contact (Fig. 5.14). The benches were 15 m high, and at the end of each sublevel a 2.1 x 2.1 m raise was opened to the sublevel above and then slotted out to give a free-face slot for blasting. Holes 75 mm in diameter were drilled downward vertical to the strike but angled sideways to match the slope of the walls. Holes with a 1.35 m spacing and a 1.50 m burden conformed most closely to fragmentation requirements. The choice of the most suitable explosive was a significant feature in these tests, consequently, determinations of the most important parameters were made using sizeable rock samples, as summarized in Table 5.12. Table 5.6 lists the explosives used, along with their main features. High energy concentration requirements precluded the consideration of ANFO explosives. The results of pilot stope blasts were favorable so the leaching experiment commenced.

In 1978, the Agnew Lake operation produced 181,437 kg U_3O_8, 113,398 kg from the surface heaps and the balance from underground stopes. Underground

Table 5.12

Physical properties of two samples of Agnew Lake mine ore

Ores	Density, $g \cdot cm^{-3}$	Compression wave velocity, $m \cdot s^{-1}$	Shear wave velocity $m \cdot s^{-1}$	Poisson ratio	Young modulus, GPa	Porosity, %
Coarse-grain conglomerate	2.77	3410	1475	0.38	16.7	0.8
Medium-size brecciated	2.69	3930	2230	0.26	33.8	0.8

For the readers who are not specialists in Mining Engineering, details about the above expressions can be obtained from References [59, 67, 143].

production apparently did not reach expected levels, however, due to incomplete wetting of the ore broken in the stopes. A company statement attributed this shortfall to the fact that the underground stopes being mined did not dip as steeply as anticipated, due to faulting [145]. Although no further data have been reported, the above study is nevertheless considered a valid case history.

Other methods of in-situ rock fragmentation worthy of coverage are hydrofracturing and nuclear-charge detonation.

Hydrofracturing involves the injection of a fairly viscous fluid with the appropriate flow rate through a borehole to generate increasing pressure within the rock formation to be fractured. The rock on the borehole wall begins breaking when the pressure reaches a value p_i, defined as "pressure of fracture initiation". Once fracturing starts, it is propagated at a rate dependent on the flow rate of the pumped fluid, the characteristics of the geological formation and the properties of the fluid [6, 46]. This technique has become widespread in the oil and gas industry since 1949, its value demonstrated by the approximately 750,000 fracturing treatments carried out worldwide by the oil industry up to 1974 [46].

The subsequent use of hydrofracturing wells for leaching represents an important benefit; however, the rock-mechanical properties of the orebodies must be examined carefully. The required hydraulic power formula is injection flow-rate times pressure at the top of the well, which depends on the fracturation gradient for a given flow rate. In certain instances, for flow rates ranging from 70 to $80 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$, the hydraulic power required may be as high as 3,000 to 3,700 kW [105]. For more details on this technique excellent handbooks are available [106].

Potential roles for nuclear explosives have been studied extensively, particularly in the United States at the Lawrence Livermore Laboratory, often in cooperation with the technical staff of private mining companies and sponsored by the U.S. Atomic Energy Commission. In the 1970's an intensive campaign was embarked upon aimed at developing models to deal with the in-situ leaching and bioleaching of submarginal copper orebodies.

Both fission and thermonuclear explosives were considered; as in the latter case, for which a fission priming mechanism was developed, the detonation of numerous nuclear charges at the Nevada test site and elsewhere during the 1970's using various types of rocks (granite, granodiorite, basalt, andesite, trachite, rhyolite, dolomite and volcanic tuffs) proved enlightening. The small size of nuclear charges allows the use of ordinary drill holes at low cost. Also advantageous is the broad-scale effect of the explosion, which not only fragments the rock adjacent to the charge but fractures the surrounding rock as well, forming a permeable envelope. Instantly after the detonation of a depth charge, a cylindrical chimney of fragmented rock forms, surrounded by a zone of rock containing a dense network of fractures, cracks and microcracks. These were the focus of detailed investigations using rock samples from various nuclear test sites, resulting in the design of a fracturation index apparently related to distance from the explosion center. Data from four nuclear tests, as shown in Table 5.13, can be utilized for the layout of in-situ leaching projects.

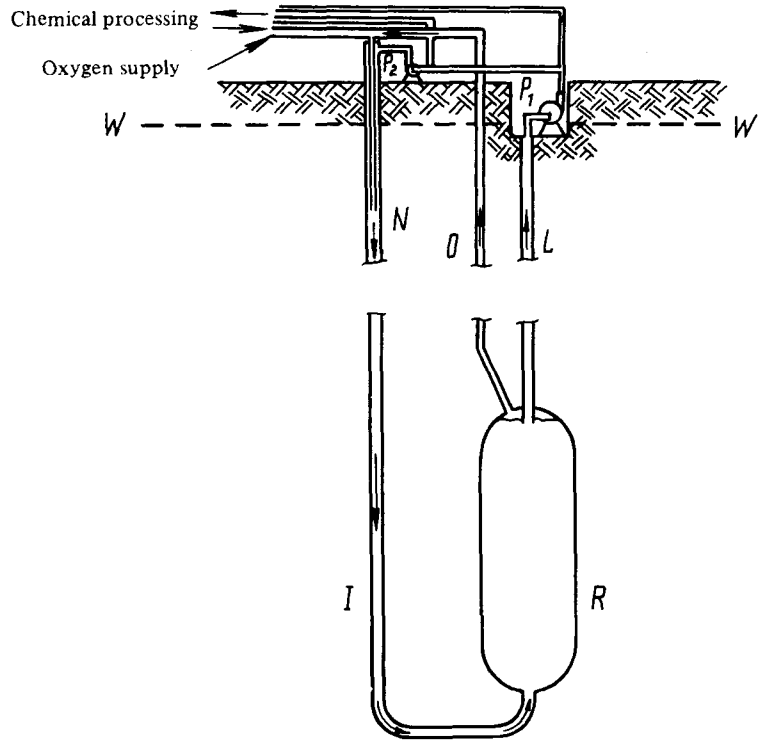


Fig. 5.15. Schematic of rubble column flow:
R – rubble column; I – injection pipes; N – oxygen sparging nozzle; L – liquid discharge; O – oxygen discharge; P_1 – discharge pump; W – water table; P_2 – injection pump

Table 5.13

Nuclear explosions produced in deep rock masses [107]

Tests	Explosive		Date of blast	Rock	Depth, m	Dimensions of chimney, m	
	Type	Power, Gg				Height	Diam.
HARDHAT	Nuclear	4.9	09.15.1962	Granodiorite	282	84	63
HANDCAR	Nuclear	12.0	11.05.1964	Dolomite	400	67	21
PILEDRIVER	Nuclear	61.0	06.02.1966	Granodiorite	460	27	48
SHOAL	Nuclear	12.5		Granite	360	107	25

The broken rock comprising the chimney is physically similar to the product of a big jaw crusher, with more than 80 % of the particles smaller than one meter. Its permeability is higher than 1 $cm \cdot s^{-1}$, with a void ratio ranging from 15 % to 30 %. The chimney walls are easily recognizable and are surrounded by a permeable envelope consisting of two zones: a spherical zone, fractured by the shock front, with a radius three to four times larger than that of the cavity around the blast center, and a zone with enhanced permeability surrounding and overlying the upper part of the chimney. The latter zone forms as tensile fractures occur, with gravity collapse causing the upward progression of the chimney. The whole permeable envelope consists of these two zones which merge to form a pearshaped region. For granite, typical permeability in the core of the zone ranges from 10^{-4} to 10^{-5} $cm \cdot s^{-1}$, decreasing outwards. In the lower portion of the chimney, the melted rock may form an impervious seal, preventing the downward seepage of the leach solutions [72]. Since the permeability of the crushed rock in the chimney far exceeds that of the surrounding fractured rock, the whole chimney zone will probably have to be flooded periodically to ensure adequate wetting of the outermost region. If the nuclear charge is blasted close enough to the topographic surface to produce a crater, the fragmented rock mass can be fashioned into a dump. The lixiviants are then sprayed over the mass by spray nozzles or sprinklers, similar to the method used for heaps or dumps. When the blasted rock fills a deep-buried chimney, it can function as a reactor and the process can proceed as depicted in Fig. 5.15, from a paper by Braun and Mallon [19].

Large mineralized areas located at considerable depth and well below the water table (class II or III according to Wadsworth) were investigated by Livermore Laboratory researchers, who proposed the use of nuclear blasts [107] to produce a system of chimneys as described above, arranged in a geometrical pattern over the entire mineralized area. Such chimneys are prepared, fractured and leached according to plans, such as that shown in Fig. 5.16, that account for their influence on one another [108]. Each row of ten chimneys is blasted as a unit; the blasting sequence ensures that new cavities are either isolated or adjacent to two pre-existing chimneys, guaranteeing symmetrical caving of the fractured rock. The single rows are blasted according to the following schedules. Initially, row 1 is blasted, followed three years later by row 2. Six years after the first blast, row 1 is considered depleted and row 3 is blasted. After another three years, row 4 is blasted.

A ten-day interval between two blasts in one row is considered adequate. The fragmented rock chimneys are flooded, restoring the primitive water table and concomitantly elevating hydrostatic pressure, thus enhancing the dissolution of oxygen injected into the bottom. The swarm of bubbles rising through the lixiviants then circulates through the chimney, dispersing the dissolved oxygen to all regions. Oxygen oxidizes the primary sulfide minerals to sulfuric acid, while releasing heat, lowering the pH and increasing the temperature. The net result is the rapid dissolution of minerals, such as copper sulfides, aided by suitable microbial strains.

Using data collected from suitable investigations, Lewis and Braun [107] determined the typical chimney characteristics shown in Table 5.14. They then

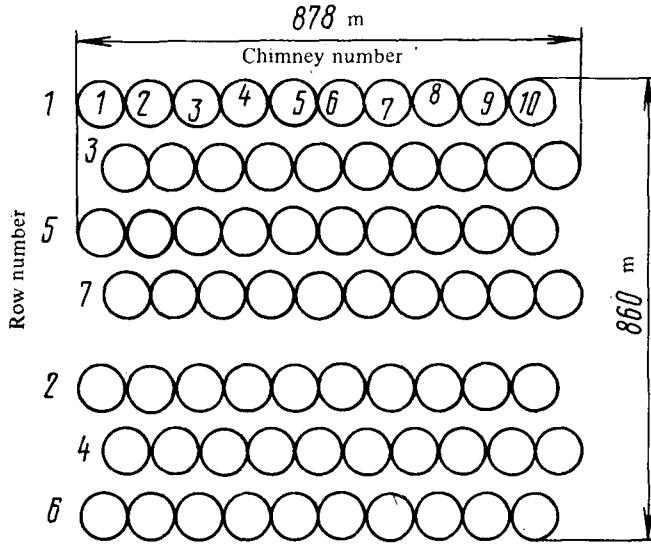


Fig. 5.16. Proposed nuclear chimney arrangement for rubblization of an orebody

developed a pilot plant consisting of a reactor with the capacity to handle 5.8 tons of ore and 1.4 m^3 of distilled water. It operated at 90 ^{0}C and 2,756 kPa O_2 with O_2 flow rates of $23.6 \cdot 10^{-3}$ $Nm^3 \cdot s^{-1}$ during the first three test months and $11.8 \cdot 10^{-3}$ $Nm^3 \cdot s^{-1}$ subsequently. These tests supplied useful data and

Table 5.14

Chimney properties of nuclear blasting [108]

Property		Unit
Yield of nuclear device	10^5	Mg
Depth of burial	750	m
Depth of water table	50	m
Water content of rock	3	wt %
In-situ density of rock	2700	$kg \cdot m^{-3}$
Bulk porosity	0.17	
Radius of cavity	41	m
Height of chimney	300	m
Volume of water in chimney	$2.9 \cdot 10^5$	m^3
Volume of rock in chimney	$1.4 \cdot 10^6$	m^3
Mass of rock in chimney	$3.8 \cdot 10^{12}$	g
Hydrostatic pressure at depth of burial	6890	kPa
Hydrostatic pressure at top of chimney	3927	kPa

allowed the evaluation of several parameters. In 160 days the copper recovery was 16 %, consistent with earlier predictions, and its dependence on the following factors was established:

1) Particle-size distribution of the rock fragments, which depends, in turn on the rock RQD,
2) Starting temperature inside the chimney;
3) Heat generated by the oxidation of sulfide minerals;
4) Heat lost by thermal conductivity;
5) Oxygen concentration in the leach solution.

Using these data, Lewis and Braun developed a model for the chimney of Table 5.14, predicting 25 % recovery in five years. One limitation of this model is related to the separate treatment of leaching and circulation. A more rigorous model proposed by Lewis et al. [108] did not, however, overcome this limitation.

In the bioleaching of deeper orebodies, the microorganisms adhering to the rock surfaces are subjected to pressures higher than atmospheric and the microorganisms suspended in the lixiviants undergo pressure gradients. The pressure increase of the lixiviants increases the dissolved oxygen mass and should therefore enhance the leaching process, provided that the pressure gradients do not inhibit microbial activity.

According to Bosecker et al. [16] the exposure of **T. ferrooxidans** to pressures of 700 kPa (corresponding to a water column of 70 m) for as long as three hours should not significantly affect their respiratory activity. Pressure variation however, may possibly have a detrimental effect.

Experimental evidence presented by Davidson et al. [48] shows that a 48-hour exposure of washed suspensions of **T. ferrooxidans** to hydrostatic pressures ranging from atmospheric to 68.93 MPa, had an almost negligible effect on iron oxidation activity, even in the presence of divalent copper (5,000 ppm). The ability to fix carbon dioxide, however, was almost lost. Furthermore, cell suspensions held at 68.93 MPa for 48 hours retained, upon decompression, nearly 80 % of their respiratory activity exhibited by the non-pressurized controls. The depth limit for microbial leaching seems to be about 3,000 m, since growth of **T. ferrooxidans** is inhibited by a hydrostatic pressure of 40.53 MPa, while the organism grows at pressures as high as 30.40 MPa, provided that dissolved oxygen does not exceed the 8 ppm concentration ordinarily encountered by the organisms under natural conditions.

In-situ uranium mining underwent extensive research during the 1970's; currently it is viewed as a special branch of in-situ mining. Many uranium orebodies consist of sandstone layers impregnated with uranium minerals sufficiently permeable that blasting is not required prior to leaching. The in-situ leaching of these orebodies can be accomplished either by the surface in-situ leaching (SI-SL) method or the underground in-situ leaching (UI-SL) method. Fig. 5.17 illustrates the SI-SL method, which consists of drilling a system of adequately cased and cemented injection wells, through which the lixiviants are forced into rock pores or fractures of the productive formation, using pressure greater than hydrostatic pressure in the deposit. In addition, production wells are drilled to create a low-pressure sump where metal-enriched solutions are collected, using

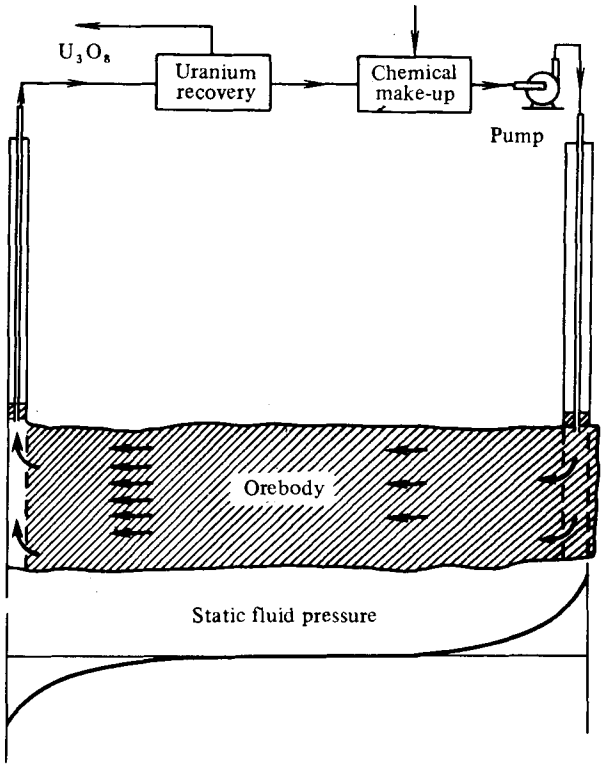


Fig. 5.17. Overview of uranium orebody in-situ leaching:

SI–SL method

downhole production equipment, including pumps, tubing and packers, specially designed for this corrosive environment. The pregnant liquors are finally pumped to surface extraction plants, where uranium is recovered as U_3O_8. Further details on this method can be obtained from a paper by Huff et al. [80].

The UI-SL method, proposed by Érickson [58] is shown in Fig. 5.18 and consists of the following main steps:

1. Sinking a shaft.
2. Driving a drift into the middle of the orebody parallel to its length.
3. Drilling almost-horizontal long holes the width of the orebody.
4. Oxidizing and leaching the uranium drawn from these holes.

The appropriate design of both methods requires information on depth, ore grade, total metal content, ore thickness, flow conductivity (permeability), void space in rock (porosity), mineral type and distribution, natural groundwater flow and structural features. According to Erickson, the UI-SL method has several advantages over the SI-SL method, and is preferred for orebodies deeper

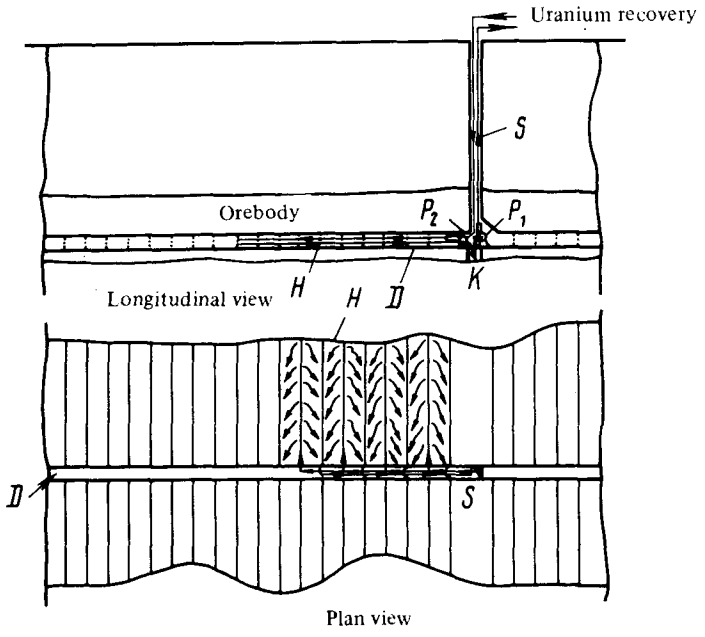


Fig. 5.18. Overview of uranium orebody in-situ leaching:
UI–SL method:
S – shaft; P_1, P_2 – pumps; D – drift; H – solution injection holes; K – sump

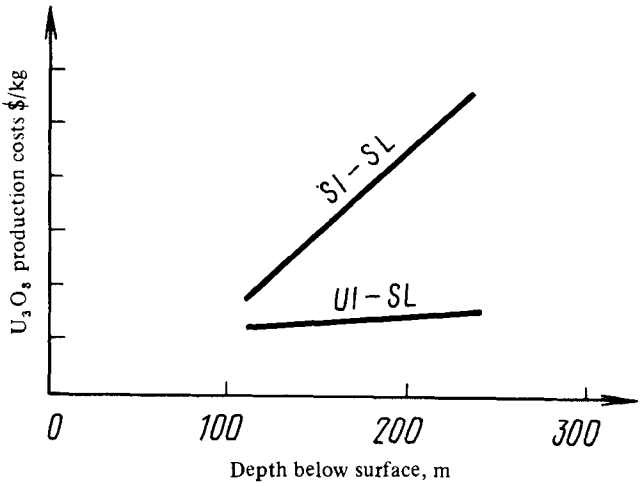


Fig. 5.19. Diagram based on a series of case-studies, showing that beyond a 90-m depth underground in-situ leaching (UI–SL) is more favorable than leaching from surface (SI–SL)

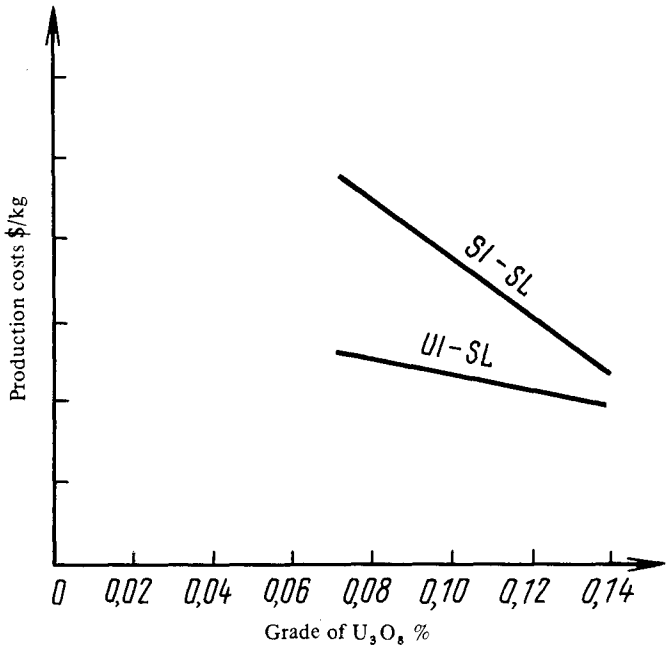


Fig. 5.20. Diagram based on a series of case-studies, showing that higher recovery rates should be expected when using the underground in-situ leaching (UI–SL) method

than 90 m, as shown in Figs. 5.19 and 5.20, which are based on a series of case studies.

A major drawback of in-situ leaching and bioleaching is the prediction of metal extraction. For as-blasted uranium ore bioleaching, a prediction method has been proposed by Rio Algom Mines Ltd., a company with considerable experience in this field. A semi-empirical calculation procedure, incorporating information from laboratory tests under simulated stope leaching conditions, led to the following approximate correlation of extraction and time [69]:

$$E = k \cdot t^{0.25} \tag{5.25}$$

where:

E is the uranium extraction, k is a constant depending on ore, particle size, temperature, etc. and t is the time.

In 1979, 8 % of U.S. uranium production resulted from in-situ leaching, and for 1980 the estimated fraction was 10 % [142]. Additional details on recent progress in-situ leaching research are given in the sequel.

5.5.3. In-Situ Leaching and the Environment

Several pollution sources are responsible for the environmental impact of in-situ leaching operations. They include:

1. Leach solutions seepage losses.
2. Chemical residues of the extraction plant, consisting of residual slimes, effluents from the periodical regeneration of ion-exchange columns, such as the carbon columns of uranium extraction plants, and volatile substances.

The risk of pollution by seepage loss may be eliminated in a number of ways:

1. Closing the solution circuits between the orebody and the extraction plant, and ensuring a watertight piping network.
2. Installing flowmeters in both the injection and production lines, in order to check daily the solution flow rates.
3. Drilling monitor wells around the leaching area, to detect any seepage losses of injected solutions (if losses should occur, the injected and produced flow rates must be balanced).
4. Casing and cementing all the wells up to the surface, to prevent the upward migration of solution.
5. Drilling shallow monitor wells in the leaching area and sampling them to detect any upward solution flow.

The slimy chemical rejects, particularly hazardous in the case of uranium mining, must be collected in settling ponds, thickened and subsequently pumped to deep disposal wells. The sand-containing solution coming from the regeneration columns is decanted in concrete tanks and recycled. The filter-cake drying section of the uranium recovery plants is the only source of dangerous volatile matter: effluent scrubbing towers trap any dangerous matter in the gaseous effluents. The disposal wells should be deep enough and adequately isolated from the overlying formations to prevent losses to the surrounding area.

Following depletion of the orebody, continued production will reduce the metal ion concentrations to acceptable levels. The products of this washing, which uses fresh water rather than active lixiviants, are discarded in the deep disposal wells. Extensive monitoring of the shallow wells is required following cessation of the leaching operations to prevent pollution of freshwater aquifers.

According to Erickson [58], with the SI-SL method, chemical and physical contaminants released by the flow of reagents can partially clog the production wells, creating problems in the final cleaning stage. In general, however, in-situ leaching is considered as a fairly "clean" method; since only metal is removed with minimal gangue, the amount of waste to be disposed of is relatively small. In addition, the landscape remains practically undisturbed due to the absence of waste dumps and subsidence.

5.5.4. Dump Leaching

Growth of open-pit mining since the 1940's has led to the formation, in the vicinity of the pits, of large dumps often containing several hundred million tons of rock. Most of these orebodies are copper deposits, and the dumps themselves

have contained minimal values, ranging from 0.1 % to 0.4 % copper, too little to justify beneficiation with conventional processes. For instance, at Kennecott's Bingham Pit in 1979, 37,803,900 tons of ore and 123,181,836 tons of waste were mined, a daily average of 452,305 tons. On February 11, 1981 a new world record was set when a total of 638,649 tons (536,393 tons of waste and 102,256 tons of ore) were mined [146].

It had long been observed that, under certain conditions, the rain water entering copper mine dumps emerged as rivulets of copper-containing acidic solutions. Only after the importance of microbial activity in the oxidation processes of metallic sulfides became apparent in the 1950's, however, research began to focus on bioleaching processes for recovering copper, or other metal values, from the dumps.

It is arguable that no fundamental differences exist among in-situ, dump and heap leaching, since all these processes are carried out in coarse unsized fragmented rock. However, dump and heap leaching offer two basic advantages over the in-situ technique. First, the former are surface operations and thus escape the often adverse influence of local geology. Second, at least for present and future sites, it is possible to design and locate projects so as to maximize metal recovery. Finally, since the rock in the dumps has no added value, in contrast with the case of in-situ mining of virgin deposits, a metal values extraction process can be justified on the grounds of current commercial economy, even if recovery is relatively small. Rock particle-size distribution, however, must be free of constraints, subject only to requirements of the blasting and haulage operations. Despite this stipulation, blasts can be designed to keep the maximum fragment size within limits consistent with economic constraints.

Considerable benefit can result, on the other hand, from careful choice of emplacement method, dump geometry and solution introduction procedure by accurate regulation of the operating parameters.

The emplacement method and dump geometry are closely related. In densely populated areas the choice of dumping area considers primarily the mine location and nearby topography, such as small valleys, canyons, and slopes in areas devoid of vegetation. In addition, consideration should be given to location characterized by gently sloping and impermeable terrain from which the dump effluents can gravitate to one or two collecting points, to ensure maximum recovery of metal values.

Recent experience has supplied a wealth of information on dump geometry. One configuration expected to greatly improve leaching efficiency is that described by Robinson and already mentioned in Section 5.3.1.4 [137]. The dumps, positioned parallel to each other and greater in length than in width and height, are called "finger dumps". A finger dump about 800 m long, 35 m high and 200 m wide at Anaconda's Butte mine, Montana, was tested in order to improve air diffusion through the rock mass. Air compressed at 7 kPa was supplied at 0.283 $m^3 \cdot s^{-1}$ by a network of lines placed underneath the dumps. The cost of this additional air supply is fully justified by the fact that the oxygen required by the leaching process penetrates the surface of a dump for approximately 60 cm [27].

As discussed in Section 5.3.2.2, the permeability of the fragmented rock

mass is also critical for satisfactory metal values extraction. As the mining schedule permits, the dumps should be raised in lifts 4.5 m thick, alternating layers of coarse lumps and fine-grained rock. The test finger dump at Butte was constructed according to this guideline. In addition, the big dumpers running on the lifts during dump construction can cause compaction and the formation of intermediate layers with very low permeability, detrimental to leaching efficiency. Naturally, waste haulage organization cannot be governed by too many constraints: one way to obviate this difficulty is the thorough deep ripping of each lift surface. In his paper Robinson [137] reports as common practice in the Butte dumps the use of blasting to disaggregate lift waste. It must be recognized, however, that both ripping and blasting result in the comminution of the rock and consequently increase the percentage of fines: literature data reports increments ranging from 5 % to 20 % minus 6 mm. One particularly detrimental long-term effect of these fines is the irregular distribution of flow paths within the dump.

5.5.4.1. Solution Management

Process efficiency can be affected to a great extent by leach solution application methods, which raise technological problems common to both dump and heap leaching. Therefore, the conclusions presented below are applicable to both types of leaching. The main methods used are:

1. Formation, using small dikes, of adjacent, shallow square or rectangular ponds covering the whole upper dump surface. The leach solutions are pumped to the ponds through a network of acid-resistant plastic distribution lines. This method was used at Rio Tinto [146] and at the Fenice Capanne mine, Italy [65], where pond remains are still recognizable on top of the dumps some fifty years after the cessation of activity. In addition, this procedure was employed at Rum Jungle, Australia [2], and is currently being used by several mining companies. One of these, ASARCO, at its Silver Bell Mine, Arizona [3, 148] utilizes square ponds with 18 meter sides and depths ranging from 0.46 to 0.61 m. The ponds of a given section are flooded with solution and remain filled until the copper content of the pregnant effluent leaving that section falls below a predetermined value. Flooding is then stopped, and the section is allowed to dry. Thus, leaching is conducted intermittently, alternating wetting with rest periods. The operators of the dumps at Fenice Capanne and Rio Tinto understood the efficiency of this procedure, which Van Arsdale endeavored to explain, suggesting that during the rest periods the dissolved copper was transported from the core to the surface of the grains by "reverse capillarity" [169]. Actually it is more likely that the alternation of wetting and rest periods is not only critical in the chemical leaching process, but also permits the diffusion of oxygen throughout the entire dump. This condition is particularly favorable to the growth and activity of **T. ferrooxidans.**

According to Brunesteyn and Duncan, "the leach dump operator should utilize rest cycles that last only as long as sufficient moisture remains in the dump to support bacterial activity". Their laboratory investigation into the optimum rest cycle-leach cycle, in columns containing 45 kg charges of minus

Data on dump

Company or operation	Country	Country rock	Copper minerals		Amount of ore, 10^3 Mg	Size of surface, 10^3 m^2
			Main	Secondary		
Asarco Silver Bell	Arizona U.S.A	Alaskite, monzonite, porphyry, dacite	Chalcocite, chrysocolla	Chalcopyrite, azurite, malachite, cuprite	130000	525
Anaconda Co. Butte	Montana U.S.A	Quartz, monzonite	Chalcocite	Chalcopyrite, bornite, azurite, malachite	10000 23000	36 80 37
Bagdad Copper Co.– Bagdad	Arizona U.S.A	Monzonite, porphyry	Chrysocolla, malachite, azurite	Tenorite and cuprite	40000 (two dumps)	222
Duval Corp. Esperanza Mine	Arizona U.S.A	Quartz monzonite, rhyolite, quartz diorite	Chalcocite, chalcopyrite	Cuprite, malachite, azurite, tenorite	19000	77
Duval Corp. Mineral Park	Arizona U.S.A	Quartz porphyry, quartz monzonite	Chalcocite	Chalcopyrite, covellite, turquois	5500 (two dumps)	31
Kennecott Copper Co. Bingham Canyon	Utah U.S.A	Quartz monzonite	Chalcopyrite	Chalcocite, covellite, bornite, copper oxides	4000000	2880
Chino Mines Div. Santa Rita	New Mexico U.S.A	Granodiorite porphyry	Chalcocite	Chalcopyrite and non-sulphide copper minerals	425000	2600
Miami Copper Co. Castle Dome	Arizona U.S.A	Quartz monzonite and porphyry granite	Chalcopyrite, chalcocite	Covellite, cuprite, azurite, malachite, chalcantite, turquois	48000	n.i.
Miami Copper Co. Copper Cities Unit	Arizona U.S.A	Quartz monzonite	Chalcopyrite, chalcocite	Covellite, turquois, malachite, azurite	n.i.	n.i.
Phelps Dodge Corp.–Bisbee	Arizona U.S.A	Quartz monzonite, conglomerate	Chalcocite, azurite, malachite	Bornite, turquois	47000	358
Phelps Dodge Corp.–Morenci	Arizona U.S.A	Quartz porphyry, monzonite porphyry	Chalcocite	Chalcopyrite, covellite, copper oxides	n.i.	n.i.
Vlaikov Vrah	Bulgaria	Andesite and diorite porphyry	Chalcopyrite, pyrite	Covellite, chalcocite	600 (six dumps)	16500

leaching operations

Dump maximum height, m	Method of ore handling	Method of solution introduction	H_2SO_4 addition to lixiviant, kg·m^{-3}	Lixiviant injection rate, m^3·s^{-1}·10^{-3}	Characteristics of Solutions						Reference
					Injected			Extracted			
					pH	Cu, kg·m^{-3}	Temperature, °C	pH	Cu, kg·m^{-3}	Temperature, °C	
60	Trucks, bulldozers	Ponds and trenches	–	63.09	3.4	<0.01	n.i.	2.4	1.09	n.i.	148
60 53 15	Trucks, bulldozers	Perforated plastics pipes	0.1	315	1.9	0.11	29.4	2.2	0.80	19.4	148
73	Trucks, bulldozers	Spray nozzles	n.i.	208	1.4	0.02	21.1	2.1	1.18	21.1	148
67	Trucks, bulldozers	Spray nozzles	0.4	100	2.5	<0.1	7.8	2.3	1.32	17.2	148
76	Trucks, bulldozers	Spray nozzles	2.0	107	2.6	0.10	n.i.	>2.0	1.10	n.i.	148
365	Trucks and trains, bulldozers	Channels	0.1	2200	2.8	0.12	34	2.5	1.80	38–52	148
91	Trucks and trains, bulldozers	Ponds and trenches	variable	820	3.5	0.08	21–26	2.5	1.40	32–35	148
n.i.	Trucks, bulldozers	Ponds and spray nozzles	n.i.	n.i.	2.7	0.04	n.i.	2.3	0.85	n.i.	148
46	Trucks, bulldozers	Spray nozzles	0.4	113	2.6	0.01	23	2.5	0.75	23–27	148
52	Trucks, bulldozers	Ponds	–	145	2.8	0.03	32–35	2.0	0.96	38	148
n.i.	Side-dumping railroad cars	Ponds	–	315	3.8	0.01	n.i.	2.8	1.0	n.i.	148
25	n.i.	spray nozzles, trenches, channels	–	0.1	3.5	0.10	16–19	2.5	1.8	11–13	71,87

Company or operation	Country	Country rock	Copper minerals		Amount of ore, 10^3 Mg	Size of surface, 10^3 m^2
			Main	Secondary		
Compania Minera de Cananea Cananea	Mexico	Andesites, porphyrites	Chalcocite, pyrite and chalcopyrite	Covellite	10000	n.i.
Phelps Dodge Corp. – Tyrone Branch	New Mexico U.S.A	Quartz monzonites	Chalcocite	Pyrite, copper sulfides	41000	40000
Inspiration Consolidated Copper Co. - Miami	Arizona U.S.A	Porphyry coppers	Chalcocite	Chalcopyrite, bornite covellite	49000 10000 31600	1000 700 700

n.i. – not indicated in the consulted literature.

50-mm copper waste, shows that the cycle using 25 % rest time had the best extraction rate, even though cycles with 80 % and 92.5 % rest periods also gave high extractions. Similar results were obtained by Grote et al. [70].

2. Spraying. The leach liquors are distributed on the upper surfaces of the dumps with various devices, such as metal or plastic lawn sprinkler heads commercially available. The most common method uses perforated plastic pipes (trickle leaching). At Alum Creek, where Bagdad Copper Co. began operating in 1961 [3], the main lines for leach solution transport are either 350-mm stainless steel pipes with inner plastic lining, or 250-mm polythene pipes through which 0.208 $m^3 \cdot s^{-1}$ of solution are pumped. This company also employs sprinkler heads made of surgical tubing [148]. The tubing is attached with reducers to the plastic pipes conveying the leach solutions from the main lines to the dump tops. Being a very flexible material it reacts vigorously to the solution flow. When the pressurized leach solution flows through the tubing, usually 0.5 m long, it swirls at random and creates a circular pattern of spray coverage. Inspiration Consolidated Copper Co., Inspiration, Arizona and Anaconda Copper Co., Weed Heights, Nevada [148] successfully pumped leach solutions through main lines of 200-mm and 250-mm cement asbestos pipes lined with plastic to which 100-mm and 50-mm perforated plastic pipes were joined. The pressure in the pipelines forced the lixiviants out of the holes in the form of jets to distances that varied with pressure. Sheffer and Evans [148] report that metal or plastic sprinkler heads were successfully employed at the Esperanza operation of Duval Corporation, Sahuarita, Arizona "to distribute the leach solutions in an overlapping circular pattern". Sprinkler leach systems unfortunately tend to become blocked with coarse trash entrained in precipitation plant tail water.

Table 5.15, continued

Dump maximum height, m	Method of ore handling	Method of solution introduction	H_2SO_4 addition to lixiviant, kg·m^{-3}	Lixiviant injection rate, m^3·s^{-1}·10^{-3}	Characteristics of Solutions						Reference
					Injected			Extracted			
					pH	Cu, kg·m^{-3}	Temperature, °C	pH	Cu, kg·m^{-3}	Temperature, °C	
n.i.	Dumpers	Spray nozzles	–	n.i.	3.5	0.5	n.i.	2.1	2.5	n.i.	17
60	Dumpers	60 m x x 30 m – ponds	–	0.22	4.0	0.10	n.i.	3.5	0.18	n.i.	17
50 50 50	Dumpers, bulldozers	Spray nozzles	4–5	0.14 0.08 0.08	n.i.	n.i.	n.i.	n.i.	1.3 1.3 1.4	n.i.	17

According to Jackson and Ream [84], a field test run at Kennecott's Bingham Canyon Mine indicated that sprinkler leaching resulted in a 15 % higher concentration in dump effluents than did trickle leaching.

3. Liquor injection methods. This method, used on the dumps of Anaconda's Butte operation [137] consists of drilling vertical holes on 30 or 7.6 m centers or at intersections of a 15 by 7.5 m pattern from the surface of the dump. The holes, usually drilled 12 m deep, are cased with 100-mm PVC pipe perforated at 0.3 m intervals with four 12-mm diameter holes at 90° spacing on the pipe circumference. Robinson [137] reports that pit expansion forced the removal of half of a dump, called St. Helena, where solution had been dispersed by this method. An inspection of the dump's interior revealed that solution had reached the entire dump including the triangle between the crest and the toe. It was also apparent that the solutions followed the angle of repose of the rock in the dump. At Silver Bell, epoxy resins were successfully used as protective pipe lining [3].

Of the lixiviant distribution methods listed above, flooding is the only one that warrants any criticism. In fact, a liquid layer a few decimeters thick acts as a veritable seal against the penetration of oxygen into a dump, detracting from process efficiency. In addition, the ponds function as settling ponds for colloidal iron, which plugs their bottom, further reducing porosity. This criticism is justified by the fact that, at Alum Creek, monthly production increased from 106 to 408 tons, resulting mainly from the adoption of the spray-nozzle method. At Chino the bottom of the ponds had to be periodically bulldozed and ripped to restore permeability which had been seriously reduced by colloidal iron precipitates [3]. The only significant drawback in the sprinkling method is the evaporation loss, which can be considerable, particularly in hot climates.

Due to their emplacement, dumps must be classified, according to Harris' definition, as "macroparticles". Hence, permeability becomes significant; values of this parameter range from $14 \cdot 10^{-3}$ $m \cdot s^{-1}$, in thirty-year old dumps containing slimes, to $116 \cdot 10^{-3}$ $m \cdot s^{-1}$ in more recently emplaced dumps, not yet compacted [74]. Solution acidity increases permeability. The residence time of the solution within the dumps, beginning when the lixiviants are introduced into the top and ending the moment they flow out of their toe, is an important measure. It depends in part on the meandering paths followed by the lixiviants within the broken rock aggregate. Too long a residence time may mean extremely low flow rates and consequently a reduced extension of the wetting front with a drop in metal recovery. A very short residence time can mean either an exceedingly high flow rate or the occurrence of channeling. In the first case, the microbial activity is upset due to the purging or washing-out of the microbes, which then do not have sufficient time to act; furthermore, the temperature inside the dump would decrease. In the second case, which is very likely more difficult to deal with, various regions of the dump would become isolated from the dissolution process. The residence times of leach solutions range from a few hours for dumps with limited height, to three days for dumps 80 m high, and to as much as twelve days for 150 m high dumps [127]. Table 5.15 gives some data concerning the operation of dumps at several mines [1, 3, 9, 70, 71, 156, 169].

Flow rate control is important, particularly from the standpoint of kinetics in the conversion of ferrous to ferric sulfate. At flow rates which allow the formation of a thin, mobile liquid film on the solid surfaces, this conversion is maximally effective, as demonstrated by the Bacfox process developed by Goldblatt et al. [109]. In addition, Groudev [71] states that, in the dumps of Vlaikov Vrah mine, Bulgaria, the rate of the process is so fast that it can be justified only by a flow regime of the above-mentioned type.

5.5.4.2. Recovery of Pregnant Solutions

Pregnant solutions flow, aided by gravity and slope, into collecting gullies dug along the toes of the dumps. The solutions are then conveyed to either the so-called "pregnant liquor reservoirs" or to the hydrometallurgical plants. Pregnant liquor reservoirs are usually rendered impermeable with appropriate linings, such as tar, plastic, cement and even clay. The reservoir's storage capacity should be able to accommodate the volume of pregnant liquor produced in 24 hours of operation, plus a cushion based on maximum estimated rainfall in the event of extended power failure. At Bagdad mine, northwest of Miami, Arizona, operated by Cyprus Bagdad Copper Co., a 3,800-m^3 reservoir was lined with a 12-mm thick PVC sheet, and another 5,500-m^3 reservoir, 80 m long, 35 m wide and 2.1 m deep accommodating an 0.208 $m^3 \cdot s^{-1}$ circulating load, was completely lined with an 8-mm thick aluminium coated, acid proof PVC sheet known as "Koroseal" [3].

5.5.4.3. Characteristics of Pregnant Liquors

General data cannot be provided, since each operation is unique especially in its ferric to ferrous iron ratio. Copper content [148] ranges from 0.75 to 2.16 $kg \cdot m^{-3}$ and total iron from 0.20 to 6.60 $kg \cdot m^{-3}$.

5.5.4.4. Water Losses

Even though the surfaces are sealed carefully, particularly in the case of new dumps, there are unavoidable seepage as well as evaporation losses, the latter moreso in the tropics and during summer. In addition, up to several hundred cubic meters per day of solution are periodically drawn off for iron bleed-off. Such losses must be replenished periodically. While seepage losses are difficult to predict, evaporation can be accurately estimated using meteorological data specific to the area or with reliable estimation methods. At Rum Jungle, for instance, in the leaching circuit of about 4 mln tons of ore, losses amounted to 136 m^3 per day during the wet season, 378 m^3 per day during the dry season, when iron bleed-off was in progress. The reliable "evaporation pan" method has long been used for estimating the evaporation of lakes and reservoirs. In the U.S.A. and Canada, the use of the U.S. Weather Bureau Class A Pan is widespread; it is made of galvanized and unpainted iron, 1.20 m in diameter and 254 mm deep, and is exposed on a wooden frame to permit air circulation underneath. A 230 mm deep water layer is maintained by daily additions. Methods for estimating the evaporation from Class A pans and lakes are based on solar radiation, air temperature, dew point and wind movement [50].

5.5.5. Heap Leaching

Heap leaching is considered a beneficiation method for oxide ores [149], although it has also been used for oxide-sulfide copper ores [87] and gold recovery. Bioleaching of sulfide minerals (pyrite and iron sulfides), when they accompany oxide ores, may prove very profitable, due to the reduced acid addition costs. Following the discovery of the catalytic potential of **Thiobacillus** microorganisms, use of this genus was extended to copper sulfides and pyrite-bearing uranium ores. A thorough understanding of the process led to the development of laboratory and pilot operations aimed at optimizing the techniques. Malouf stated that heap leaching is applied to ores too low in grade to bear the transportation and operating costs associated with conventional beneficiation operations. Commercial applications of this method are relatively few, since preference has usually been given to the much simpler, though less effective, dump leaching method for submarginal ores and to conventional concentration by flotation for richer run-of-mine ores. While it is conceptually simple, as Fig. 5.21 shows, this method does raise some practical problems as far as the following areas are concerned:

1. Topographic and geomorphologic features of the areas where the heaps are sited.

2. Chemical, mineralogical, structural and physical properties of the mineralized rock.

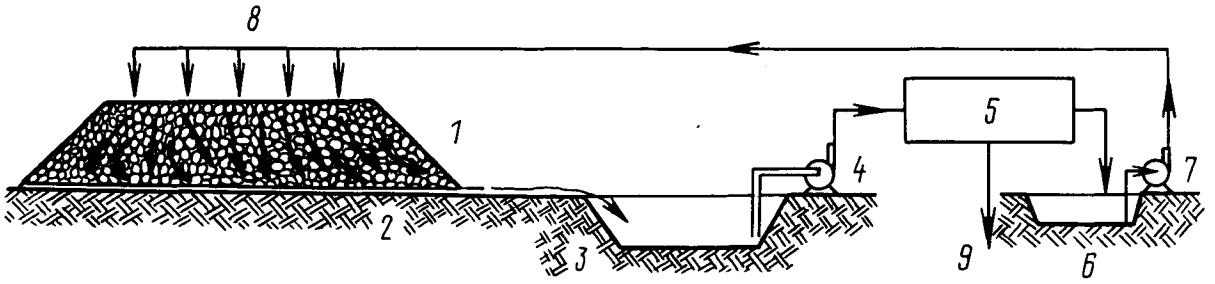


Fig. 5.21. Schematic of heap leaching operation:
1 – heap; 2 – ground surface; 3 – pregnant solution collecting pond; 4 – pump; 5 – cementation launders; 6 – spent solution pond; 7 – pump; 8 – dump irrigation system; 9 – metal

3. Particle-size distribution of the run-of-mine ore.
4. The way the heaps are built.
5. Operational procedures.

5.5.5.1. Topographic and Geomorphologic Features

Obviously the area has to be in the immediate vicinity of the mine or pit. It is advantageous to erect the heaps halfway up any hillsides, so that only the spent or very lean solutions must be pumped; pregnant solutions then flow downhill by gravity and, when necessary, several heaps may be leached in series. In each case, the local geology should be carefully investigated, since fractures, faults or even simple cracks and cleavages may cause unacceptable solution losses and aquifer pollution. When the detailed geological survey cannot assure ground impermeability, a non-porous pad must be created in the areas where the heaps are to be erected. The preliminary operations involve the thorough eradication of all vegetation, grading of the soil surface and accurate compaction using the best available techniques for earth-work foundations with the pad slopes generally at least 3^{o} in two directions. At Rum Jungle the slope was 2^{o} [1] and at Bisby it ranged from 5^{o} to 10^{o} [3]. Steeper slopes might cause pad erosion. If, as is the norm, several heaps are juxtaposed, it can be helpful to isolate them with curbs, thus increasing the reliability of solution sampling. Pads can be built with any appropriate locally available material. Design factors include available area, length of leaching cycle, maximum practical heap height, availability of local clays, local construction costs and availability of tailings storage areas.

The type of pad depends primarily on the type of operation planned. Pads can be built for one-time or repeated use, depending on surface availability, environmental regulations and costs of heap formation and exhausted-ore movement. Following completion of metal values recovery from the heap, the exhausted ore is moved to a tailings storage area and a new heap is erected on the free pad. In this procedure, sometimes called "cyclic operation", front-end loaders are customarily used for materials handling and the pad must be able to support the heaviest load to be carried per vehicle tire. Potter reports that a 50-mm layer of asphalt, followed by a rubberized seal coat and 100–150 mm of hot rolled asphalt, has been successful for loaders up to 6 m^3 [131]. For single-use pads, impermeability can be obtained by:

1. Cemented soil covered by a layer of diluted tar for curing and sealing purposes. This procedure was used at the Bluebird mine, Ranchers Exploration and Development Corp., Arizona [148].

2. Emplacement of a fine sand layer 20 to 30 cm thick on the levelled ground, followed by a layer of 10-mm polyethylene plastic and by a final top layer of another 30 cm of fine sand. This procedure was adopted by Zontelli Western Mining Co. [148].

3. Stabilization (after final compaction) of a layer of top-soil a few centimeters thick by a soil stabilizer, such as a slow-curing bitumen emulsion [2]. With this procedure, a total of 25,000 m^2 was sealed at a rate of 500 m^2 per hour at Rum Jungle.

4. Placement of a clay lining about half a meter thick on a prepared earth foundation. The clay under consideration should be laboratory-tested using the same water and under the same physico-chemical conditions as during production [131].

5. Emplacement of an impermeable sheet about 1 mm thick, for example, of butyl plastic, reinforced with fibreglass, and consisting of strips joined together on site [131]. To avoid puncture of the plastic sheet, there should be a protective covering of fine sand [131]. Plastic lining is advisable for drainage ditches as well. Monitoring drillholes should be angled under each pad for the prompt detection of leakages.

5.5.5.2. Chemical, Mineralogical, Structural and Physical Properties of the Rock and Its Particle-Size Distribution

The conclusions reached for in-situ and dump leaching are also valid here. It is therefore essential, in evaluating the amenability of the ore to heap bioleaching, to ascertain:

1. Amenability to bioleaching of the mineralogical species containing the desired metal values.

2. Potential toxicity of gangue components, which could be detrimental to microbial growth.

3. That rock porosity is sufficient to allow the leach solutions to reach even the most inaccessible mineral grains.

4. Particle-size distribution and porosity of heap-forming fragmented rock enable the leach solutions, and hence the dissolved oxygen, to reach all the mineral grains. In particular, clay contents should be as low as possible; when clays are wetted, they swell and increase in volume by 25 % to 30 %, depending on both their hydrophilic nature and colloidal activity. The increase in volume leads to reduced porosity of the rock mass and therefore to a drop in lixiviant flow rate. On the basis of laboratory filtration experiments, Stroganov et al. [157] deduced that an increase in the clay content of minerals from 15 % to 60 % may cause filtration rate decreases from 25 to 0.4 cm per day and increments in leaching time for heaps 2.5 to 3.0 m high, ranging from 15 to 120 days. Dudas et al. [54] discovered that clays also cause considerable copper loss from leach solutions; i.e. copper ions are removed from the leach solutions to "impregnate" the clays.

Clearly, in building a heap, the goal is to approximate Harris' particulate leaching. However, this objective is difficult to attain if run-of-mine ore with its original particle-size distribution is employed. The ore may contain lumps of 150 cm and larger, particularly if mining is of the open-cast type, as well as clays and fines. Subsequent treatments, to enhance the suitability of the run-of-mine ores, depend upon economic considerations. These procedures can include further fragmentation of the ore and separation, by screening, of its fine and clayey fractions and/or the agglomeration of the latter. At Rio Tinto, the heaps were formed with a run-of-mine ore containing about 4 % −100-mm and about 25 % −19-mm fines [159], much like Harris' "prevailing particulate leaching" [75]. At Rum Jungle the run-of-mine ore contained "significant amounts of fines

and about 28 % voids" [75, 86] and exhibited a pronounced tendency to form fine slimes having a great affinity for water. After an initial, high extraction rate phase, Harris' pseudo-particulate leaching behavior was observed. According to Karavaiko et al. [91] the fine fraction should not exceed 10 % to 20 %. In one section of the J.H. Trigg Co. mine, the heaps are formed with 150-mm ore, while in another section, 50-mm ore predominates. The largest lumps in the heaps operated by Ranchers Exploration and Development Corp. are about 300 mm in size, but most of the particles are smaller than 100 mm. Finally, at the Zontelli Western Mining Co. operation, the run-of-mine ore is crushed to −100 mm [148].

When the run-of-mine ore has to be finely crushed in order to achieve adequate leaching efficiency high proportions of fines are generated. This drawback can be overcome, nature of the ore permitting, by resorting to their agglomeration. This has been done successfully by the Sociedad Minera Pudahuel at its Lo Aguirre Mine, in Chile, where the copper oxide run-of-mine was chemically leached with the so-called Thin Layer (TL) heap leaching process. The run-of-mine was crushed to 100 % −4.75 mm and "cured" by moistening it with 25 kg/ton of H_2SO_4 and 40 kg/ton of water in a mixing drum where the fines underwent physical agglomeration, considerably enhancing permeability [31, 51, 52].

5.5.5.3. Heap Construction

To attain adequate bioleaching efficiency, it is crucial that a high level of oxygen be maintained in the lixiviants throughout the rock mass. Taylor and Whelan [159] point out that a strong natural draft was present in the heaps at Rio Tinto, causing a sharp rise in the copper extraction rate. Bruynesteyn and Duncan [28] reporting on a test defined as "dump leaching", but also qualifying as "heap leaching", confirm the importance of aeration. To control this factor, they placed the heaps on pads consisting of asbestos air pipes 100 mm across, spaced on 0.46 m centers; the section of air pipe under the dump was perforated every 50 mm with holes 12 mm in diameter. Air was supplied by two 0.236 $m^3 \cdot s^{-1}$ blowers at 1.12 kPa pressure. The role of aeration in the construction of the Mangula mine heaps is also stressed by Karavaiko et al. [91], even though in this case the air distribution system was not so sophisticated. In agreement with Harris' theory, extractions conditions should be optimal in heaps longer than they are wide or tall.

At Rum Jungle and Rio Tinto, where the dumps were constructed empirically and these conditions were thus only partially satisfied, dump porosities were 28 % and 40 % respectively.

The heaps constructed by Cortez Gold Mines [55], Smoky Valley Mining [174] and at Saint Pierre, France, [17] summarized in Table 5.16, are typical and deserve mention, even though these operations involve chemical leaching.

The heaps construction procedure also affects its height. If compaction can be reduced, then there will be only minimum deviation from the particulate leaching condition. Dumpers should therefore not be used for ore delivery, as they increase the undesirable compaction of the heap. Ripping the upper surface

Table 5.16

Characteristics of typical heap leaching operations

Operation	Ore	Size of heap			Amount of ore, Mg	Slope of pad, %	Flow-rate of lixiviant, $m^3 \cdot s^{-1} \cdot 10^{-3}$	Recovery, %	Reference
		Height, m	Length, m	Width, m					
Gold Acres	Oxidized limestone gold ore	6.0	137.0	107	170 000	5	19	50	55
Smoky Valley	Welded rhyolite tuff with gold-bearing pyrite	3.0	122.0	76	40 000	3	25.2	n.i.	183
Saint Pierre (Cantal, France)	Uranium vanadates and phosphates in argillaceous sands and green clayey formations	4.0	n.i.	n.i.	1 500	n.i.	n.i.	90	17

n.i. – not indicated.

of the heap does not appear to effectively counteract compaction, as the literature demonstrates [2, 27]. When compaction is unavoidable, the use of suitably spaced vertical drillholes, in addition to ripping to below two meters deep, may be expedient. Trucks or conveyor belts are advised for transporting ore to the heap area, depending on available capital and operation costs. Bulldozers, front-end loaders or even draglines can then move the ore to form the lifts. With bulldozers, it is possible to increase heap height to 3.5–4 m or more, but this procedure also causes some packing. With front-end loaders compaction is virtually nil, but it is difficult to obtain heaps higher than 3 m. The use of conveyor belts and tripper distribution from a bridge unit is more expensive but more advantageous in terms of the regularity of lift formation and the total absence of packing.

In ores with high clay content Kappes [88] suggests controlled segregation within the heap to ensure permeability. One method involves using a mobile stacking conveyor to pile the ore in a series of cones 1 meter high. This method segregates fines, surrounding them with a very permeable coarse layer. Heaps as high as 5 m can be formed in this way. The need to prevent compaction while trying to limit heap height, is contrasted by the need to maximize use of the available area. Experience would recommend heights of about 4 m, but the optimum height must be verified using laboratory and pilot plant tests.

In some cases, emphasis was given to the proper shape of the heap surfaces. At Rum Jungle, for example, deep trenches were dug along the entire length of the heap and the ore removed was piled between them [75].

5.5.5.4. Heap Operation

Concerning the introduction of leach solutions, recovery of pregnant liquor and compensation for water loss, the approach to problems is identical to that taken in the case of dumps. However, the copper content of the pregnant solutions flowing out of the heaps may be higher than for dumps, as much as 8 $kg \cdot m^{-3}$, because heaps are constructed of higher grade ore.

5.6. CASE HISTORIES

The following case histories, necessarily limited in number, are reported as operating examples. In no way are they to be taken as general examples, since it is almost impossible that two orebodies have identical geologic, topographic, geometric, environmental and physico-chemical features. They may represent, however, a kind of guideline and a memo for the planning of a feasibility study.

5.6.1. Dump Leaching of Copper at Nikolayev Mine (USSR)

5.6.1.1. Characteristics of a Commercial Installation

Fig. 5.22 [92] shows a flow chart of dump leaching of low-grade copper-zinc ores at Nikolayev Mine. The dump was formed on a natural clayey bedding with

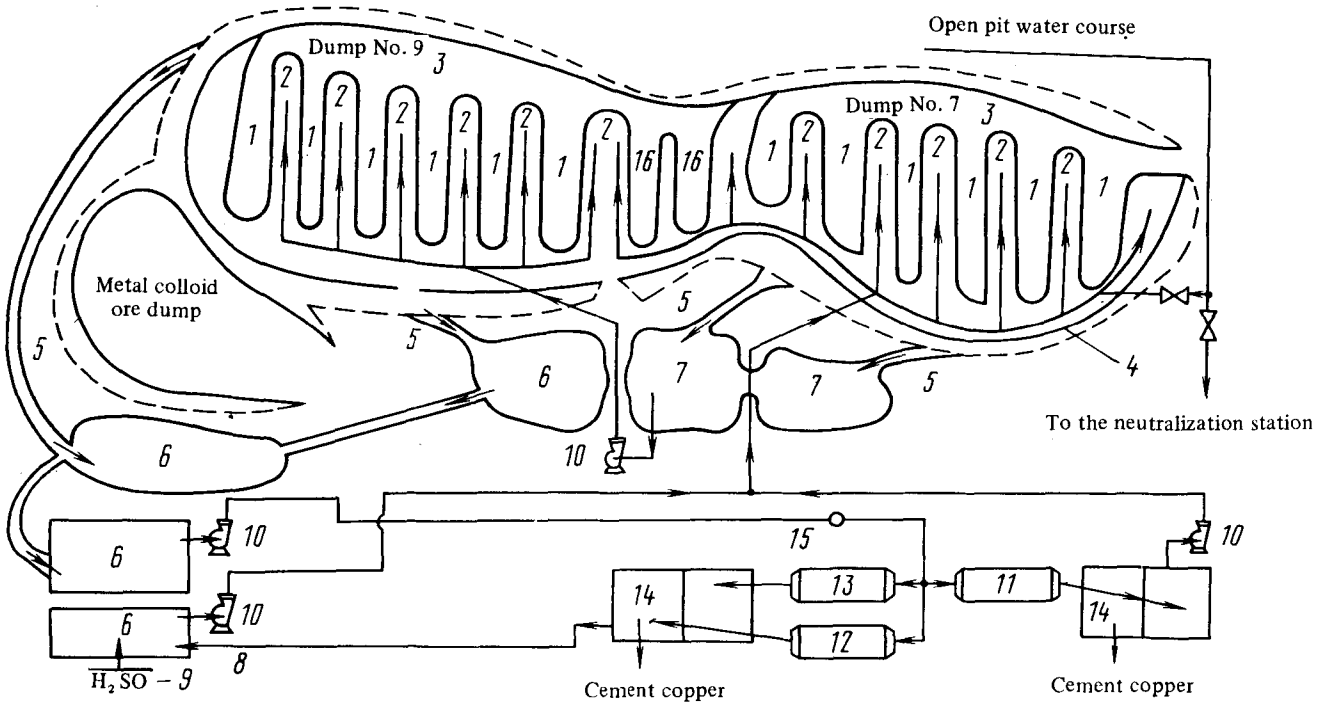


Fig. 5.22. Process flow diagram of dump leaching operation at Nikolayev Mine:
1 – spraying ponds on the dump surface; 2 – dividing ridges; 3 – roads; 4 – irrigation pipes; 5 – solution collecting canal; 6 – pond with head solution No. 2; 7 – pond with head solution No. 1; 8 – pond for tailing solution; 9 – sulfuric acid tank; 10 – pumps; 11 – cementation unit No. 1; 12 – cementation unit No. 2; 13 – cementation unit No. 3; 14 – cement copper settling basins; 15 – flow-gauges; 16 – ponds on the ore surface between the dumps

a 2–8° gradient. The clay layer is 6–14 m thick. After ripping off the vegetation layer and compacting the ground with dump trucks with a specific weight of 5 kg/cm^2 a 30–50 cm layer of fine pyrite ore, "pyrite fines", was formed and compacted with rollers. As a result there was formed an inexpensive and reliable site which precludes any filtration of solutions into the ground and prevents washing of the clayey bedding by pregnant solutions flowing from under the dump during leaching. Because of the relief, the height of the dump varies within 15–25 m, the natural slope gradient being 40–50°. The top area of the dump was 10,400 m^2, the base area was 17,600 m^2. The ore volume of the dump was 304,500 m^3.

To increase percolation capacity of the ore mass, holes spaced at 8 x 8 m and 5–6 m deep, 189 mm in diameter were drilled in the dump and the ore was blasted. The dump surface was divided into 8 ponds of 400–800 m^2 each and equipped with a pipeline to feed solutions. Around the dump, canals were dug out and a settling basin for head solutions of 1500 m^3 capacity was erected for accumulating solutions and settling sludges prior to cementation. An extensive network of pressure pipelines for PE pipes of 150 mm in diameter and 2 km long was arranged. Two water-proof concrete settling tanks for cement copper with dimensions of 10 x 10 x 25 m were constructed using the thickener design with central feeding of solutions through a loading cup and periphery draining of the clarified solution. The area of both tanks was 200 m^2

To unload the precipitated copper from the settling tanks a crane and tightly closing grab bucket were erected. A rotating cementation machine 2.2 m in diameter and 11 m long was installed for copper cementation. The angle of the drum was about 2°. The inner surface of the cementation machine was lined with the X18H10T stainless steel. The cementation machine output capacity was 83 m^3/h, the rotation rate was 6 rpm.

The leaching solution consumption was 15 dm$^3 \cdot$Mg^{-1} of ore, the interval between irrigations 2–3 days. On the average 1,354 m^3 of solutions (900 m^3 of tailing solutions after cementation and 454 m^3 of solutions from the quarry) were fed to the dump daily. Average solution losses by evaporation, for ore soaking and via dump base were 44 % in the initial period and 21.8 % at the end of the season.

It was determined that for the given type of cementation machine the scrap load should be about 10 Mg. The best scrap is detinned tin cans, waste of transformator or roofing iron.

Capital outlays for the installation paid off in less than half of the operating season.

An analysis of the site operation indicates that, in leaching complex ores, not only the basic valuable components (copper and zinc) may be extracted, but also cadmium, indium, gold, silver and other metals [93]. Copper, zinc and cadmium are solubilized most intensively, namely 7–11 (Cu), 8–10 (Zn, Cd) % in 210 days (one season). Arsenic, antimony, selenium and tellurium are solubilized less intensively. Gold and silver content of the solution did not exceed 0.0015 mg·dm^{-3}. It can be increased to 0.035 mg·dm^{-3} using during leaching small amounts of sulfuric acid wastes (up to 1 g·dm^{-3}), containing chlorine and hydrochloric acid admixtures. Copper is extracted from solution by cementation.

During cementation up to 44 %of gold and part of silver, arsenic and antimony precipitate from the solution simultaneously with copper. During zinc sedimentation cadmium, cobalt, nickel and indium are released into the concentrates, while gold, silver and copper are incompletely extracted during cementation.

5.6.1.2. Microorganisms

It was established that in oxidative processes during metal leaching from dumps, four types of microorganisms are involved, namely **T. ferrooxidans, T. thiooxidans, L. ferrooxidans** and **S. thermosulfidooxidans** [90]. **T. ferrooxidans** ($10^6 - 10^8$ cells·cm^{-3}) and **L. ferrooxidans** (up to 2.5×10^6 cells·cm^{-3}) are contained in solutions flowing from under the dumps.

At 22–25 °C solutions were found to contain the moderately thermophilic microorganism **S. thermosulfidooxidans** ($2.5 \times 10^2 - 6 \times 10^5$ cells·cm^{-3}).

In tail solutions, the number of cells sharply declines because of unfavourable conditions for their vital activity during copper cementation [89, 121]. After the solutions had passed through the ore, the number of bacteria is two or three orders of magnitude higher which is indicative of their development at the expense of oxidation both of Fe^{2+} and sulfide minerals.

Moistened fine ore was found to contain the highest number of microorganisms (10^6–10^8 cells·cm^{-3}). The activity of microorganisms, measured in solutions by $^{14}CO_2$ fixation, despite their relatively high number, is quite low. Distinct results were obtained only after three-day exposure, which is indicative of the fact that bacterial development was considerably inhibited.

It should be noted that bacteria in the solutions flowing from under the dump of high-grade metacolloid ore have an activity one order of magnitude lower than in those flowing from under the dumps of lower-grade ores (No. 7; 9).

Apparently, activity of microorganisms during passage of solutions through metacolloid ore is suppressed by a sharp increase in zinc content (from 4–5 to 10–15 g·dm^{-3}). Naturally, activity of microorganisms measured by fixation of $^{14}CO_2$ rises 5–10-fold when temperature is increased from 15 to 22–26 °C. In this case the Fe^{2+} to Fe^{3+} oxidation rate is 2–6 times higher. An increase in the activity of microorganisms was observed when adding KH_2PO_4 (0.5 g·dm^{-3}) and $(NH_4)_2SO_4$ (3 g·dm^{-3}) to the solutions. Correlation is also observed between increase in activity of microorganisms and increase in their number. Investigations revealed that the number of bacteria in solutions and their activity are insufficient to ensure high oxidation rates of Fe^{2+} to Fe^{3+} and sulfide minerals.

Bacterial activity is inhibited by high iron content of solutions (Fe^{2+}, 20–30 g·dm^{-3}) at the total cation and anion content of 70–120 g·dm^{-3}.

It was shown that regeneration of recycle solutions increases the intensity of the process of copper and zinc leaching [82].

To intensify the activity of microorganisms and, hence, to increase the metal leaching rate from ore, it is necessary to extract from the solutions zinc in addition to copper [97]; to add phosphorus and nitrogen salts to the solutions, if appropriate; and, finally, to remove excessive iron and other elements from

recycle solutions [96]. To regenerate recycle solutions, some of the solutions should be subjected to induced aeration with air in the sump of the settler of cementation machine No. 1 and, after that, solutions should be fed to the barren rock dump situated between dump No. 7 and No. 9 (see Fig. 5.22). During passage through the barren rock dump, the solution pH rises. In this case, most of the elements are precipitated in the dump body, and the purified solutions are collected in a separate pond, where they are again aerated to ensure oxidation of Fe^{2+} remaining in the solutions. These solutions, saturated with atmospheric oxygen and containing 2–5 g·dm^{-3} of Fe^{3+}, are supplied together with other recycle solutions for leaching ore dumps.

5.6.2. Dump Leaching of Copper at Kounrad Mine

5.6.2.1. Characteristics of a Commercial Installation

At Kounrad mine a pilot site for dump leaching of copper from low-grade ores was arranged.

The dump is located on a natural site with a 1° gradient. The upper layer of the site is 0.5–3 m thick and is constituted of sand/clay eluvium with coefficient of percolation of 0.07 m/day. The underlying rock is represented by granodiorites. Six meter deep ponds for collecting pregnant solutions are arranged around the dump as a drainage ditch (see Fig. 5.23).

The dump length from west to east is 700 m, height from 15 to 20 m, and width over the upper section from 10 to 45 m. Maximum ore size in the dump is 1–1.5 m, and the average size is 300–400 mm. The dump slope angle is 35–37° on the southern side and 42–45° on the northern side.

Seventy-seven per cent of copper is represented by oxides, 12 % by secondary sulfides, and 11 % by primary sulfides. The major copper minerals are malachite, azurite and, less frequently, brochantite, chrysocolla, chalcocite, chalcanthite and covellite. Of the other ore minerals, special note should be given to the presence of iron hydroxide (hydrogoethite $FeO(OH)\cdot 2H_2O$ and goethite FeO(OH), which under the effect of acid solutions dissolve to form Fe^{3+}.

The rock-forming minerals, namely quartz (55–60 %) and sericite(35–40 %) are broken by numerous fissures and are inert to mild sulfuric acid solutions. The ore structures facilitate penetration of solutions inside ore dumps and better contact of solutions with valuable minerals.

In the process of leaching, the ores decrepitate, but do not lose their percolating properties, which facilitates natural dump aeration and development of microbiological oxidative processes.

The dump is divided into four blocks, each block containing about 90,000 Mg of ore. The blocks were put into operation consecutively, at one year intervals. In the third year of operation, three blocks were under irrigation. The flowchart may be presented as follows. The leaching solutions (sulfuric acid concentration 2–3 g·dm^{-3}) were supplied to block No. 1 which was in operation for the third consecutive year. The obtained solutions were collected in a settler pond, supplemented with sulfuric acid (to 4 g·dm^{-3}) and supplied to block No. 2 which was in operation for the second consecutive year. The ob-

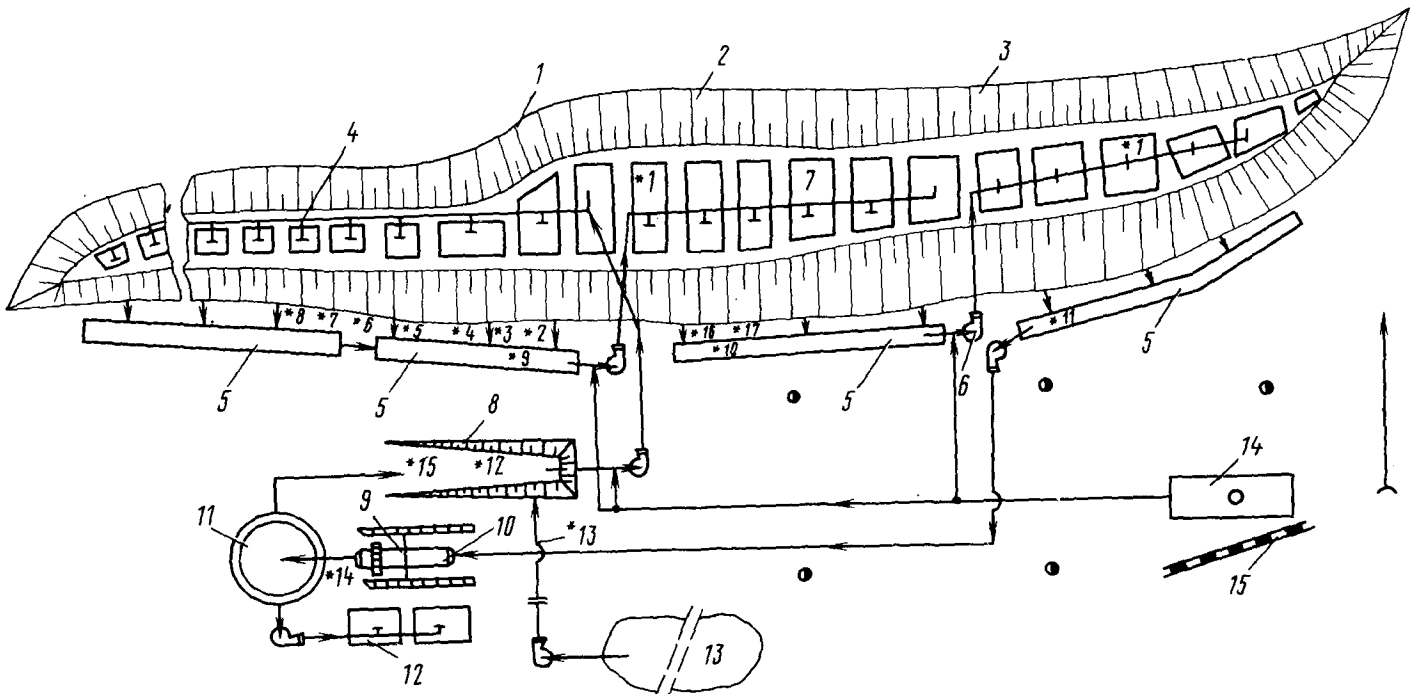


Fig. 5.23. Diagram of dump leaching operation at Kounrad Mine:
1 – 3 – ore dumps; 4 – pipes; 5 – ponds with head solutions; 6 – pumps; 7 – ponds on the dump surface; 8 – pond for tailing solution; 9 – overhead travelling crane; 10 – cementation unit; 11 – thickener; 12 – settling basins; 13 – fresh water basins; 14 – sulfuric acid tank; 15 – railroad; ◑ – surveillance boreholes; * – microbiological monitoring points

tained solutions were further supplemented with sulfuric acid (to 5–6 $g \cdot dm^{-3}$) and supplied to block No. 3 of fresh ore, put into operation for the first year. The solutions obtained from block No. 3 were subjected to cementation [92].

After cementation and settling of cement copper, the tail solutions were collected in a pond, supplemented with sulfuric acid (to 2–3 $g \cdot dm^{-3}$) and recycled to block No. 1.

Implementation of the above flow charts ensured constant 1.5–2.0 $g \cdot dm^{-3}$ copper content of pregnant solutions, a three-fold increase in the amount of ore leached using the same amount of leach solutions and, correspondingly, the same number of cementing units for processing pregnant solutions. Copper production was increased about 2.5–3 times. The pause in irrigation varied within quite narrow limits, 2–3 days. Irrigation density varied from 40 to 50 dm^3 per 1 ton of ore.

After three years of operation, the next (fourth) block of fresh ore was introduced, while block No. 1 was utilized as a purifying installation [96, 160].

After cementation the solutions with pH 2–3 were supplied without acidification to the leached ore block No. 1 where 50–90 % of iron and other admixtures were sedimented in the dump body. The purified solutions were then acidified with sulfuric acid and used for leaching. Without such purification of solutions, the intensity of copper extraction from the ore was found to drop at least 10–15 %, because repeatedly recycled solutions are enriched in iron (to 10–15 $g \cdot dm^{-3}$) and other elements which, in partially sedimenting over the ore of the leached blocks, blind the copper minerals surface [92].

During three leaching seasons (one leaching season equalled 180 days per year), copper extraction reached 70 % (30–35 % in the first year, 20–25 % in the second year, and 10–15 % in the third year). The desirability of a higher degree of copper extraction is determined by the economic factor. For instance, in the fourth season, not more than 5–10 % of copper is extracted additionally from an ore block. The solution coefficient of percolation through the ore depends on its size and changes during 3–4 years from 0.5–1.2 $m \cdot h^{-1}$ to 0.2–0.8 $m \cdot h^{-1}$. For Kounrad mine, it is important to maintain the solution coefficient of percolation at 0.4–0.6 $m \cdot h^{-1}$, because at higher rates it is difficult to create a solution "table" in the ponds. For example, blocks with increased coefficients of percolation were irrigated not by pouring solutions into ponds, but by sprinkling through perforated hoses, which increased the cost of the process and impaired the uniformity of ore moistening in the dump.

The sulfuric acid consumption during leaching was 2.2 kg/kg of copper. As much as 92–95 % of copper was extracted in drum cementation machines [92, 94, 95].

The cement copper precipitate was separated from the solution in an acid-resistant thickener 12 m in diameter. At sedimentation rate of the finest copper particles (~10 μm) being equal to 0.72 $m \cdot h^{-1}$, the thickener productivity in terms of solution was $Q = 113 \times 0.72 \times 0.8 = 65\ m^3 \cdot h^{-1}$, where 113 is the thickener area, m^2; 0.72 the particle sedimentation rate, $m \cdot h^{-1}$; and 0.8 the thickener area utilization coefficient. In fact, the amount of solutions fed to the thickener reached 80 $m^3 \cdot h^{-1}$, and in some periods this led to loss with decantation of as much as 8 % of copper.

The copper precipitate (S:l=1:5) was removed from the thickener by gravity three times per day and settled to 40–50 % humidity in settling tanks. The resultant moist precipitate was granulated together with the flotation concentrate [177] and forwarded to smelting. The copper content of the commercial cementation sediment equalled 76.5 %, of which one-third of copper was in the form of oxides formed in the process of dehydration and transportation of the precipitate.

It is also important to determine the solution losses owing to evaporation and seepage. To that end, account was taken of the amount of fresh water added to the leach solutions, the amount of water evaporated from the pond surface on the dump, solution collecting ponds, thickener and from other open surfaces.

According to the Weather Service, 1,332.7 mm·m^{-2} of open surface had evaporated during five years of operation. Knowing the total evaporation area, it was calculated that the water losses due to evaporation amounted to 67.35 thousand m^3. During this period, 113.7 thousand m^3 of fresh water were used for process needs, and 33.5 thousand m^3 of process solutions were removed from the scheme during the start-up period. As a result of solution balance, we obtain: 113.7 thousand m^3 – 67.35 thousand m^3 – 33.5 thousand m^3 = = 4.85 thousand m^3.

Thus the balance between the water supply and water consumption differs by only 4.85 thousand m^3 (4.3 %), which is due to inaccurate account of the solutions. It follows from the above-mentioned data that evaporation-caused water losses in Northern Kazakhstan (USSR) at the dump leaching site, in relation to the amount of process solutions circulating daily in the scheme, amounted to:

$$\frac{67{,}000 \times 100}{2{,}000 \times 180 \times 5} = 4\ \%, \text{ where}$$

67,000 is the amount of water evaporated during 5 years, m^3; 2,000 is the amount of solutions circulating in the scheme, m^3/day;
180 is the annual duration of the operation period, days; and 5 is the duration of site operation, years.

The obtained data were used in planning a further increase of dump leaching at the mine to commercial scale.

To control solution leakage, twenty-six boreholes were drilled near the dump, and also in the direction of ground water flow. The water in the boreholes was constantly mildly alkaline at the outset (pH=7.1–8.0) and no copper was found therein. Two times in five years estimations were made for the balance between the amount of commercial copper obtained and the residual copper content of the dump. The ore in the dump was sampled by drilling holes and taking core samples [150, 161]. The amount of obtained copper and its residual content of the dump corresponded to the initial amount of copper in the ore. This again confirmed that process solutions are not lost in the soil.

5.6.2.2. Microorganisms

The maximum number of **T. ferrooxidans** bacteria (7×10^5 – 1.3×10^6 cells·cm^{-3} was observed in solutions flowing from under the dump (their num-

ber in irrigating solutions was 10^2-10^3 cells·cm^{-3}). Hence, conditions in the ore are more favorable for development and growth of these microorganisms.

At the end of the leaching season (in November), the dump was drilled using the column method. Drilling was conducted without supplying water, and this allowed to select more representative samples for microbiological analysis. An analysis of samples selected at 1 -2 m intervals showed the presence of microorganisms only in the upper section of the dump (6–8 m), which is apparently due to sufficiently intensive irrigation of that section. This is confirmed by analysis of a sample from a hole drilled in the dump section not subjected to active irrigation. In that site, microorganisms were present only to a depth of 4 m. The bacterial cell concentration in the dump approximately coincides with that in the solutions that flow from under these sites in corresponding spots at the dump base. Thus, the analysis of microflora contained in solutions flowing from under the dump, is also indicative of the number and specific composition of microorganisms contained inside the dump.

Experience demonstrated that copper production using the leaching technique is profitable even on a small scale, while copper prime cost is even 1.5 times lower than in the traditional processing of high-grade ores from this deposit.

5.6.3. Underground Leaching at Blyavinsk Mine

The orebody at this mine is constituted of four lenses, called Northern, Southern, Small and Eastern lenses. The country rock is liparite porphyries and their tuffs. The ore is characterized by a fine-grained structure with abundance of sooty and finely disseminated cryptocrystalline pyrite in particles ranging in size from 0.001 to 0.01 mm; its porosity is suitable for leaching, being higher than 3 %. Chalcopyrite is uniformly distributed throughout the ore in the form of pockets and veinlets ranging in size from 0.01 to 2–5 mm. Covellite develops over chalcopyrite in the form of fine crystals and pockets. Chalcocite is associated to covellite and forms on it elongated, lamellar encrustations; it also occurs as the sooty variety and develops over pyrite. The latter, accompanied by marcasite, is incompletely crystallized and exhibits a defective crystal structure. Both these varieties of iron disulfide have a strong tendency to oxidation.

All the lenses were mined with conventional stoping methods and in-situ bioleaching has been carried out in the stope remnants. Bioleaching was first undertaken in the Northern and Southern lenses and with the experience gained has been extended to the Eastern lens. Acid (pH from 2 to 3) mine drainage solutions and partly fresh water from the natural water body were used for leaching.

5.6.3.1. The Northern Lens

This lens is the thickest and its ore contains up to 80 % pyrite, the remaining 20 % being constituted of copper minerals, of which 11.7 % sulfates, 3.3 % oxide minerals, 67 % secondary sulfides and 18 % primary sulfides. The rock is highly fissured, thus ensuring the penetration of leach solutions and access of atmospheric oxygen throughout. Leaching is carried out in the rock forming a pillar

damaged by the mining of adjacent stopes. The outcropping part of this pillar is overlain by a 80 meters high dump. An appendix to this orebody, called block No. 45, forms part of the open pit where conventional mining was carried out earlier. As the rock here is relatively undisturbed, it had to be fragmented prior to leaching. This was achieved by blasting explosive in forty 14-m long vertical and 20-m long inclined 105 mm diameter blastholes, the latter at 2 x 3 centers.

Bioleaching was also performed in a 7-m high dump located on a slope of the pit and formed with sub-marginal ore produced by overburden stripping during pit excavation.

5.6.3.2. The Southern Lens

Top slicing was the conventional mining method used in this lens, whose thickness ranges from 2 to 16 m. The mined-out stopes are therefore filled with caved country rock which strongly diluted the ore, bringing its average grade down to 0.035 % Cu.

5.6.3.3. The Eastern Lens

The orebody is located 300 m southeast of the Northern lens, has dip of 55^{o} and thickness ranging from 10 to 20 m. The top of the lens is buried at a depth of 40 m under the topographic surface. The zone where bioleaching is planned, some 50 m high above the 373 m level requires some development work consisting of the excavation of a drift, where pregnant solutions will be collected; some crosscuts to convey atmospheric air from the drift to the rock masses undergoing bioleaching, and of blasting some explosives. Since the cost of these preparation works may amount from 70 to 80 % of total capital outlay involved in bioleaching, this zone has been subdivided into various blocks, where different preparation methods will be tested in order to find the least expensive.

One block has been fragmented by blasting eighteen 95-m deep blastholes, designed in such a way that swelling (15 %) filled the existing old drifts. The explosive charges were placed in the lower sections of the blasthole, so that the upper part would not be damaged by the blast. In this way, the upper parts of these holes, lined with stainless steel pipes, could be further utilized for leach solutions injection. The average explosive charge was 1120 kg per blasthole and the powder factor was 0.7 $kg \cdot m^{-3}$.

5.6.3.4. Leach Solutions Management and Composition

In the Northern lens the solution is distributed by means of the method of ponds (see Section 5.5.4.1) prepared on top of the dump overlaying the pillar, as well as on the tops of block No. 45 and of the lean ore dump.

In the Southern lens leach solutions are injected into the broken rock mass via 52 holes drilled from the surface at 10 m centers. These holes are cased with polyethylene-lined pipes and range in depth from 40 to 87 m. Solutions are also introduced into the Eastern lens by injection through drillholes.

The Southern and Northern lenses as well as block No. 45 are leached all year round. The lean ore dump is leached for seven months of the year (from May to November).

Pregnant solutions from the underground operations, with pH ranging from 2.5 to 1.0, drain through cracks and cavities into underlying drifts (old drifts or drifts purposely excavated, like in the Eastern lens) and are finally collected in underground pools and are then pumped either to the Cu contact precipitation ("cementation") plant or to a head tank for recycling.

The specific flow rate of leach solutions ranges from $50 \cdot 10^{-3}$ to $60 \cdot 10^{-3}$ $m^3 \cdot Mg^{-1}$ of ore in the Northern and Southern lenses and experience has shown that in the Eastern lens it is optimum at about 0.14 $m^3 \cdot Mg^{-1}$ of ore. Slightly different rest cycles were adopted in the different zones: in the lean ore dump and block No. 45 of the Northern lens rest time is 10 days; in the Northern lens proper and in the Eastern lens the rest cycle is 20 days.

The coefficients of percolation are $20.5 \cdot 10^{-3}$ $cm \cdot s^{-1}$ through the Southern lens, $5.6 \cdot 10^{-3}$ $cm \cdot s^{-1}$ through the dump and $1.25 \cdot 10^{-3}$ $cm \cdot s^{-1}$ through the Northern lens.

In the Eastern lens it was observed that after 2–3 years operation solution coefficients of percolation in some injection holes fell sharply. This drop was due to self-grinding of the leached ore resulting in the formation of fines and clays and to the precipitation of iron hydrates which all contributed to reducing the permeability of the upper layers of the broken rock mass. Short-term acidification of leach solutions to 2–3 $kg \cdot m^{-3}$ H_2SO_4 partly offsets this drawback, producing an increase in coefficients of percolation and in copper concentration in the pregnant liquors. Acid consumption is 3 kg per kg of copper.

The effect of temperature on microbial activity was investigated with surveys carried out underground in summer and winter during the 2nd and 3rd year of operation in the Eastern lens. Table 5.17 shows the findings of this survey. It emerged that low temperature of solutions and of the ore (from 6 to 12 °C) is the main cause of inhibition of bacterial activity. For instance, a drop in temperature from 22 to 12 °C results in a decrease in ferrous iron daily oxidation rate from 224 to 120 $g \cdot m^{-3}$. It should be noted that, in the absence of bacteria, Fe^{2+} was not oxidized at such temperatures.

It is thought that improving aeration of the ore mass enhances ore oxidation processes and the temperature inside the broken rock mass may thus reach 25 °C or more.

Natural aeration is active in block No. 45 and in the dump, as proved by the temperature, which attained from 40 to 60 °C in the former and from 25 to 30 °C in the latter, whereas the temperature of the influent leach liquors ranged from 10 to 14 °C.

Owing to the generally unfavorable environmental conditions (scarce or non-existent rock aeration and low temperatures of the orebodies, ranging from 8 to 14 °C) the number of **T. ferrooxidans** cells in the effluent liquors did not exceed 10^4–10^5 $cells \cdot cm^{-3}$. From 90 to 98 % of iron in solution is ferrous. The highest bacterial activity – 3.9 $counts \cdot s^{-1} \cdot cm^{-3}$ and 4.1 $counts \cdot s^{-1} \cdot cm^{-3}$ respectively – was found in the effluent liquors from the Northern lens and the dump; the lowest activity was recorded in the leach liquors from the Southern lens (2.0 $counts \cdot s^{-1} \cdot cm^{-3}$).

Table 5.17

Results of the microbiological survey of underground leaching site in Blyavinsk deposit

Solution	Time of investigation	Temperature, °C	pH	Redox potential, mV	Fe^{2+}, $g \cdot dm^{-3}$	Fe^{3+}, $g \cdot dm^{-3}$	Cu, $g \cdot dm^{-3}$	Bacterial concentration, $cells \cdot cm^{-3}$
Drainage solutions	August	12.5	2.5	412	1.14	0.11	0.23	2×10^5
	November–December	6	2.76	390	1.93	0.07	0.25	2×10^4
Solutions from 301 m level	August	12	2.02	445	3.74	0.11	0.47	2.5×10^5
	November–December	9	2.11	415	2.63	0.17	0.40	1.5×10^5
Injected solutions	August	12	2.66	417	1.40	0.12	0.26	2×10^5
	November–December	6	2.70	410	2.23	0.17	0.28	1.5×10^5
Pregnant solutions from Eastern lens	August	12	2.54	440	2.91	0.26	0.69	1.5×10^5
	November–December	6	2.39	420	3.73	0.32	0.58	9.5×10^4
Solutions prior to cementation	August	11	2.66	425	2.34	0.26	0.60	9.5×10^4
	November–December	3	2.86	417	3.01	0.34	0.45	10^5
Solutions after cementation	August	11	2.85	395	3.60	0.04	0.006	9.5×10^4
	November–December	3	3.73	380	4.00	0.05	0.004	9.5×10^4

The concentrations in the pregnant liquors ranged from 3 to 5 $kg \cdot m^{-3}$ Cu in the dump and in block No. 45, 1.5 to 2.0 $kg \cdot m^{-3}$ in the Northern lens and 0.15 to 0.25 $kg \cdot m^{-3}$ in the Southern lens. The average concentration of pregnant liquors not diluted by natural solutions ranged from 1.7 to 2.0 $kg \cdot m^{-3}$. Mixing with natural solutions lowered the average concentration to between 1.1 and 1.3 $kg \cdot m^{-3}$ of Cu.

The average yearly copper recovery was 15.1 % from the Southern lens and 17.4 % from the Northern lens.

In the Eastern lens, recovery after four years operation was 62 %, on average 12.4 % per year. During the first three years the yearly copper recovery was 15 % and dropped to 8.5 % in the fourth year. Copper concentrations in leach liquors are twice as high in summer than in winter, corresponding to temperature variations from 12 to 6 °C.

Pregnant liquors flowing out of the rock masses are collected in underground sumps and pumped to the outside.

5.6.4. The Avoca Underground In-Situ Commercial-Scale Bioleaching Test [32, 33, 63]

This test was recently carried out with the financial support of the European Economic Community and it provided a wealth of useful advice on this technology.

5.6.4.1. The Orebody

The Avoca copper sulfide deposit is located in the southern part of County Wickham, approximately 6 miles north-west of Arklow, in Ireland. The mining area, which strikes NE/SW is divided into east and west sectors by the Avoca river valley. The orebody consists of lenses of quite highly mineralized rock in little mineralized chloritic host rock. The location selected for the bioleaching test was a block of ore approximately 25 m long, 9 m wide and 12 m high situated in the hanging wall of the partly mined 14 stope on the 850 level at East Avoca. The average ore density is 2941 $kg \cdot m^{-3}$.

The orebody, approximately 20 m wide, is bordered on the footwall by pyritized shale and on the hanging wall by sericitic tuffs. Both of these rock types are reasonably impervious except in areas of intense jointing. The joint structure was recognized as having considerable significance in fragmentation and liquor recovery from the ore zone. Two sets of semi-vertical and a single set of horizontal joints intersect the general foliations at high angles. The intersection of these joints results in a prismatic fracture pattern, which is significant in two respects. If they are not naturally or artificially sealed, the joints at the base of the ore zone will act as conduits for the passage of leach solutions to lower inaccessible levels. Extensive jointing also inhibits transmission of the compressive energy wave during blasting, as each joint effectively acts as a free face. To obtain maximum fragmentation, the design of the blasthole drilling pattern had to take into account joint direction.

Table 5.18

Quantitative mineralogical analysis of crosscut ore [32]

Mineral	%	Mineral	%
Quartz	69.0	Chalcopyrite	2.74
Amesite	14.7	Pyrite	4.01
Magnesium Chamosite	3.7	Sphalerite + 10 % Fe	1.10
Sericite	1.7	Galena	0.46
		Others	2.59

Major sulfides present are pyrite, chalcopyrite, iron-rich sphalerite and galena and occur in veins or stringers varying in thickness between 13 cm and 60 cm and parallel to the foliation. Each stringer has a well defined structure normally comprising a core of crystalline pyrite, separated by nodular white quartz from a peripherally zoned region of chalcopyrite, which in turn is in contact with the chlorite/sericite gangue. Sphalerite and galena are associated with the stringers, but occasionally form crosscutting veins.

The intervening gangue between ore stringers contains only small amounts of sulfide mineralizations. It is composed mainly of fine cryptocrystalline quartz penetrated by fine contorted layers of sheet silicate. The main sheet silicate, amesite, magnesium-chamosite and sericite, are associated with any sulfides present.

Table 5.18 shows a quantitative mineralogical analysis of samples taken from a crosscut driven in the block.

The natural porosity in the main quartz/chlorite/sericite rock is in the order of one per cent and water permeability in the order of 10^{-12} $m \cdot s^{-1}$, i.e. this type of rock is highly impervious (Section 5.3.2.2). Fragmentation by blasting is therefore essential for the in-situ leaching of this ore.

5.6.4.2. Microorganisms

The species existing in the natural mine waters at Avoca are diverse and well adapted to the ore environment. Strains of **T. ferrooxidans, T. thiooxidans** and other thionine bacteria and heterotrophs were isolated from mine water at various pH's.

5.6.4.3. Ore Fragmentation by Blasting

From the crosscut driven into the block a series of holes was drilled in a fan-shaped pattern. These were filled with ANFO (Ammonium Nitrate/Fuel Oil) or Frangex (ANFO base + nitroglycerine), which were considered the most suitable explosives, despite their relative low energy characteristics. Powder factor (expressed in terms of kg per m of hole) ranged from 2.4 to 4.0.

Fragment size ranged from 30 to 100 cm at the easterly end of the stope to 95 % smaller than 15 cm in the drawpoints close to the crosscut. The results of blasting were therefore difficult to quantify. The swell amounted to 33 %. The overall cost of fragmentation was 3.12 Irish pounds per tonne.

5.6.4.4. The Main Leaching Circuit

The appropriate selection of the stope produced a veritable textbook leaching circuit. Leach solutions are pumped from the sump to the top of the broken rock mass and irrigation is carried out by means of Bagdad-type surgical tubing sprinkler heads or "wigglers" (section 5.5.4.1). After percolation through the ore, the pregnant solution is collected in the sump. Part of it is delivered to the cementation plant, the rest is recirculated together with the required amount of make-up minewater, which also compensates for the liquor lost through cracks etc. at the base of the broken rock mass. One major feature of this circuit is the automatic pH control circuit whose main objective is to prevent the rise of leach solution pH produced by cementation and by acid-consuming material in the ore. The pH meter is connected, through a transducer, to a pump which feeds H_2SO_4 to the sump automatically maintaining the leach solution pH at any desired preset value.

Another remarkable feature of this test is the monitoring of oxygen potential of leach solutions in various points of the circuit. These measurements revealed or confirmed several facts, among which:

a) Oxygen is subtracted to the leach liquor in the cementation section and during its percolation through the 15-m thick broken rock mass, attaining levels as low as 2 ppm at the bottom. Since the activity of oxidizing autotrophic bacteria is inhibited below 1 ppm O_2, the conclusion can be drawn that the depth limit during active percolation leaching is in the order of 22 m for a leach liquor flow rate of $2.8 \cdot 10^{-6}$ $m^3 \cdot s^{-1} \cdot m^{-2}$. Below this depth a sulfide ore will only be leached during rest periods.

b) O_2 levels increase rapidly to approximately 5 ppm in the delivery liquor, as the spent solution cascades through the sump system, the barren liquor falls into the mixing sump and oxygenated make-up mine water is added. A further increase, up to a maximum O_2 level of 9 ppm, takes place when the leach solution is pumped up to the wiggler distribution system and is sprayed on the surface of the fragmented rock mass. Air diffusion through the latter contributes to compensate the oxygen loss and, in agreement with Harris [75] and Brimhall and Wadsworth [25] (see Section 5.3.1.); in this respect drainage of all but pellicular water and therefore the correct alternation of rest and wetting periods is of fundamental importance.

Loss of leach liquor through fractures in the ground at the base of the stope were as high as 40 % at the beginning of the operation, but dropped to less than 15 % during the first 250 days, presumably as a consequence of the sealing of some openings with fine material.

Over days 200–250 the average rate of extraction remained fairly constant at about 17 $mg \cdot s^{-1}$ which, according to the authors of the reports on this test, should correspond to approximately 8 % total Cu contained in the run-of-mine ore per annum.

A major disadvantage of the rest/leach cycle is that the metal extraction rate determined during the leaching cycle, must be discounted to cover the rest period as well, if an overall rate of extraction is to be determined. At Avoca, where the ratio between rest and leach cycles has been established at 3:1, this would imply that in order to obtain an overall annual extraction rate of 10 %, the extraction rate during the leach cycle must be in the order of 40 %. In mining terms this means that return on invested capital will be longer than for conventional mining processes. The low capital and operating costs could however, still outweigh this disadvantage.

5.6.5. An Engineering Prefeasibility Study for In-Place Bacterial Leaching (IPBL) of Copper [83]

Although this is not, strictly speaking, a case history, since it does not concern the analysis of an ongoing operation, it appears to be a very significant example of a realistic, stepwise, technico-economic approach to the solution of the problem of assessing the feasibility of in-situ underground bacterial leaching applied to a deposit within the domain of an existing mine. It must be stressed that this is only apparently a particular case; in fact, the parts of orebodies which are too small and/or contain ore too lean or complex to be mined profitably with conventional mining and milling techniques, but where hoisting facilities and drilling equipment are available, are represented by this example. These orebodies present the advantage that capital investments are limited to the leach and recovery plants.

For the problem which arises in the case where some development work is needed and the metal values contained in the ore as well as the swell have to be extracted, two alternatives exist: either the ore is beneficiated in a concentrator at or in the vicinity of the mine and sold as metal concentrate, or the metal value is extracted via heap leaching. The heaps can be formed either at the surface (the case of Agnew Lake Mine [143, 145]) or even underground if large enough cavities exist and are in stable rock.

The project entails four phases: mine site selection, prefeasibility of underground operation, prefeasibility of the metallurgical circuit and overall assessment and sensitivity analysis.

5.6.5.1. Mine Selection

Seven copper sulfide mines operated by the Noranda Company were evaluated through a ranking process that took into account mine configuration, geology, grade of copper and other metals, existence of surface facilities and site-specific factors, such as potential damage to the environment.

The choice fell on the Geco Mine, in Manitouwadge, Ontario. The orebody is lens-shaped, about 730 m long and 20 m wide and some 36 M Mg of ore have been mined since production commenced in 1957. The ore in the zone selected for the study is constituted of 2.1 M Mg of disseminated sulfides (1.5 % pyrite, 2.9 % pyrrhotite, 2.6 % chalcopyrite and 93 % quartz) in sericite schist at a grade of 0.9 % Cu. The mineralized rock is competent and can be classified as impervious, its original coefficient of permeability ranging from 10^{-11} to 10^{-10} $m \cdot s^{-1}$.

5.6.5.2. Rock Preparation for IPBL

The very low permeability of the rock required preliminary fracturing by blasting. It was therefore necessary to select the stoping method most suitable for ore preparation. After an exhaustive comparative analysis of the applicable methods, sublevel stoping was selected for the following reasons:

a) availability of equipment and expertise at the mine and hence no need for capital investment for new equipment;

b) moderate costs; no capital requirement for the mine shaft and concentrator;

c) possibility of achieving the desired particle size distribution of the broken rock mass;

d) possibility of forming the broken rock masses in such a way as to enable access to their upper surface and the installation of a sprinkler leach solution distribution system.

For fracturing the ore to an average size of −150 mm a powder factor of 0.7 $kg \cdot Mg^{-1}$ of run-of-mine (r.o.m.) ore was assumed. 25 % of the r.o.m. ore would be removed to allow for swelling during blasting.

5.6.5.3. Leaching Process

The technico-economic calculations were based upon 55 % Cu recovery from chalcopyrite leaching over a 10-year period and an average annual production of 1000 Mg of copper. The specific rate of solutions was set at $2.78 \cdot 10^{-6}$ $m^3 \cdot s^{-1} \cdot m^{-2}$ (corresponding to 10 $dm^3 \cdot h^{-1} \cdot m^{-2}$), with a pregnant solutions flow rate of $31.6 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$.

The pregnant solutions, containing 1 $kg \cdot m^{-3}$ copper, are pumped to a surface cementation plant through a 150-mm diameter rubber-lined carbon steel (RLCS) pipe with a geometric head of 1000 m which is overcome with three pumping stages each of which provided with two 110 kW pumps (one standby). The cement cake assays 85 % Cu at 15–20 % moisture. Spent solution from the cementation plant is sent underground through a 150-mm RLCS pipe with pressure let-down valves every 70 m to reduce pressure to 345 kPa required for the sprinkler system.

The ore produced in stope development (drivage of drifts, crosscuts and raises, as well as the swell after blasting, which must be removed in order to create the required clearance on top of the broken rock mass) is treated by the concentrator. All mining development costs are considered operating costs and the revenue obtained from treating the r.o.m. thus produced is deduced therefrom.

The cost analysis of the ore produced in development work is shown in Table 5.19.

To the total of Table 5.19 the incidence of the cost of concentrate transportation (38.50 $/Mg) and smelting and refining charges (0.85 $/kg of copper in concentrate) referred to one Mg of r.o.m. ore must be added. The sum S is the total cost of extracting copper from one Mg of r.o.m. The credit C from development r.o.m. ore is calculated on the basis of a copper price of 2.20 $/kg and therefore the difference

$$D = S - C$$

is the credit from development r.o.m. ore and amounts to 1.35 $/Mg.

Table 5.19

Cost analysis of r.o.m. produced in development work

Item	Mine transportation from face to shaft	Hoisting	Concentration	Total
$·Mg^{-1} of r.o.m. ore	0.5	1.1	5.5	7.1

Table 5.20

Mining development costs

Development item	Access	Over-cuts	Under-cuts	Raise	Sublevel develop-ment	Slot drilling blasting	Stope	
							Drilling	Blasting
$/Mg of leach-able r.o.m. ore	3.45	2.34	0.20	2.20	1.84	1.74	1.71	2.61

Total Mining Development Cost	16.09 $/Mg
Credit from Development r.o.m. Ore	1.35 $/Mg
Net Cost of Mining	14.74 $/Mg

Table 5.21

Capital costs for in-place bacterial leaching ($ x 1000)

Leaching Section		Cementation Section	
	Direct Costs		Direct Costs
1. Piping	490	1. Sitework, structure foundations and building	670
2. Pumps	233	2. Equipment	370
3. Bleed Circuit	40	3. Electrical	100
Installed equipment	763	4. Instrumentation	25
4. Iron residue pond	150	5. Piping	75
5. Electrical (6 % of I.E.)	46	6. Miscellaneous	25
6. Instrumentation (4 % of I.E.)	30	Total Direct Costs	1,265
7. Process control (5 % of I.E.)	38		
8. Laboratory (8 % of I.E.)	61		
Fixed Capital	1,088		
9. Start-up costs (10 % FC)	108		
Total Direct Costs	1,196	Indirect Costs	467
Indirect Costs	440		
Total	1,636	Total	1,732

Table 5.22

Operating costs for in-place bacterial leaching (¢/kg copper)

Leaching Section		Cementation Plant	
Cost Component	Cementation	Cost Component	Cementation
Labour	4.51	Labour	17.95
Reagents	4.82	Reagents	44.99
Supplies	0.55	Supplies	6.18
Power	16.70	Power	1.50
Residue Disposal	1.30		
	27.90		79.62
		Supervision	5.84
			85.4

Unit Costs of Reagents On-Site		Other Unit Costs	
	$/Mg		
Sulfuric Acid	50	Electricity	4 ¢ kWh
Lime	80	Residue Disposal	3 $/Mg
Scrap Iron	180	Labour	45,000 $/year

Table 5.20 gives details of mining development costs.

Tables 5.21 and 5.22 show the capital and operating costs respectively for IPBL.

The economic analysis which, for the sake of brevity, is not reported in detail here, shows that for the conditions existing in the Geco mine, neither IPBL nor conventional mining and concentration are economically viable for producing 1000 Mg per year of copper from 3.8 million Mg of r.o.m. ore grading 0.9 % Cu. In the case of IPBL only a reduction of 87 % of all operating costs or an increase in the price of copper to 9.22 $/kg would render this solution economically acceptable.

A sensitivity analysis reveals that development costs condition the economic viability of the process and that such costs depend to a great extent on fracturing costs. It is therefore imperative that particular care be taken in every feasibility study on IPBL, as far as preparation costs are concerned, especially blasting costs.

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Chapter 6

ROLE OF MICROORGANISMS IN LEACHING OF NON-SULFIDE ORES

6.1. LEACHING AND BENEFICIATION OF MANGANESE ORES

In processing manganese ores, microorganisms could be used to reduce Mn^{4+} to Mn^{2+}, to solubilize manganese carbonates, and to decrease the silicon and phosphorus content of the ores. In such cases the grade of ores can be considerably improved.

6.1.1. Characteristics of Manganese Minerals

In nature, manganese occurs as an element in over a hundred minerals. The major accumulations, at present, are in the form of oxides, carbonates and silicates. The most important oxides are: psilomelane ($mMnO \cdot nMnO_2 \cdot pH_2O$), birnessite ($\delta\ MnO_2$), pyrolusite ($\beta MnO_2$), manganite ($Mn_2O_3 \cdot H_2O$ or MnOOH), todorokite [$(Mn^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, K^+, Na^+)_2 Mn_5^{4+} O_{12} \cdot 3H_2O$], hausmanite ($Mn_3O_4$) and braunite ($Mn^{2+} \cdot Mn^{4+} O_3$). Rhodochrosite and rhodonite are the most important minerals among carbonates and silicates, respectively. These are either secondary minerals resulting from degradation of the primary igneous minerals, or authigenic minerals resulting from the precipitation of dissolved manganese. Manganese is an element of industrial importance.

From the point of view of microbial action on manganese the most important property is that manganese shows a variable valency character. This element occurs in the oxidation states of 0, +2, +3, +4, +6 or +7. In nature, however, +2 and +4 states are prevalent. The Mn^{2+} from manganese ion occurs at pH below 5.5 while the Mn^{4+} form occurs at pH above 5.5, if the Eh is around 0.8 V. At Eh below 0.5 V, the Mn^{2+} form prevails in the pH range of 7.8 to 8.0. At pH above 8.0, autooxidation occurs when an aqueous solution of Mn^{2+} ions is exposed to air [8].

It is reasonable to discuss only diagrams of equilibrium for pyrolusite (MnO_2), which represents the bulk of manganese containing sedimentary rocks, and is the most stable of the listed manganese compounds.

Some of the reaction equations, showing the relationship of manganese ions and pyrolusite are shown in Table 6.1. The stability of manganese ions in aqueous solutions is shown in Fig. 6.1 and approximate position of some natural media in Eh-pH coordinates is superimposed on this diagram.

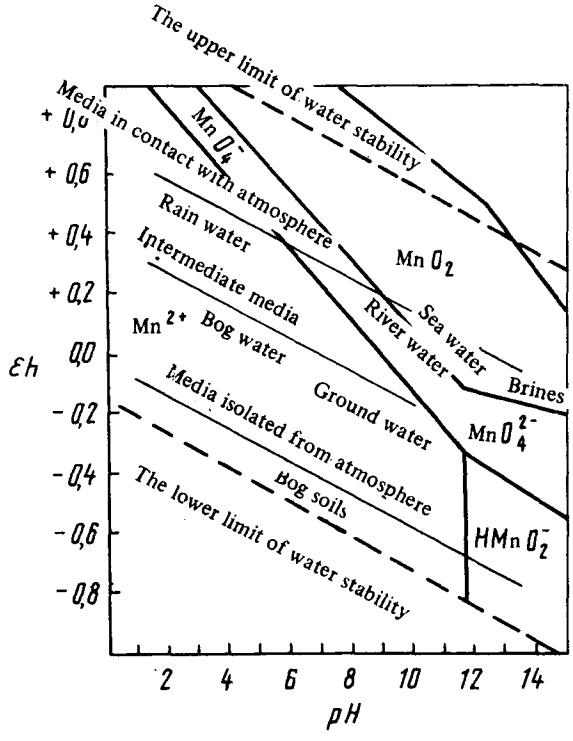

Fig. 6.1. Diagram of manganese ion and pyrolusite stability in natural media

Table 6.1.

Reactions & equations showing relationship between activities of manganese ions & pyrolusite

Reactions	Equations
1. $HMnO_2^- + 3H^+ = Mn^{2+} + 2H_2O$	pH = 11.4
2. $Mn^{2+} + 4H_2O = MnO_4^{2-} + 8H^+ + 4\bar{e}$	Eh = 1.15 — 0.118 pH
3. $HMnO_2^- + 2H_2O = MnO_4^{2-} + 5H^+ + 4\bar{e}$	Eh = 0.65 – 0.074 pH
4. $MnO_4^{2-} = MnO_4^- + \bar{e}$	Eh = 0.56
5. $Mn^{2+} + 2H_2O = MnO_2 + 4H^+ + 2\bar{e}$	Eh = 1.22 – 0.028 lg $[Mn^{2+}]$ – 0.118 pH
6. $HMnO_2^- = MnO_2 + H^+ + 2\bar{e}$	Eh = 0.203 – 0.028 lg $[HMnO_2^-]$ – 0.028 pH
7. $MnO_2 + 2H_2O = MnO_4^- + 4H^+ + 3\bar{e}$	Eh = 1.695 – 0.019 lg $[MnO_4^-]$ – 0.078 pH
8. $MnO_2 + 2H_2O = MnO_4^{2-} + 4H^+ + 2\bar{e}$	Eh = 2.26 – 0.028 lg $[MnO_4^{2-}]$ – 0.118 pH

It can be seen from the diagram (Fig. 6.1) that the Mn^{2+} ion occupies a considerable area of stability and can occur in acid, neutral and alkaline media. However, as per equation 2, with the increase in the redox potential, the area of its stability shifts toward low pH values. The $HMnO_2^-$ ion is stable only in the area of highly alkaline solutions with extremely low Eh values. The MnO_4^{2-} ion has a considerable field of stability in the range from alkaline to neutral and even low acid solutions. At Eh above +0.56 V, all manganese ions are converted to MnO_4^-. The Mn^{2+} ion is metastable in the Eh and pH range typical of natural waters and does not possess a field of stability of its own.

The diagram of stability of manganese ions can be superimposed on the diagram showing an approximate position of some natural media in Eh-pH coordinates. Fig. 6.1 shows that the Mn^{2+} ions can occur only in waters which are partially or completely isolated from the atmosphere. In the area of high Eh values these are mine waters while in the area of low and negative Eh values, marsh and water soils could be included.

In well aerated waters, only MnO_4^- or MnO_4^{2-} ions can be relatively stable depending on Eh and pH. According to Garrels and Christ [9], these two ions were not stable with respect to water and were gradually converted to MnO_2. From Fig. 6.1 it is obvious that this mineral is stable only under conditions obtained in the well aerated surface waters. The diagram of equilibria indicates that with lower redox potential and higher hydrogen ion concentration in the aqueous environment, pyrolusite becomes thermodynamically unstable and will be degraded with release of the Mn^{2+} ions.

6.1.2. Leaching of Manganese

Heterotrophic bacteria. The US Bureau of Mines [22, 27] used in their experiments a 500 g sample of Manganese ore containing 4 % Manganese in the form of oxides. The ore was placed in a 2 litre bottle and supplemented with 1 litre of water and a small amount of organic material (leaves, yeast, manure, soil, etc.) containing various types of microorganisms. Similar flasks with ore but without bacteria served as controls. After 90 days the solution in the bottle with microorganisms contained 5 g/l Mn while in controls Mn was not detected. Low-grade ores from Boulder City, Nevada (3 % Mn) and Kuyuna Range (5 % Mn) deposits were also used in the experiments. To one litre of water, four cultures of microorganisms were added separately, to which 8 g of nutrient substances (beef extract and peptone) were added along with 100 g of ore. The cultures of microorganisms were isolated from manganese ore and dumps containing industrial wastes. As manganese was leached out the solution was partially replaced at regular intervals to prevent the Mn concentration from exceeding 3 g/l. The pH of the medium was adjusted to pH 5–6 with glacial acetic acid. The experiments were conducted at 21–29 °C. After 60 days, on an average about 97.5 % of manganese was leached from the ore. In the controls without bacteria, only traces of manganese were detected.

Manganese was extracted from the solution by precipitation with adding alkali to the medium to raise the pH to exceed 7.0. The microorganisms simulta-

neously leached iron. Thus, the precipitate from the solution after leaching of ore from Kuyuna Range deposit contained 39 % manganese and 12.4 % iron, and from the Boulder City deposit, 33.8 % manganese and 2.5 % iron.

In India, using cultures of **Pseudomonas sp.** and **Basillus sp.** manganese leaching was carried out at all the 3 levels, i.e. flasks, columns and tanks from an ore containing 44 % manganese [1]. The solubilized manganese was precipitated by addition of lime for quantitative recovery. At the end of 90 days nearly 90 % manganese was recovered for both the species used [5].

In the USSR Gvilava used heterotrophic bacteria belonging to **Aeromonas sp.** and **Pseudomonas sp.**, adapted to the process conditions to leach manganese from wastes containing 12–14 % Mn . The experiments were conducted in a liquid medium: solid to liquid ratio (S:L) = 1:20, pH 7.45, ore size – 0.15 mm with active aeration and using 10^8–10^9 bacterial cells/ml. The recovery of manganese was 90–96 % in 12 days.

Autotrophic bacteria. In Japan another method was developed of leaching manganese from manganese ores using **T. thiooxidans** [15, 23, 24]. These bacteria were grown on a sulfur containing medium. After 1.5–1.7 g/l sulfuric acid accumulated, the medium was supplemented with ground manganese ore with iron, zinc and other sulfides, gaseous hydrogen sulfide or sulfur dioxide. When sphalerite (ZnS) was added the rate of manganese leaching increased almost 20-fold; and similar results were obtained with addition of pyrite (10-fold) and covellite (5-fold increase). MnO_2 does not dissolve in weak solutions of sulfuric acid, another does, **T. thiooxidans**, on it's own, as ineffective in manganese leaching. However, addition of hydrogen sulfide to the medium contributed to the growth of **T. thiooxidans** and considerably increased the rate of manganese leaching.

Another method of manganese leaching was based on converting MnO_2 to manganese dithionate (MnS_2O_6) with the addition of sulfur dioxide. Chemically, the process proceeds easily. Manganese dithionate is comparatively resistant to chemical oxidation. However, in presence of **T. thiooxidans** MnS_2O_6 readily is oxidized to $MnSO_4$. Following addition of **T. thiooxidans** to the solution with agitation at pH 3–4 and at 30 °C the major part of MnS_2O_6 was converted to $MnSO_4$ in 24 hours. The experiment was conducted as follows. A 50 g sample of crushed ore containing MnO_2 (22 %) and 200 ml of water was placed in a chamber through which sulfur dioxide (100 % concentration) was blown from the chamber bottom at the rate of 300 ml/min for 20 min. Manganese dioxide was almost completely converted to manganese dithionate. In another vessel, **T. thiooxidans** was grown with agitation for 3 days at 30 °C. Then, 100 ml of the solution separated from the solid phase and containing manganese dithionate, was neutralized with Na_2CO_3 to pH 4.0 and diluted with water to 200 ml. 70 ml of **T. thiooxidans** culture was added to the solution and aerated for 20 minutes at 30 °C. Then 97 % of MnS_2O_6 was found to be converted to $MnSO_4$.

6.1.3. Technological Aspects of Manganese Leaching

A description of dump and tank techniques of manganese leaching is given below.

Dump leaching

The dump (percolation) leaching of manganese should be carried out in transparent columns made of corrosion-resistant organic material. The height of the columns must ensure complete consumption of nutrient substances by the sorbate microflora under conditions that diameter of the columns should be adequate to preclude the "wall effect" and to mount pipes for sampling, etc. The diagrammatic presentation of laboratory installation is shown in Fig. 6.2.

Since the slurry after enrichment is a mixture of particles of different size, its percolation ability is governed by the law of Darcy and depends on the effective diameter of the particles. Nutrient medium fed into the slurry percolates between the grains. The correlations between the percolation rate of nutrient medium and depth of slurry layer in the columns must be such as to minimize the washout of nutrient substances (around 0.02 % of reducing sugars). Simultaneously, the following principal processes will take place in the slurry:

consumption of nutrient substances;

reduction of manganese oxides and hydroxides.

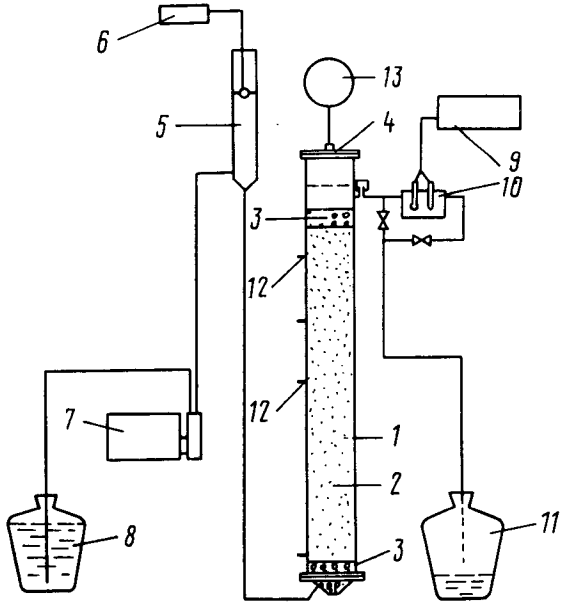

Fig. 6.2. Graphic presentation of an installation for dump (percolation) leaching of manganese:

1 – acrylic plastic column; 2 – tailings of manganese ore dressing; 3 – sand and gravel filter; 4 – connection for gas removal; 5 – pressure regulator; 6 – automatic regulator; 7 – pump; 8 – tank for nutrient medium; 9 – pH meter; 10 – chamber with pH-meter electrodes; 11 – receiving tank for filtrate; 12 – connections for taking samples; 13 – gas meter

According to experimental data, consumption of nutrient substances is linear with height and depends on the activity of the bacterial culture. The height to which nutrient substances penetrate in sufficient quantities also depends on the hydraulic characteristics and their concentration. Oxidation of nutrient substances is accompanied by generation of organic acids and depends on the height of ore layer in the column. The glucose content in the column section was sharply reduced up to 0.5 m ht. However, higher concentration of dissolved manganese in the filtrate was observed in the column section from 0.5 m to 1.4 m. This can be attributed to both the generation of active reducing agents and more favorable redox conditions in this section.

At higher levels in the column, the concentration of dissolved manganese decreases. Probably in this section, as a result of intensive liberation of carbon dioxide and high activity of carbonic acid, manganese carbonates were formed and precipitated in the porous spaces of the slurry. Partially, dissolved manganese was adsorbed by the upper layer of slurry.

Increase in the concentration of dissolved manganese was linked with the increase in consumption of nutrient medium in the column. However, a constant high rate of consumption of nutrient medium was possible only in the first days of the operation of the column. Due to the reasons described below, the percolation ability of slurry in the column was continuously found to be decreasing, and, therefore, to maintain the high rate of consumption of nutrient medium, considerable pressure had to be applied. Any decrease in the consumption of nutrient medium would cause an intensive oxidation of organic acids to carbon dioxide in the upper part of the column. Comparison between the percolation characteristic of the slurry and degree of oxidation of carbohydrates shows a clear inverse relationship between the percolation coefficient and specific yield of gas. At a sufficiently high rate of percolation, 3–6 % of carbohydrates were oxidized to CO_2, while at lower percolation rates production of CO_2 sharply increased, when as much as 33 % of carbohydrates were oxidized to CO_2. This type of considerable gas production resulted in gas entrapment in the column and formation of gas discontinuities. To reduce the influence of the gaseous phase on the percolation ability of the slurry, an upward flow of nutrient medium in the column was considered.

Changes in the granular composition of the slurry were observed in all cases. Manganese leaching was accompanied by the increase in size of fractions. The effective diameter of the particles, which controls the percolation ability of the material, also changes in the process.

When the percolation properties of finegrained and mediumgrained sands for use in the columns were investigated by recording the percolation coefficient at different time intervals, followed by granulometric analysis of the various fractions of slurries before and after microbiological treatment it was observed that the percolation coefficient of finegrained sand decreased more quickly*) than that of mediumgrained sand. This was noted to be the main limiting factor

* The granulometric analysis showed that, concomitantly, the effective diameter of finegrained sand slurries also decreased with time.

for the application of dump leaching technique for manganese ores. It was observed that during the period of active percolation, microbes could not solubilize more than 25 % manganese contained in the ore initially. Therefore, dump leaching could be recommended only in few cases to treat only the extremely low, coarsegrained ores.

Tank leaching

The advantages of tank leaching are as follows: possibility to actively control the mass transfer of nutrients, oxygen, various reagents, and to maintain the required temperature.

Disadvantages of tank leaching include difficulty in maintaining sufficient degree of pulp agitation in high capacity tanks, high abrasiveness of the pulp and intensive washout of highly dispersed materials in continuous flow systems.

Because of the presence of solid phase (ore), apparatus of complete displacement occurs such as corridor-type aerotanks, cannot be used for manganese leaching. Practically, only apparatus like chemostat, tanks with stirrers of various designs or battery of chemostats can be used.

The rate of manganese leaching under conditions of intensive agitation depends on the following factors: pulp density, amount of bacterial biomass, mass transfer, temperature, redox potential and pH of the medium. Examples of 2 types of bacterial tank leaching of manganese are discussed below.

A batch process. The aim of the experiment was to determine the main parameters of the leaching process, namely, dynamics of manganese leaching and consumption of nutrients.

The initial conditions. Tailings of enriched manganese ores containing 9.9 % manganese and nutrient medium (aqueous solution of molasses containing 0.5 % reducing sugars in term of glucose) were used. The inoculum was introduced into the tank at 10 % level. Cell concentration of microorganisms in the inoculum is 10^8 cells/ml. Experiments were carried out with a solid-to-liquid ratio of 1:10 and temperature of 26 °C, under aerobic conditions, achieved by means of pulp agitation, without forced aeration. Agitation was carried out on a 24-hour basis using a mechanical stirrer at 200–300 rpm.

The experiment. In a previously calibrated leaching reactor with an effective capacity of 180 l through a hatch in the lid 18 kg of manganese ore dressing tailings were loaded, then 162 l of nutrient medium were pumped in, and finally 18 l of inoculum were added. Then agitation was started. Sampling was carried out 2–3 times a day.

After 24 hours, concentration of reducing sugars in the culture medium dropped to a value, at which the leaching process might cease. Therefore, it was necessary to add concentrated molasses to the culture medium in order to increase the sugar content. Table 6.2 presents the results of determining concentration of manganese and reducing sugars, and the calculation of the main features of the leaching process.

It was observed from Table 6.2 that a high rate of manganese solubilization (over 47 mg/l/h) maintained after first addition of molasses (24 h) when the ratio of sugar used to manganese produced was maintained on an average of 3.3.

Table 6.2

Main features of the process of manganese leaching

Duration, h	Concentration of dissolved* manganese, mg/l	Concentration of reducing sugars, %	Consumption of sugars, g/l	Rate of manganese solubilization, mg/l/h
0	30	0.50	0	
8	410	0.40	1	15.8
24	1170**	0.15/0.5**	3.5	47.5
32	1560	0.37	4.8	48.8
48	2310	0.1	7.5	47.5
			Average	47.5

* Concentration of manganese solubilized due to acidic products of molasses.

** After introduction of an additional quantity of molasses.

A continuous process. The aim of the experiment was to obtain a sample of the concentrate.

The initial conditions were similar to those in batch process. Here, the flow rate was kept at 6 l/h and manganese hydroxide was precipitated using 25 % ammonia solution at pH 10.

The experiment. The experiment was carried out in an scale-up laboratory installation. Manganese was leached in a reactor with an effective capacity of 180 l. The reactor, 18 kg of manganese ore tailings were loaded, then 162 l of nutrient medium and 18 l of inoculum were pumped in and the agitation was started.

During the first day of the experiment, the leaching of manganese was carried out at zero flow rate to ensure accumulation of dissolved manganese in the culture liquid.

After 24 hours, the pump was switched on to feed the nutrient medium prepared beforehand in a separate vessel, and the valve, which ensures the outflow of excessive culture medium into the settling tank (capacity of 60 l) was opened. After this tank was filled, settled water was fed to the reactor with the capacity of 30 l, where the liquid was automatically made alkaline to pH 10 with addition of ammonia solution with a constant agitation. In this reactor, under the impact of alkali, the manganese hydroxide precipitates and was suspended in the solution under conditions of constant agitation. From the reactor, the pulp was fed to the settling tank where it was separated into the precipitate of manganese hydroxide, which was the target useful product, and supernatant which was subjected to further processing. The precipitate was poured on to the filter or was centrifuged, dehydrated, and sent to chemical analysis or other investigations.

The technological scheme of manganese concentrate production in an scale-up laboratory installation is shown in Fig. 6.3.

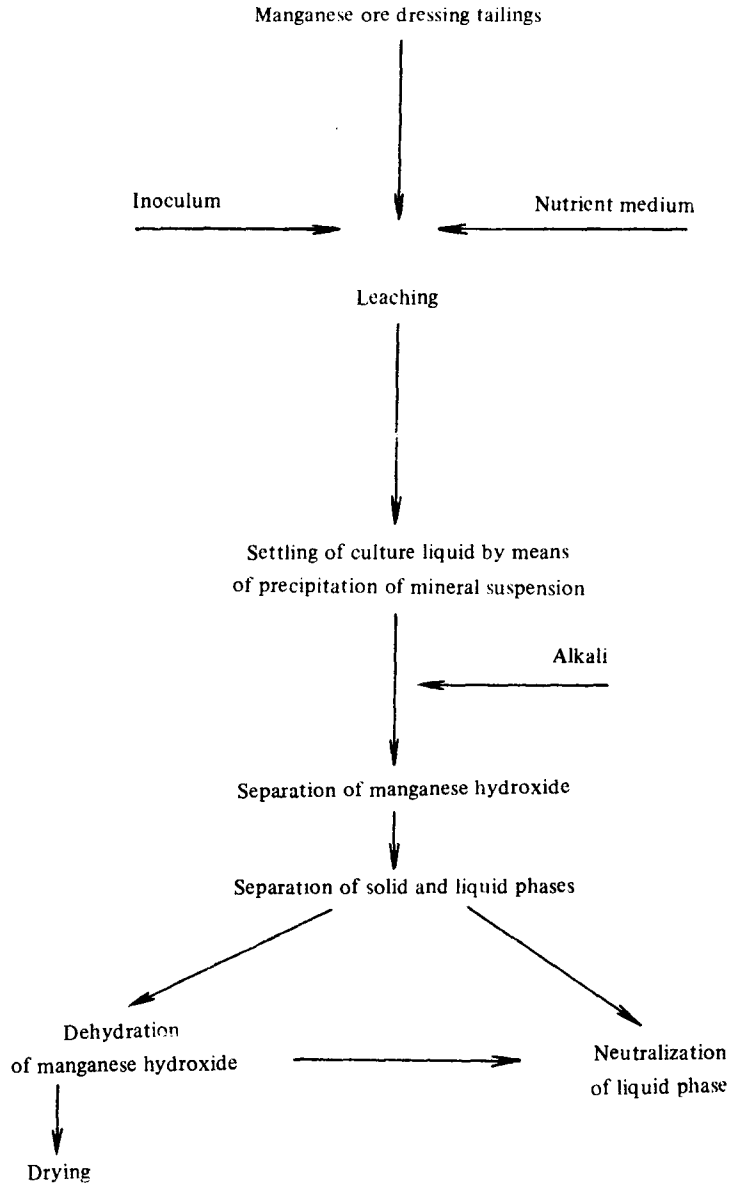

Fig. 6.3. Basic flowsheet for production of manganese concentrate in an up-scale laboratory installation

6.1.4. Beneficiation of Manganese Ores. Bioconversion of Hausmanite

Using strong manganese oxidizing cultures of **Hyphomicrobium** and **Arthrobacter species**, isolated from various manganese containing ecosystems such as pile deposits from Australia, soils from Mn-mining regions in India [3] and adapted to withstand high concentrations of ore, it was possible to convert Mn_3O_4 form of ore (hausmanite) to MnO_2form. This was first carried out in flasks and the converted product was first checked using the Eh-pH diagram and confirmed later by X-ray crystallography to ascertain the presence of desired species of manganese (γ type). These experiments were scaled-up to column level and then to 100 kg tank level. In 15 days time, 78.4 % conversion was obtained using an **Arthrobacter sp.** culture and 85.2 % conversion, using **Hyphomicrobium sp.** This bioconversion was found to be economically feasible under Indian conditions [2].

6.1.5. Removal of Phosphorus and Silicon from Manganese Ores

As presence of phosphorus was not considered desirable over 0.1 % in manganese ores which are to be used for making ferromanganese alloys and it was not possible to reduce this using known physico-chemical processes, phosphorus removal was successfully tried using **A.niger** and **Hyphomicrobium sp.** The latter was 3 times better for phosphorus removal than **A.niger sp.** (30 % removal) [4].

In the USSR phosphorus was leached from manganese ore containing 40.0 % Mn, 0.35 % P and 18.8 % SiO_2. The ore was placed in 2-litre vessels filled with modified Surman medium containing per litre: molasses, 5 g; KNO_3, 1.5 g; $CaCO_3$, 1.5 g. The molasses concentration in some experiments was reduced to 50 mg/l. Culture of **B.mucilaginosus** with a cell count of up to 10^9/ml was introduced into these vessels.

The solid-to-liquid ratio was 1:1 to 2:1. The ore was ground to particle size −10 +7 mm; −7 +5 mm; −3 +2 mm; −2 +1 mm; −1,0 mm. The pH was adjusted to 7.2 – 8.0. Solutions were periodically replaced with fresh ones, enhancing the leaching of phosphorus. However, the best results were achieved in conditions of intensive agitation of pulp in shakers or in tanks and with intensive aeration. There was no significant differences in the degree of phosphorus extraction from the above mentioned classes of ore size.

As much as 30 % of phosphorus was leached in these experiments. Scale-up experiments on removal of phosphorus from manganese were conducted in tanks with S:L = 1:1. Owe to the action of **B.mucilaginosus.**, the P:Mn ratio decreased from 0.09 to 0.004, which corresponds to the concentration of the 1st grade manganese. As a result of bacterial impact, the SiO_2 content of the ore also fell from 18.8 % to 11.0 %, i.e., in the process of leaching, the phosphorus content of the ore decreased, on the average, by 35 % and that of silica by 40 %.

Fig. 6.4 shows a flowsheet for bacterial leaching of phosphorus from manganese ore (by Rapava A.V.).

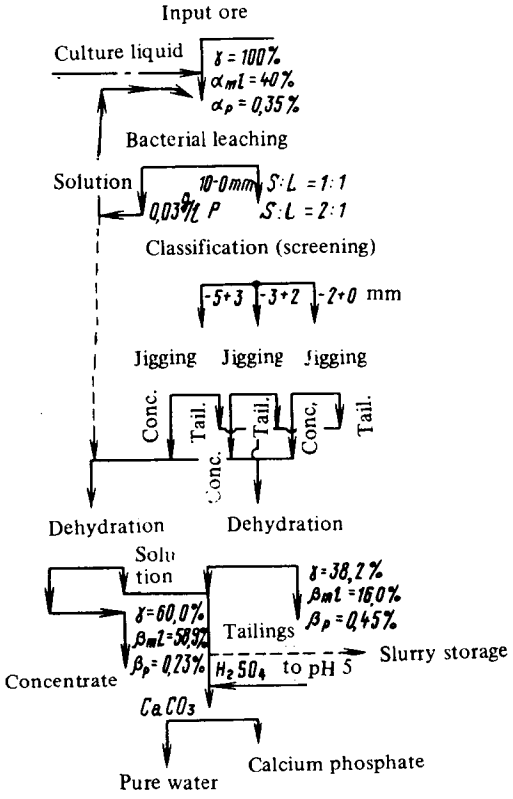

Fig. 6.4. Flowsheet for bacterial leaching of phosphorus and silica from Mtsvari manganese ore

6.2. MICROBIAL LEACHING OF ALUMINUM FROM MINERAL RAW MATERIALS

6.2.1. Characteristics of Aluminum-Bearing Raw Materials

Different raw materials can be targets for aluminum extraction by microbial leaching processes [10, 12, 19, 29].

The aluminosilicates are the largest and most important group of aluminum-bearing minerals. In principle, the susceptibility to degradation decreases with the increase of the amount of Si-O-Si bonding. The substitution of Al^{3+} for Si^{4+} in tetrahedral sites weakens the silicate lattice by producing an asymmetrical charge distribution and reducing the amount of Si-O-Si bonding [18]. Furthermore, any isomorphous substitution in general will weaken structures by increasing the variety of bonds present and further distorting the electronic charge

distribution, giving localized excesses or deficits of charge that can provide sites for attack by reagents.

Different clays are very suitable to be targets for aluminum bioleaching. They are abundant and relatively cheap, the costs for their comminution are low, and some of them are characterized by a relatively high aluminum content. The susceptibility of the different clays to biodegradation is quite different. In principle, the dioctahedral clay minerals (kaolinite, halloysite, illite, montmorillonite, etc.) are more stable than the trioctahedral clay minerals (vermiculite, serpentine, chrysotile, genthite, etc.). Chlorites, which may be either, are intermediate in behaviour. The susceptibility can be increased considerably by heating the clays at 600–650 ^{o}C for 1–2 hours [10]. This treatment causes amorphication of the raw material due to the separation of water from the hydroxylic groups in the crystalline structure of clay minerals. The heat treatment not only enhances the aluminum leaching but also inhibits the iron leaching. The latter effect is considered very important as iron impedes the subsequent extraction of aluminum from pregnant solutions.

Dressed kaolins, kaolin rocks and kaolin-containing rocks may also be targets for aluminum leaching. However, the dressed kaolins are expensive raw materials, whereas the aluminum content of the kaolin rocks and, especially, of the kaolin-containing rocks is relatively low (the kaolin rocks contain at least 50 % minerals of kaolinite group; rocks containing less than 50 % kaolinite minerals are referred to as kaolin-containing rocks).

Low-grade bauxites and some aluminum-bearing waste products, e.g. fly ashes can also be targets for bacterial leaching.

6.2.2. Technological Scheme

A flowsheet for aluminum leaching from mineral raw materials has been proposed [12, 14]. The flow sheet includes the following major steps (Fig. 6.5):

– preparation of leaching solution;
– leaching of aluminum;
– extraction of aluminum from pregnant solution.

Preparation of leaching solution. The leaching solution is obtained by cultivation of an appropriate strain of **A. niger** capable of producing substantial amounts of citric and oxalic acids under certain conditions (see Chapter 2, Media Nos 28–33).

In general only sugars which are rapidly taken up by the fungus are useful carbon sources for this fermentation. In laboratory experiments, pure sugar substrates such as glucose, fructose or sucrose can be used. However, in scale-up experiments, several cheaper crude carbohydrates are used. These are beet and cane molasses, unrefined sucrose, cane juice, citrus molasses and various starch hydrolysates. These substrates contain different heavy metal ions which in many strains drastically decrease acid production. Removal of the heavy metal ions from the sugar solutions is achieved by cationic ion exchange or by precipitation with hexacyanofferate. However, such removal is not always

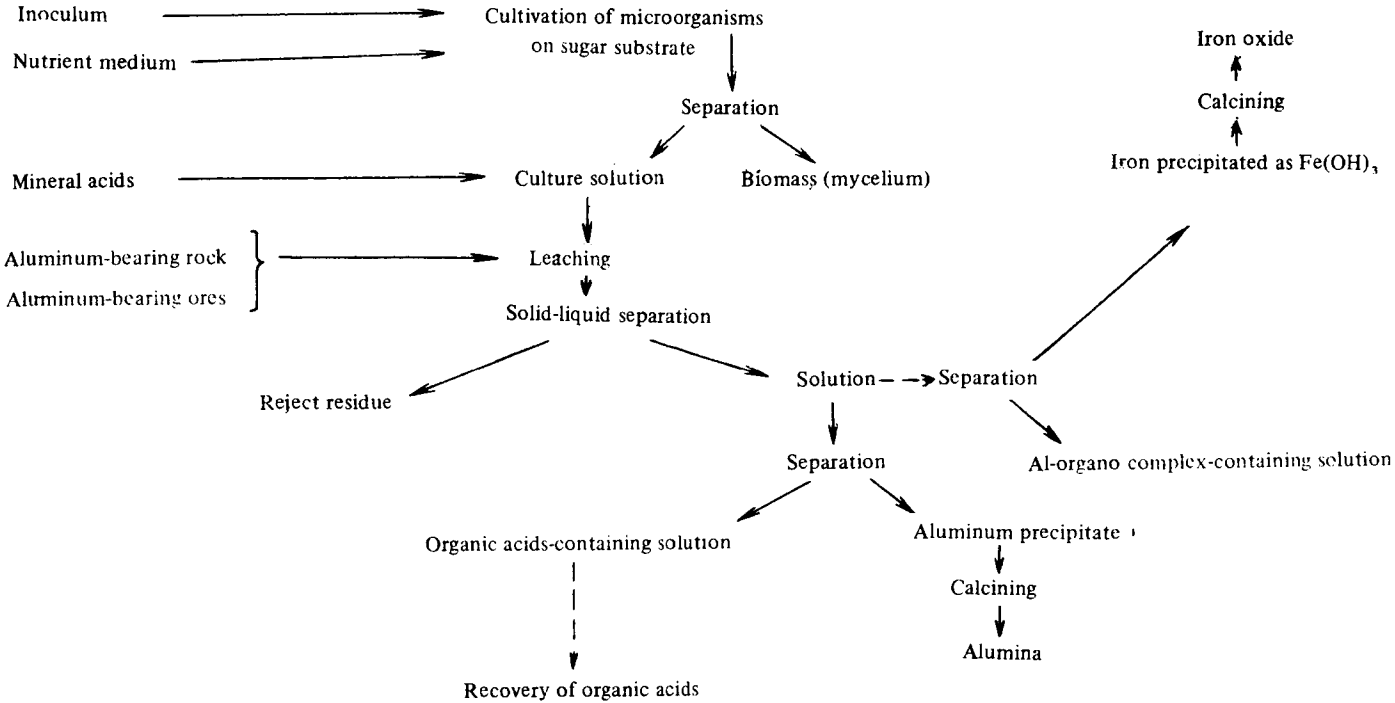

Fig. 6.5. Flowsheet of process for microbial leaching of aluminum from mineral raw materials

possible. The addition of 1–5 % (v/v) of lower alcohols (methanol, ethanol, n-propanol, isopropylalcohol or methylacetate) reverses the negative effect of trace metals on acid formation.

In addition of the problem of the metal ion content, other inhibitory substances which may be present in some substrates have to be removed prior to fermentation, e.g. acetic acid and certain peptides in molasses, sulfite, furfurole, acetic and formic acids in wood hydrolysates [28].

The concentration of sugar in the fermentation medium is as high as 10–20 % (w/v).

The pH and temperature of the medium are factors controlling the ratio of the citric and oxalic acids produced as a result of the fermentation. In order to increase the yield of oxalic acid, the initial pH of the medium is made neutral or slightly alkaline, and the incubation temperature is lower than the optimum temperature for citric acid formation, i.e. lower than 28–30 °C.

The acids-producing strains can be cultivated as surface or submerged cultures. In laboratory experiments, the cultivation of **A.niger** is performed in static or shaken flasks, respectively. Spore suspensions or mycelial fragments are used as inocula. Spores usually are obtained by growing the mould on a suitable agar medium; upon attaining abundant spore production spores are harvested by rinsing out with a suitable solution, e.g. saline. The inoculum usually contains about 10^7–10^8 spores/ml and is added to the nutrient medium at 10 % (v/v) concentration. The process is followed by periodic determinations of the acidity in aliquots taken from the nutrient medium. The acidity is determined by titration with sodium hydroxide (phenolphtalein as indicator). The acid products are identified and their proportions estimated by partition chromatograph, with different solvent systems, e.g. butanol + water containing 2 N-formic acid. Residual sugar is estimated by an appropriate method, e.g. by Cole's ferricyanide method. The amount of mould growth is estimated by determining the dry weight of mycelium formed. The fermentation time depends mainly on the way of cultivation (surface or submerged culture), the type and concentration of the sugar substrate and on the strain being used.

In scale-up experiments, the submerged process is the preferred type of fermentation owing to its shorter duration and higher yields of organic acids. The submerged fermentation may be performed using both conventional stirred reactors and tower fermentors. In submerged fermentation processes, aeration is extremely critical. The optimum aeration rates may varied from 0.1 to more than 1 volume of air per volume of fermentation medium per min.

The fermented liquid may have a total acid concentration higher than 200 g/l.

Mycelial mats from the surface process are easily separated from the fermentation fluid. The latter is drained off, the remaining mats are disintegrated carefully and flushed onto a washing vessel using only limited amounts of water. The wash water is added to the fermentation broth. This procedure is needed as the spongy mycelium retains about 15 % of the acids formed in the fermentation.

In the submerged process mycelial material is removed from the fermentation fluid usually by a vacuum filter. Under large-scale conditions, filter aids have to be employed.

The washed mycelium may be dehydrated by pressure filtration and subsequently dried to yield a protein-rich feedstuff which represents a valuable by-product.

The fermentation fluid is diluted to a total acid concentration of about 100 g/l and the pH is adjusted to 0.5 either by hydrochloric or sulfuric acid or by a mixture of mineral acids. The mixture contains sulfuric, hydrochloric and phosphoric acids in a volume ratio of 2:1:1. Potassium nitrate may also be added at a concentration of 0.5–3 %. The resulting fluid is used as leaching solution. The extraction efficiency of this solution is similar to that of solution containing commercial organic acids of equal molarity. However, the solution containing microbiologically-produced organic acids is much cheaper.

Leaching of aluminum. The leaching process is performed in acid-resistant reactors with mechanical stirring, at a temperature of about 90–100 °C. The pulp density is 20–25 %.

The leaching may be performed as batch or continuous process. A countercurrent leach unit consisting of 2–4 reactors may be very suitable. Under such conditions, as much as 90–95 % of aluminum are extracted within 5 hours from some thermically pretreated clays.

After leaching, the pregnant solution containing dissolved aluminum is separated from the solid residue by filtration. Some impurities such as iron, silicon, etc., can be separated from the aluminum-containing solution by precipitation at a pH other than that for aluminum. The purified aluminum-containing solution is used for extraction of aluminum.

Extraction of aluminum from pregnant solution. Aluminum is recovered from the pregnant solution by using various techniques.

The aluminum may be recovered as an aluminum hydroxide precipitate by increasing the pH of the pregnant solution after heating at 170 °C for about 2–3 hours. An aluminum hydroxide precipitation may be also achieved by increasing the redox potential of the pregnant solution. The precipitation reaction may also yield the original complexing reagent, i.e. the citric and oxalic acids, which may then be recycled for further use in leaching additional raw materials. By calcining the aluminum hydroxide the alumina final product may be obtained.

The aluminum may be also recovered as potassium aluminum sulfate (alum) by adding potassium sulfate and sulfuric acid to the pregnant solution, heating the solution to 90 °C for 15 min and subsequent cooling.

The aluminum may be also recovered as an $AlCl_3 \cdot 6H_2O$ precipitate by saturation of the pregnant solution with hydrogen chloride. The precipitate is separated by filtration and dissolved in 20 % hydrochloric acid. This solution is saturated with hydrogen chloride and the aluminum is reprecipitated as purified $AlCl_3 \cdot 6H_2O$. The $AlCl_3 \cdot 6H_2O$ may be treated by calcination to obtain alumina.

6.3. MICROBIAL REMOVAL OF IRON FROM MINERAL RAW MATERIALS

The iron oxides contained as impurities in different raw materials such as quartz sands, kaolins, clays, etc. can greatly lower their quality. Various non-

biological methods are used independently or in different combinations to remove the iron. However, some of these methods, e.g. magnetic separation and flotation, are not fit for universal application and their efficiency greatly depends on the properties of the mineral raw material being treated. The chemical methods consisting in leaching with mineral or organic acids and treatment with reducers usually are suitable for achieving a higher extent of iron removal but at the same time they are more expensive, the operating conditions are very complicated, and the processes are environmentally dangerous.

The leaching of iron from oxide minerals by means of microorganisms is well-known. Solubilization is connected with the formation of organic acids and other metabolites acting as complexing agents [7], as well as with enzymatic and non-enzymatic iron reduction.

The ability of microorganisms to leach iron from oxide minerals can be used for the removal of this element from different mineral raw materials containing iron oxides as impurities.

6.3.1. Mineral Raw Materials

Quartz sands are largely used as a raw material in the glass industry. However, for the preparation of high-quality glass, quartz sands possessing particular qualities are needed (Table 6.3). Such sands are obtained as a result of the beneficiation of low-grade sands. As a rule, these low-grade sands are rust-coloured due to the presence of different impurities – mainly iron compounds. Large quantities of such sands are recovered during the dressing of kaolin. The iron

Table 6.3

Qualities of quartz sands used for the preparation of high-quality glass [31]

Property	Content, %
Chemical:	
SiO_2	more than 99.0
Al_2O_3	not more than 0.5
Fe_2O_3	not more than 0.012
Cr_2O_3	traces
TiO_2	not more than 0.05
CaO + MgO	not more than 0.6
kaolin	not more than 1.0
Particle size:	
more than 0.63 mm	not more than 3.0
less than 0.1 mm	not more than 5.0
Loss on ignition (1000 °C)	not more than 0.5
Humidity	not more than 0.5

content of these sands usually varies in the range 0.03–0.08 % Fe_2O_3. Some iron is present in the form of different iron minerals such as biotite, magnetite, hematite, limonite, pyroxene, etc., which occur as single grains or form accretions with silica. The rest of the iron is present as a coating on the surface of quartz grains. This iron coating consists of different iron hydroxides – mainly limonite and goethite.

Kaolins with a low iron content (less than 0.6 % Fe_2O_3) and a high level of whiteness (more than 88 %) are used for the preparation of high-quality chinaware as well as in the paper industry. Such kaolins are obtained as a result of the treatment of dressed kaolins. The iron content of dressed kaolins usually varies in the range 0.8–1.5 % Fe_2O_3. Some iron is included in the crystal lattice of the kaolinite, whereas the rest of the iron (different iron hydroxides) impregnates the kaolin mycelium.

Clays always contain different iron compounds as impurities (the iron content may be as high as 10–15 % Fe_2O_3). Most of the iron usually is present as hydroxides which impregnate the clay minerals. The removal of iron from rich-in-iron clays may increase considerably their fireproofness. Such clays can be used for the preparation of fireproof materials.

Bauxite and manganese ores containing iron impurities also can be targets for the microbial leaching process.

6.3.2. Technological Scheme

Under laboratory conditions, leaching in shaken or static flasks as well as in mechanically stirred reactors and columns can be performed by using either growing cultures or fermentation fluids obtained as a result of prior cultivation of microorganisms in the absence of mineral raw material. The leaching rates achieved by means of growing cultures are relatively low, e.g. a continuous flow system employing iron reducing bacteria in a battery of five columns was able to remove all the iron from bauxite in 40 days [30].

The leaching by means of appropriately adjusted fermentation fluids takes place at much higher rates. A flow sheet for microbial removal of iron from mineral raw materials has been proposed [11, 13]. The flow sheet is similar to that for aluminum leaching and includes the same major steps (Fig. 6.6):

- preparation of leaching solution;
- leaching of iron;
- removal of iron from leaching solution.

Preparation of leaching solution. The procedures are very similar to those used for preparation of leaching solution for aluminum leaching (Chapter 6.2.2). However, optimum nutrient media are those that ensure high yield of oxalic acid during cultivation of appropriate strains (of **A. niger**). Such media contain physiologically alkaline mineral salts and their initial pH is in the range 7–8. The composition of a typical nutrient medium for microbial production of oxalic acid is given in Chapter 2 (Medium No. 33). The incubation temperature is about 20 °C.

The strains can be cultivated as surface or submerged cultures. After cultiva-

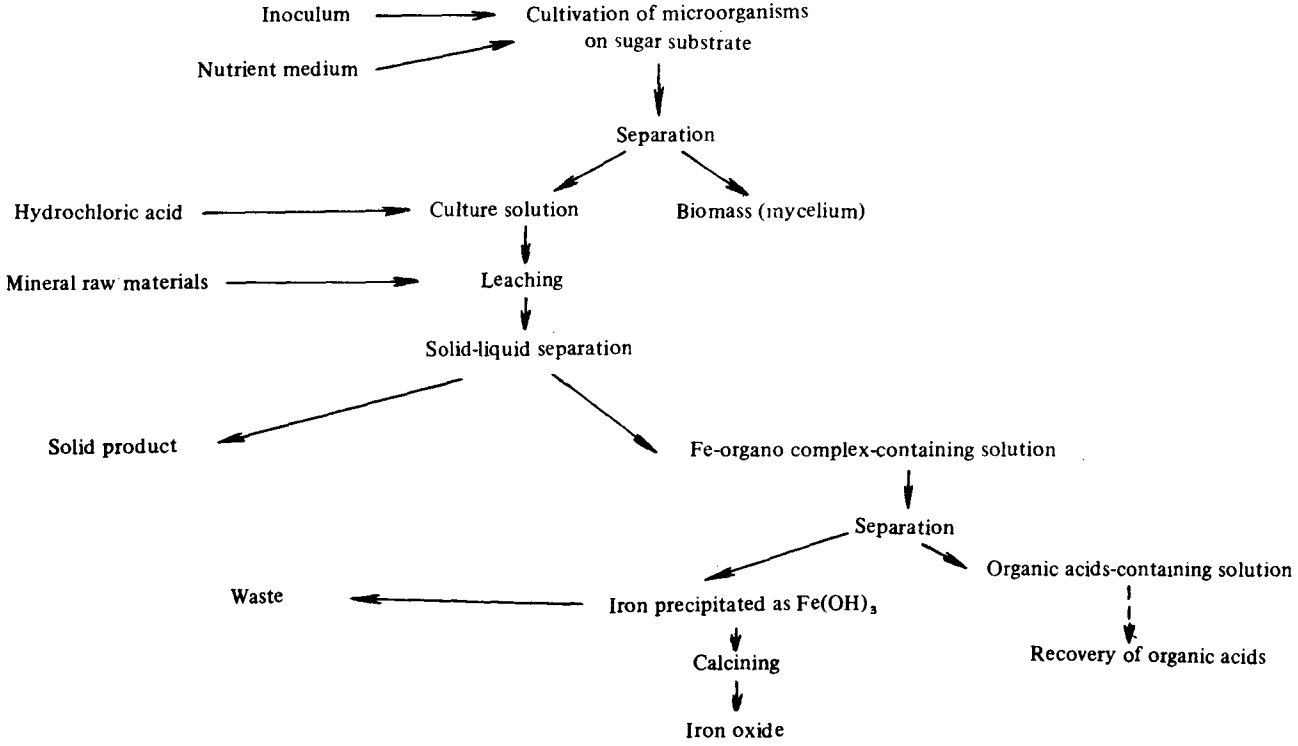

Fig. 6.6. Process flow sheet for microbial removal of iron from mineral raw materials

tion, the fermentation fluid is separated from the mycelium by filtration, diluted with water and acidified to a pH of 0.5 with hydrochloric acid. This solution is used for leaching of iron. The concentration of organic acids in the solution, i.e. the extent of dilution of the fermentation fluid with water, depends on its intended use. For example, to remove iron from sands, which normally contain less than 0.1 % Fe_2O_3, solutions containing 5–10 g/l organic acids are found to be very efficient. Such solutions may also be used for treatment of kaolins, which normally contain about 1 % Fe_2O_3. However, for removal of iron from some rich-in-iron clays, bauxites, etc., the concentration of organic acids may be higher than 50 g/l. The extraction efficiency of these solutions is similar to that of solutions containing commercial organic acids of equal molarity.

Leaching of iron. The leaching process is performed in acid-resistant reactors with mechanical stirring, at a temperature of about 90–100 °C. The leaching of quartz, sands and kaolins usually is performed as batch process. However, the treatment of rich-in-iron clays, bauxites, etc. may be performed by using a countercurrent leach unit consisting of 2–4 reactors.

The optimum pulp density is about 35–40 % for the quartz sands and 20–25 % for the kaolins and clays. The treatment of the sands needs a more intensive agitation than that of the kaolins and clays. The duration of leaching usually is in the range 1–5 hours.

Aluminosilicates (clays, kaolins, etc.) are leached without any prior heat treatment. The solubilization of iron from untreated aluminosilicates proceeds more intensively than the solubilization of aluminum, whereas the opposite is observed during the leaching of aluminosilicates, which had been subject to prior heat treatment.

The iron content of some sands treated by this method was lowered from 0.035–0.088 to below 0.012 % Fe_2O_3. The iron content of different kaolins was lowered from 0.65–1.49 to 0.44–0.75 % Fe_2O_3 and as a result of this their whiteness was increased from 55–87 to 86–92 %. The calcium was also removed from the kaolins. The iron content of a clay was lowered from 6.25 to 1.85 % Fe_2O_3 and its fireproofness was increased from 1670 to 1750 °C. Only small amounts of aluminum (about 1 %) were leached together with iron from the kaolins and the clay.

It must be noted that only the iron not included in the crystal lattice of the minerals can be removed by this method.

Prior to leaching, some quartz sands may be mechanically activated. The activation is performed in a mechanically stirred reactor, at high pulp density (60 %) and at ambient temperature. The duration of activation is 15 minutes.

The biological leaching of iron from quartz sands can be combined with such conventional methods as flotation and magnetic separation, i.e. prior to leaching, part of the iron is removed by means of flotation or magnetic separation.

Removal of iron from leaching solution. Iron is removed from the leaching solution by increasing the pH.

6.4. ROLE OF MICROORGANISMS IN SOLUBILIZATION OF NATIVE GOLD

6.4.1. Characteristics of Gold

Gold occurs in rocks either as nuggets or as inclusions into sulfide minerals, such as pyrite, arsenopyrite, pyrrhotite, galenite, sphalerite, and silicate minerals. It is one of the most stable elements. Copper and silver always accompany gold. Its active valency is +1 and +3. Despite the high chemical stability gold is rather mobile in nature as shown by the data depicted in Fig. 6.7. This given Eh-pH diagram presents data on ion activity of gold under different conditions. Comparison of thermodynamic characteristics shows that gold-organic complexes are stable in a wide range of redox potential as compared to thiosulfate ones. This suggests wider dispersion areas of gold associated with organic substances.

6.4.2. Solubilization of Gold

Several examples of the use of microorganisms for gold solubilization are given below.

Experiments on leaching of gold-bearing laterites using bacteria isolated from gold-bearing deposits of Iti were performed in 1.5 l flasks containing 800

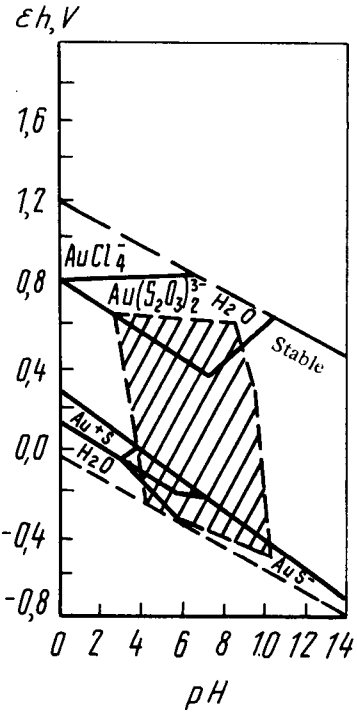

Fig. 6.7. Correlations of stability between some gold components in water at 25 °C and 1 atm pressure

– 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.

ml of medium. Gold content of laterites ranged between 0.6 and 19.8 g/t. Laterites and nutrient media were sterilized separately. After sterilization the medium was inoculated with bacteria and after 4-day's incubation the culture obtained was applied to a laterite sample. Aerobic spore forming bacteria of the genus **Bacillus** were used in these experiments.

After 21 days, solutions were analysed twice a week. Twenty ml samples were taken for the analysis. One hundred ml of 4-day-old bacterial culture was added in the flasks containing 400 ml samples. Sampling was repeated after 8 days. Experimentation was stopped after 217 days and 293 days and gold was recovered from solution by means of precipitation on activated carbon, which was subsequently roasted. The results obtained are shown in Table 6.4.

Table 6.4

Flask Nos.	Days	Au recovered, mg/l	Flask Nos.	Days	Au recovered, mg/l
1	217	51.9	4	217	10.1
2	217	31.9	5	217	65.7
3	217	51.9	6	293	82

Experiments by Pares [26] involving other types of laterites, led her to the following conclusions:

1. Different species of bacteria affect different types of laterites;
2. Nutrient medium must be selected depending on the bacterial species and type of ore to be leached;
3. Extraction percentage can be increased, but this requires longer time of leaching.

Pares et al. isolated bacteria belonging to the genus **Aeromonas** from Iti gold mines and soil samples [26].

These bacteria were effective both in solubilization of pure gold and in leaching of gold from gold-bearing ores. After leaching, gold content of the solution exceeded 10 mg/l.

Pares was of the opinion that the problem of gold solubilization could be also solved by other methods, in particular, by elucidating nature of the chemical compound which causes gold solubilization. Also, it was reported that gold was solubilized in media containing dead bacterial cells. In this case, the action of organic substances released into solution during destruction of bacterial cells was assumed. However, gold extraction was low in these experiments.

Another example is of solubilization of gold using microorganisms that actively synthesize amino acids [16, 17, 20, 21] (See Chapter 1).

Mutants of Bacillus spp. and other bacterial species release amino acids, nucleic acids, organic acids such as pyruvic, lactic, formic, and acetic acids as well as a number of enzymes: catalase, peroxidase, protease into the nutrient medium. However, gold was most actively solubilized by amino acid producers

in the presence of oxidizers. Optimum concentration of inoculum was 3–4 %. Further increase in inoculum concentration produce virtually no effect on biosynthesis of amino acids.

The rate of gold solubilization depended on a number of factors:

1. Medium type – solubilization increased on media containing carbohydrates (molasses, glucose).

2. Presence of considerable amounts of amino acids, showed not be less than 5 g/l for active solubilization;

3. Availability of oxidizers, such as peroxides, ion exchange resins. Optimum consumption of Na_2O_2 and of other oxidizers is 2–4 g/l;

4. pH of the medium; optimum pH 8.0–10.0;

5. Size of gold particles and type of ore.

Since biosynthesis of amino acids by bacteria occurs at lower values of pH compared to gold solubilization, growth of bacterial cultures and biosynthesis of amino acids should be performed in separate cycles.

Besides living microorganisms and their metabolites, reagents prepared from the microbial biomass or other reagents of biological origin may be also used for gold solubilization.

A considerable amount of amino acids could be obtained by hydrolysis of protein-containing products. Many microorganisms can form a relatively large biomass with a high protein content (Table 6.5) [6]. Some bacteria and yeasts can yield more than 60 % protein in their biomass [25].

Table 6.5

Biomass characteristics of different types of microorganisms

Type of microorganism	% content (dry weight)		Dry weight of biomass in g per 100 ml of culture
	proteins	nucleic acids	
Bacteria	40–50	13–25	0.02–2.9
Fungi	10–25	1–3	3–5
Yeasts	40–50	4–10	1–5
Algae	10–60	1–5	0.4–0.9

Alkaline treatment of microbial biomass at 80–100 °C resulted in deep destruction of proteins and accumulation in solutions of elementary amino acids – their amount totaled 3–10 g/l, i.e. of the same order as in the culture media of gold-solubilizing bacteria of the genus **Bacillus.** Alkaline hydrolyzates of microbial biomass could be used for gold leaching in the presence of an oxidizing agents as sodium peroxide, potassium permanganate, the latter being preferred. For example by treating fodder yeasts with caustic soda solution, hydrolyzates were obtained containing about 5 g/l amino acids, 0.5–0.8 g/l nucleic acids, and 1–2 g/l lipids.

For the preparation of gold-solubilizing reagents of biological origin, of great practical interest were the wastes from several industries – pharmaceutical, food, etc.

Technological aspects. Tailings of ore concentrates and washings of placer sands were used in the experiments on leaching of gold using bacterial metabolites. Experiments were conducted at pH 9–10 and at a solid-to-liquid ratio of 1:1–2 in the presence of Na_2O_2 (2–6 g/l). Gold extraction in 10 days was noted as follows: from ore concentrate tailings, 25–35 %; from placer sands – 50–80 %.

Extraction of gold in a slightly alkaline medium was 24–40 %.

Experiments were conducted with placer gold extracted from placer, sandy-gravel deposits using amino acid containing wastes of pharmaceutical and food industries [32]. To samples containing 5–10 mg of gold, 200–300 ml of leaching solution were added and during the experiment solution samples were taken and analysed for their gold content, using a Perkin-Elmer atomic-absorption spectrophotometer. Table 6.6 shows results of one such series of tests.

Table 6.6

Placer gold leaching (test duration, 30 days)

Sr. No.	Leaching solution from	Au concentration in the solution, mg/l
1	Fodder yeasts	0.6
2	Sizamicine production wastes	0.6
3	Rifamicine production wastes	0.7
4	B3 product* (7 g/l)	2.3
5	B3 product (4 g/l)	2.4

*B3 product – biomass of antibiotic producing organisms.

In experiments with the B3 product during leaching of gold of – 0.5+0.049 mm size, the metal concentration in solution was 1.0–3.4 mg/l in 7–37 days and was, on an average, two times as high as in similar tests conducted with fodder yeast biomass.

During percolation of ore of the size of –10+0 mm containing 2.4 g/t gold, as much as 71–73 % of metal was solubilized in 16 days. From an ore containing 0.75 g/t gold, 53.3 % of metal was solubilized in 9 days. Sandy ore heap (10 t) with ore size of –300+0 mm, containing 0.75 g/l gold was leached in dumps. The leaching solution was prepared by growing fodder yeasts on paraffin. Totally, 46.7 % of metal was extracted in 12 days at the average percolation rate of solutions of 12–15 l/t·day and residual content of tailings of 0.4 g/t [21]. The solvents tested are non-toxic.

Prospects for using such bioorganic products depend on the detailed investigation, of their biochemical composition and selection of the most appropriate of these products particularly in terms of quantitative and qualitative composition of the amino acids contained therein.

Inhibition of extraneous microflora. An important factor contributing to the successful leaching of gold is inhibition of extraneous microflora. This may be achieved adding small amounts of anti-microbial fiber into ore and into solution, or by periodical filtration of leaching solutions on such fiber. It is likely that the use of other anticeptics is also promising.

Gold's extraction from solutions. Leached metal may be extracted from the solution using adsorption on a resin or activated carbon. Biosorbents are also promising.

Economics. Economic calculations showed a good cost/benefit ratio for using a culture medium containing amino acids and proteins as a solvent for gold. Inexpensive substrates for bacterial growth could be used as industrial wastes etc., containing carbohydrates and hydrocarbons.

It was calculated that the use of solvents is economically expedient if the primary ore contains about 1.5 g/t gold, though the cost of ore processing is slightly higher as compared with the cyanide process.

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Chapter 7

BIOACCUMULATION AND PRECIPITATION OF METALS BY MICROORGANISMS

Hydrometallurgical recovery of metals is related to their removal from dissolved solutions. An important task is also treatment of industrial effluents. Recently new approaches emerged to solve this problem by using microorganisms. Among the most feasible are:

1) biosorption;
2) evaporative recovery;
3) metal precipitation from solutions.

Some microbiological methods of metal recovery from solutions and treatment of industrial effluents are described below.

7.1. REMOVAL OF METALS FROM INDUSTRIAL WASTEWATER

Microbiological treatment of industrial wastewater for metal recovery resorts to heterotrophic microorganisms and the addition of organic substances to the solution, which leads to the reduction (oxidation) of metals or to their precipitation in the form of sulfides.

7.1.1. Metal Precipitation in the Presence of Plant Decomposition Products [19]

Laboratory tests. In order to develop efficient processes of metal precipitation using heterotrophic microorganisms, the behaviour of metals in the presence of intermediate metabolites formed at earlier stages of plant decomposition must be studied. To this end, laboratory tests have been carried out to investigate the effect of plant decomposition products on the mobility of metal ions, in particular, copper, lead and zinc, in solution.

Crushed decomposition products of cat's-tail (10 g dry matter per 11 of water) were added to 150 ml of a solution containing 5 mg/l of copper, lead and zinc ions in a 250-ml Erlenmeyer flask. Flasks containing distilled water and cat's-tail served as controls. The flasks were kept at room temperature for 6 months; metal content in solution was measured every 10 days.

Copper precipitation. Interaction between the organic substances, accumulated in the solution due to microbiological decomposition of cat's-tail, and copper ions reduced the content of the latter by 78 % in ten days. Most of the copper (47 %) precipitated in the form of stable compounds with organic sub-

stances. By the end of the second month the copper content in solution dropped by 96 %.

Zinc precipitation. Decomposition of cat's-tail phytoresidues by natural microflora resulted in the formation, first of all, of unstable zinc complexes with organic substances, and after just three months 82 % of zinc had precipitated in the form of stable metal-organic complexes.

Lead precipitation. The most prominent fixation effect of decomposing phyto-material has been observed in lead. In ten days the lead in solution dropped by 90 %.

Thus, decomposition products of aquatic plants have been shown to affect precipitation of heavy metals, since the mineral and organic compounds formed at different stages of humification, react with heavy metals ions producing soluble and insoluble complexes. However, industrial effluents contain, besides, metals, a wide range of toxic agents; therefore, experiments have been carried out using industrial wastewater in order to verify the laboratory results.

Precipitation of metal ions from industrial wastewater. Samples from a tailings pond of a mineral dressing plant were placed at the bottom of the continuous-flow vessel. The toxic action of the samples on microorganisms was prevented by covering them with a humus-containing soil layer 1 to 2 cm thick. This layer served as habitat for microorganisms and it was herein that fixation of precipitating metal ions by organic substances took place. Water from an active tailings pond was used in the experiments. The initial content of toxic components is shown in Table 7.1. Saccharose was added to the feed solution (20 g/m^3) to enhance the activity of natural microorganisms.

Table 7.1

Purification of wastewater from a tailings pond in a continuous-flow vessel

Chemical composition of wastewater	Initial content, mg/l	Period of treatment, days			Degree of purification, %
		10	20	30	
pH	10.2	9.0	8.4	8.2	
Cyanides	14	4.8	3.5	0.5	96
Copper	5.8	1.4	0.7	0.5	91
Zinc	1.3	1.0	0.5	n.f.	100
Lead	0.25	n.f.	n.f.	n.f.	100

n.f. not found.

The table shows that the degree of wastewater purification amounted to 91 % for copper, 100 % for lead and zinc and 96 % for cyanides. No change in the content of toxic components was observed in effluents from the control flask. In the continuous-flow system the number of microorganisms in the effluent was insignificant (400 cells/ml) initially. After the addition of saccharose and due

to the organic nature of the bottom layer, it rose to 190 thousand cells/ml in the middle of the vessel and dropped to 100 cells/ml at the exit.

Purification of wastewaters in ponds. The metal-removing, self-purification process was tested in a laboratory continuous-flow system which formed the ba-

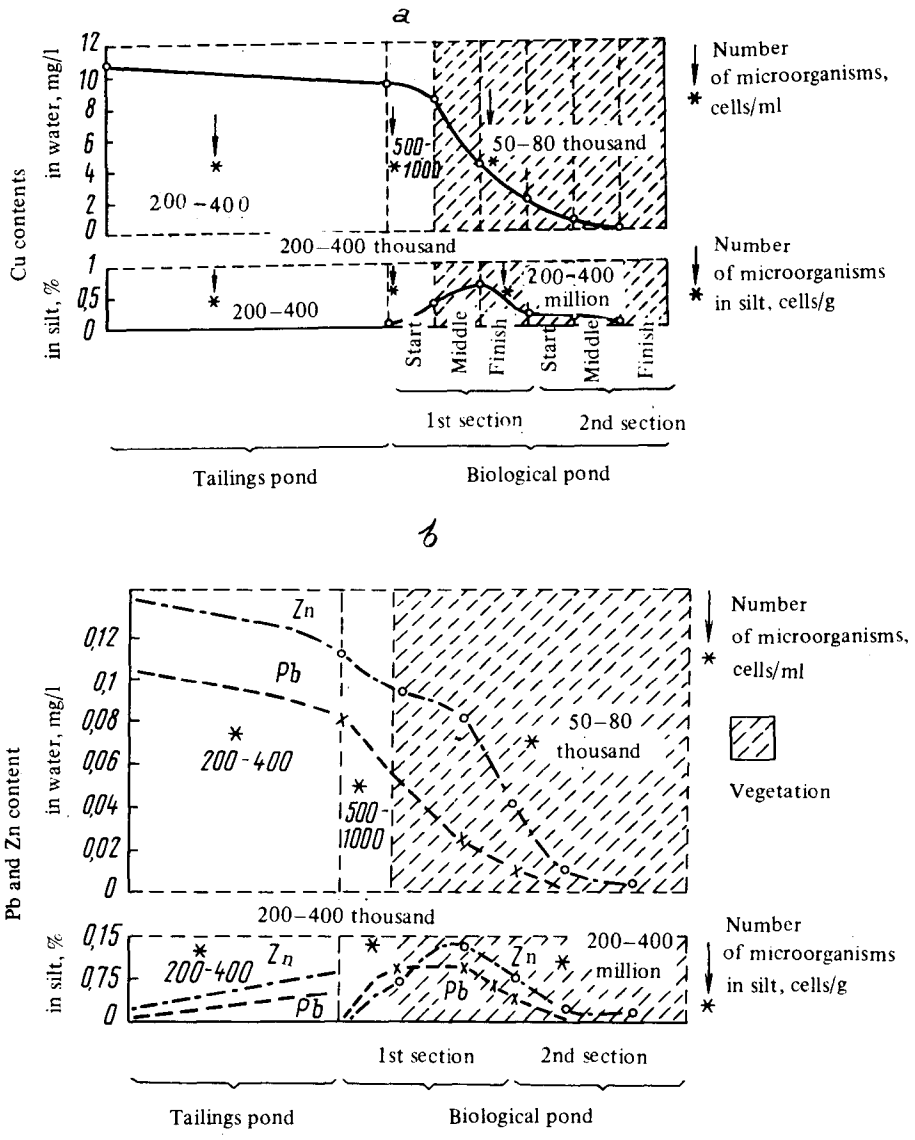

Fig. 7.1. The dynamics of copper (a), lead and zinc (b) content in water and silt of purification units in non-ferrous metallurgical plants

sis of several projects designed for eventual purification of tailings pond effluents in an ore dressing plant. Some examples are reported below. To purify a 7500 m^3/day effluent from a tailings pond with average copper and cyanide concentrations of 5 and 10 mg/l respectively, a three-section oxidizing pond was designed with a total area of 75,000 m^2. The working depth was 1 m, to be increased to 3 m so that operations can be continued throughout the winter.

Artificial aeration of wastewaters, supply of sugarbeet molasses and activation of near shore vegetation are to be introduced to enhance the activity of heterotrophic microorganisms.

In another tailings dump 34.6 ha of reed was cut off into a secondary pond, resulting in enhanced purification and a considerable drop in copper, arsenic and molybdenum concentrations in the wastewater.

The rate of precipitation of metal ions from solution depends on the growth of microorganisms in the silt and pond water and is related to presence of vegetation. At the inflow to a biological pond free of vegetation the average number of microorganisms observed ranged between 500 and 1000 cells/ml while in the area where aquatic plants are present the number rose to 50–80,000 per 1 ml. The number of microorganisms in the bottom sediments is much greater than in the aqueous phase. In the vegetation-free inflow zone of the pond, bottom sediments contain an insignificant number of microorganisms, 200–400,000 per 1 g of dry silt as compared to an average of 194 to 400 million per 1 g of silt in the upper silt layer bearing the plants' root system.

Water and silt microorganisms in a biological pond promote the removal of metal ions from wastewater. The chemical composition of wastewaters flowing through the biological pond was observed to vary due to uneven rate of metal removal in different zones of the pond (Fig. 7.1). In the initial, vegetation-free part of the pond the metal concentration in the effluent does not change significantly. Purification is markedly enhanced after metal ions enter the vegetated area where there is a greater number of microorganisms that mineralize the organic substances. Metals bound to organic substances as well as in the form of sulfides precipitate to the bottom.

7.1.2. The Use of Activated Sludge for Precipitating Metal Ions

Due to its good adsorption properties, activated sludge can be used to extract heavy metals from wastewaters. The greatest adsorption of mercury ions was observed in sludge kept for 2 and 3 hours at 100 °C. The sludge heated for 2 hours adsorbed 90 % of metal from solution in 15 minutes while that heat treated for 3 hours adsorbed 62 % of metal after 6 hours of contact (Fig. 7.2). However, activated sludge per se, without preliminary heat treatment can be used as an adsorbent of mercury ions prior to biological purification. Excess activated sludge which is usually dumped in special areas, was discharged into primary settling ponds where it interacted with wastewater. Activated sludge was supplied at a rate of 25,000 m^3/day to primary settlers in water purification units of an acetaldehyde producting plant for preliminary sorption of mercury

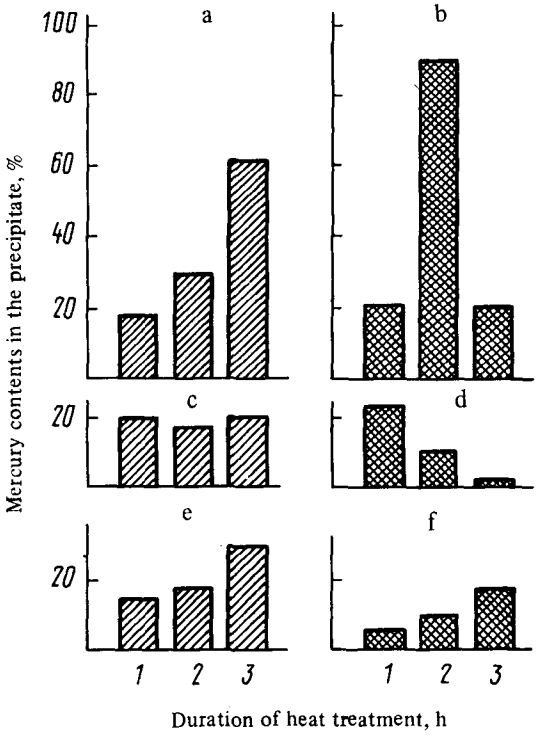

Fig. 7.2. Absorption of mercury ions from solution by active silt 6 hours (a, c, e) and 15 minutes (b, d, f) after preliminary heat treatment at 100 °C (a, b), 150 °C (c, d) and 200 °C (e, f)

ions. As a result, mercury sedimentation in primary settlers increased by 40 % and the content of organic contaminants dropped by 30 %.

Latex-aggregated **Micrococcus luteus** was used to investigate the possibility of extracting mercury ions from wastewaters with the aid of concentrated microbial biomass. Bacterial cells aggregated into artificial flocs allowed to adsorb 85 % of mercury ions during 12-hour exposure. After 15 minutes mixing of wastewater containing industrial concentrations of mercury (0.65 to 0.7 μM) with bioflocs, and a 30-minute settling period, metal adsorption attained 92 %. .

Experimental data on the adsorption of mercury ions by **M. luteus** artificial flocs were verified at the wastewater purification units of an acetaldehyde production plant. Several batches of **M. luteus** biomass were obtained in fermentors to be further flocculated with the aid of divinylstyrene latex, dried or concentrated to a paste. Biomass was tested in the laboratory on wastewaters with a mercury content of 0.638 μM. After 15 minutes mixing and 30 minutes settling a 0.7 g/l dose of biomass produced a 65–68 % reduction of mercury in wastewaters.

In pilot tests of mercury sedimentation the dose of flocs was 0.3 g/l. At the inflow to the settling pond mercury concentration in the wastewater was 2.37 μM. After 30 minutes the metal contents in the experimental settler dropped to 0.723 μM as compared to 2.24 μM in the control (without bioflocs), i.e. artificial bioflocs adsorbed 69.5 % of mercury from wastewater. The initial concentration mentioned above corresponds to a single discharge, therefore, the adsorption of 70 % of metal from wastewater after 30 minutes at biofloc dose of 0.3 g/l proves the efficiency of concentrated biomass for mercury extraction.

7.1.3. Metals Precipitation in the Form of Sulfides

Oxidizing activity of heterotrophic microorganisms that adsorb dissolved oxygen from the substrate precedes the development of an anaerobic microflora in the water and, particularly, in silt deposits. Among them, sulfate-reducing microorganisms are the most active in metal extraction.

I.V. Katayeva [23] showed that sulfate-reducing bacteria can be used to purify mine effluents. The addition of microflora contained in municipal sewage enhanced the process of biological purification which was most effective at 20 °C.

Some examples are cited below to illustrate how the hydrogen sulfide produced by bacterial reduction of sulfates can be used in removing copper from effluents [17, 18, 27, 28]. The effluent from a tailings pond settler contained 0.08 mg/l copper and 1200 mg/l sulfates. Calcium lactate (5 g/l) was used as carbon supply. This mixture served as a medium for culturing sulfate-reducing bacteria. After sterilization, the inoculum was placed in tubes with this medium; sterile medium served as control. Several weeks after inoculation of the medium with sulfate-reducing bacteria intensive release of hydrogen sulfide and the formation of a black precipitate were observed. Copper concentration in the solution dropped from 0.08 to 0.02 mg/l. Filtered precipitate contained 0.26 mg/l copper. In sterile control there was no copper precipitate.

The amount of available organic substance in the substrate is the main factor regulating the number of sulfate-reducing bacteria. Saccharose, finely crushed reed and reed with ammonium sulfate were added to the effluent from the tailings pond. In each variant a pure culture of sulfate-reducing bacteria **Desulfovibrio desulfuricans** was used.

By cutting reed into the discharge flow from a secondary settler of an ore dressing plant, the activity of heterotrophic microorganisms, including sulfate-reducing bacteria, was enhanced. The addition of organic substance to the water led to a high concentration of plant decomposition products in some areas which reduced the redox potential.

In such areas the number of heterotrophs attained 10^5 to 10^6 cells/ml, with 10^2 cells/ml of sulfate-reducing bacteria. Oxygen content dropped to 5.6 mg/l in the vegetation-free part of the pond and to 2.1 mg/l at the point of probable effluent discharge. No hydrogen sulfide was detected in the water in the vegetation-free part of the pond as compared to 5.1–6.8 mg/l in places with a high content of reed residues. The copper content in the treated effluent exiting

from the pond is 0.014 mg/l, which meets effluent purification requirements in non-ferrous metallurgical plants.

Our experimental study on the role that bacteria-produced hydrogen sulfide plays in the precipitation of copper revealed a direct correlation between the number of sulfate-reducing bacteria, the presence of available organic substance in the medium and the reduction of copper content in the solution to zero values.

In the first stages, the organic substance of plant residues is charged with mineral substances by anaerobic heterotrophic microorganisms whose metabolites react with metals causing them to precipitate. As the microbial biomass increases, water-dissolved oxygen is taken up for oxidizing the organic substance and the redox potential decreases. Significant deficit of oxygen impedes the activity of aerobic saprophytes, and an anaerobic microflora starts to develop, including sulfate-reducing bacteria. The latter produce hydrogen sulfide that precipitates metals.

Effluents from certain industrial plants (paper mills, chlorine and alkali production plants) contain from ten to a thousand times more heavy metal ions, e.g. mercury, than natural water contained in reservoirs. In the majority of cases only one or two sections of a large plant are sources of effluent contamination with heavy metal ions. Such effluents should be repeatedly diluted so that microorganisms at purification plants can perform normally. Obviously it is more convenient to treat effluents of these plants locally. Natural populations of sulfate-reducing organisms at certain points of the purification cycle of a plant may perish owing to a sudden emergency situation: a gush of toxic compounds, a sharp change in pH, Eh or effluent water temperature. In the case of effluents with an elevated heavy metal ions content it has been suggested [13, 16] that sulfate-reducing bacteria might be cultured in tanks made of semipermeable membrane, e.g. cellophane and introduced into the effluent to be purified along the flow of heavy metal ions. The semipermeable membrane will protect microorganisms against harmful external factors but not prevent the thorough mixing of hydrogen sulfide within the effluent.

Precipitation of mercury, copper, lead, cadmium and cobalt ions was studied in the laboratory with ion concentrations in solution 3 to 4 orders of magnitude higher than those of industrial effluents. Figure 7.3 shows the precipitation of mercury by sulfate-reducing bacteria cultured in the experimental cellophane test tank and in the control tank made of a triple layer of chromatographic paper with initial concentration of Hg(II) 200 mg $HgSO_4$ / 1 in 7 l of water both in the experiment and in the control. In the control tank, mercury sedimentation virtually ceased after 15 minutes as metal ions penetrated inside the tank where microorganisms were cultured. In the test tank, after 5 hours the concentration of mercury ions was 50 times less than that in the control tank after precipitation had ceased and 1000 times less than the initial concentration.

Sulfate-reducing bacteria cultured in tanks where organic substances had been added effectively purify industrial effluents containing heavy metals, e.g. molybdenum and tungsten [21]. Kerosene, oleic acid, terpenyol and sodium xanthate are used as sources of organic supply. The flowsheet of a wastewater biochemical purification unit comprises a biotank for culturing microorganisms,

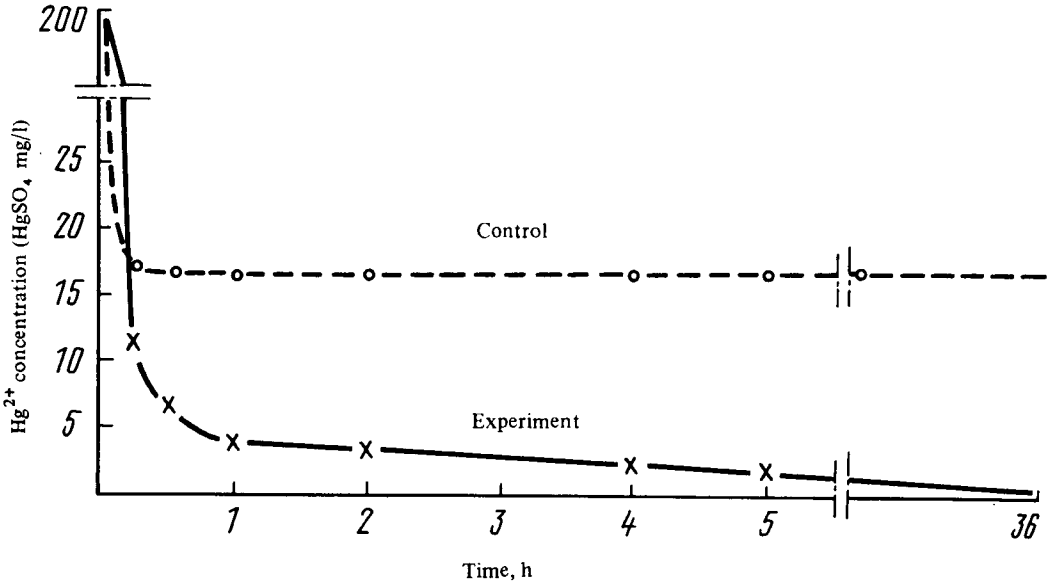

Fig. 7.3. Mercury precipitation by hydrogen sulfide of bacterial origin

a tank for raw material and a settler. The unit is operated continuously for 10 days under anaerobic conditions with constant circulation of activated sludge. The flow rate is 0.5 l/h, temperature in the biotank is 18–20 °C and the rate of agitation does not exceed 96 r.p.m. Sulfate-reducing bacterial culture isolated at the oil field of Romashkovo was first adapted to the sources of carbon supply and then to tungsten and molybdenum ions. The degree of removal of heavy metal ions attains 94–98 %.

7.1.4. Oxidation and/or Reduction of Metals

Extraction of arsenic from effluents. The effluents of many non-ferrous metallurgical plants contain three-valent arsenic in the form of arsenites which are more soluble than arsenates [7]. For complete removal arsenic must be converted into the pentavalent form, i.e. it must be oxidized.

The pyrolusite-phosphate method entails the preliminary oxidation of arsenites into arsenates mediated by MnO_2; the reaction with milk of lime and phosphate-ions produces practically insoluble precipitates.

The following procedure was adopted for arsenic removal:

1. Oxidation of arsenites by pyrolusite;
2. Precipitation of arsenite by milk of lime in the presence of phosphate ions in the form of mixed arsenites and calcium phosphates.

Oxidation of arsenites by pyrolusite. The use of pyrolusite as a chemical oxidant has some disadvantages. Fe, Cu, Zn and other cations, as well as As ions, are sorbed on the surface of pyrolusite, gradually diminishing its oxidation ability. The surface of its grains become slagged. Therefore, pyrolusite columns have to be regenerated every 72 hours of operation with 10 % sulfuric acid [14]. Pyrolusite can actively oxidize arsenite if the concentration of the latter in the effluent does not exceed 100 to 150 mg/l, otherwise the filtrate will contain three-valent arsenic. However, the initial concentration of arsenite in the effluent from a sulfuric acid plant can be as high as 2000 to 3000 mg/l, thus the effluent must be diluted to the required concentration of arsenic.

Precipitation of arsenite. Therefore, the use of microorganisms for removing arsenic from wastewaters is of great importance. In particular. **Pseudomonas putida** 18 can oxidize As(III) at mildly alkaline and neutral reaction of the medium [1, 20]. To prevent microorganisms from being washed out by the effluent, they are adsorbed on wood chips. In the experiment, solutions flowed through wood chips in oxidation columns and no organic substance was added. After a month, bacteria actively oxidized arsenite with plastics and wood chips as adsorbents at the flow rate of 1 l per day and a column working volume of 2 l. Once aeration ceased, the rate of arsenite oxidation declined sharply.

The experimental results demonstrate the possibility of using adsorbed microorganisms for oxidizing arsenite in industrial wastewaters.

Removal of chromium from effluents [25]. The main features of the biological purification of chromium-contaminated effluent are given in Table 7.2.

Table 7.2

Main features of biological purification of chromium-contaminated effluent

Parameters	Main technological features
Substrate	Chromium-contaminated water + municipal sewage as a source of organic substance
Bacterial culture	Pseudomonas dechromaticans
Reagents	No special reagents are required
Conditions of the reduction process	Anaerobic
Duration of the reaction $Cr^{6+} \rightarrow Cr^{3+}$	From 0.5 to 4 hours (depending on the initial concentration) 1 g of potassium chromate in 3 days per 1 g of dry biomass
Temperature	20–25 °C
pH	7.7 to 8.3
Corrosion-resistant equipment	Not required. Main structures are made of reinforced concrete
Electric power consumption	Insignificant
Capital investments and equipment costs	3 to 5 times lower than ordinary methods
Operating costs	5- to 7-fold reduction per 1 m^3 of decontaminated water
Purification cost per 1 m^3 of chromium-contaminated effluent	5 to 10 copecks

A pilot variant of this technique was tested at "Communar" automobile plant in Zaporodzye (Fig. 7.4). The unit, with capacity of 47 m^3/h, has been in operation since 1976. Municipal sewage with a mean BOD value of 100 mg/l enters settler 1 and then spontaneously flows to mixing chamber 3. Activated sludge as well as chromium-contaminated effluent, with large particles removed, are also delivered to the mixing chamber. The sludge comes from settler 4 of purified effluent. Flowmeter regulates the supply of chromium-containing effluent.

The effluent/activated sludge mixture in the mixing chamber is separated into two flows and delivered to tank 6 where Cr^{6+} is reduced to Cr^{3+} with constant stirring. Water with chromium hydroxide and activated sludge flow to settler 4. After precipitation, part of the activated sludge is recirculated into the mixing chamber, the excess sludge is dumped into the reservoir of pumping station 2. After removal of Cr^{6+}, water from municipal sewage settler 1 enters the same reservoir.

The effluents mixture is discharged into the municipal sewage system. Removal of chromium from effluent is stable.

Concentration of Cr^{6+} in the mixing chamber varies from 10 to 40 mg/l as compared to 0.04 to 0.2 mg/l in the settler of purified effluent.

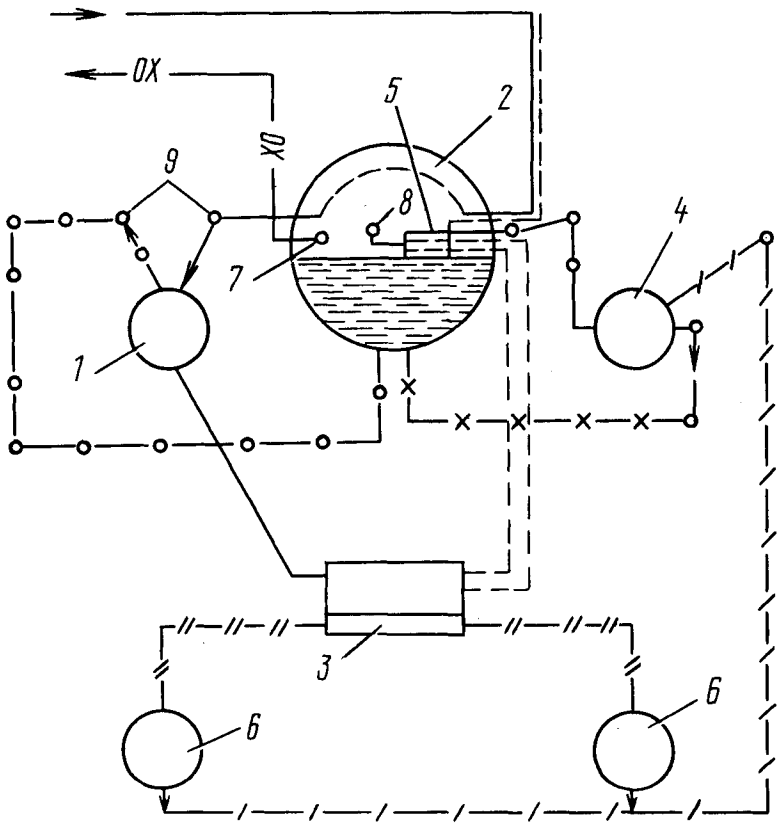

Fig. 7.4. Flowsheet for the biological purification of chromium-containing effluents:
1 – municipal sewage settler; 2 – pumping plant; 3 – mixing chamber; 4 – purified wastewater settler; 5 – activated sludge pump; 6 – bioreducer; 7 – pump for discharging waters and precipitates; 8 – tank for used chromium electrolyte; 9 – distribution chamber

——————— municipal sewage from wastewater disposal system, Q = 65 m^3/h

– – – – – – – – – – chromium-containing effluent from a metal-coating plant, Q = 47 m^3/h

——— · ——— anaerobic activated sludge

——— ,, ——— mixture of waters and activated sludge

——— / ——— effluents with chromium hydroxide and activated sludge

——— x ——— purified water

——— o ——— precipitates of municipal water

——— ox ——— mixture of waters and precipitates discharged into the city wastewater disposal system, Q = 112 m^3/h.

The main factors to be considered are:
1. Temperature;
2. Volume of water delivered to reactor, m^3/day;
3. Dose of sludge, g/l;
4. Concentration of Cr^{6+} in mixing chamber, mg/l;
5. Concentration of Cr^{6+} in settler of purified effluent, mg/l;
6. Concentration of Cr^{3+} in settler of purified effluent, mg/l;
7. pH in reactor.

A. dechromatica [24] may also be used to reduce Cr^{6+}. It is expedient to utilize microbial associations consisting of **A. dechromatica** and **Rhodococcus rubropertinica, Micrococcus varians, Pseudomonas fluorescens**, isolated from sludge of the industrial plant. They are able to utilize substrates not used by the monoculture **A. dechromatica**, for example, γ-butirolactone, which is a highly concentrated waste of the organic synthesis on the basis of natural gas. The immobilization of cells considerably increases the reduction rate of Cr^{6+} (up to 9-fold).

7.2. BIOSORPTION OF METALS

Biosorption is based on the interaction of metals with superficial cellular structures of microorganisms, with microbial metabolites and exopolymers.

7.2.1. Metal Sorption by Exopolysaccharides

Bacterial exopolysaccharides actively accumulate metals from solutions [4–6, 8–10, 11, 29–31, 33, 36]. Below are some examples of exopolysaccharides used to recover certain non-ferrous metals and radioactive elements from solutions.

Exopolysaccharides of Zoogloea ramigera [29–31]

Biomass production. The organism used was **Zoogloea ramigera** 115 (ATCC 25935) which produces substantial amounts of polysaccharide when cultured on a medium containing carbohydrates. The organism was maintained on Trypticase Soy Agar. The biomass was cultured in a fermentor on a medium of the following composition (g/l): glucose, 25; K_2HPO_4, 2; KH_2PO_4, 1; NH_4Cl, 1; $MgSO_4 \cdot 7H_2O$, 0.2; yeast extract, 0.01. Temperature was kept at 26 °C, stirring 800 rpm and aeration 0.5 vvm. (*) After 120 h of culture, the total biomass output from 3 l medium was 17.5 g/l, with 15 g/l of polysaccharides and 2.5 g/l of bacterial cells. In order to separate the biomass from the medium, the samples were centrifuged at $11.4 \cdot 10^3$ g for 10 minutes or were allowed to settle and further decanted. Precipitate was washed with distilled water and used in the experiments. The washed sample preserves its properties when frozen.

(*) "volume per volume · minute".

Sorption of copper, cadmium and uranium. The washed suspension of biomass (0.83 g dry weight) was added to metal-salt solutions containing $CuSO_4 \cdot 5H_2O$; $CdSO_4 \cdot 8/3H_2O$; $UO_2(NO_3)_2$ or a mixture thereof. The pH in the mixture was adjusted to the desired value with 0.05 M sodium borate. The mixture was then homogeneized for 15 minutes stirring continuously at 800 rpm. Biomass saturation and flocculation occurs within 15 minutes and further exposure does not affect the absorbed metal concentration.

After three successive exposures of Cu^{2+}-containing solution (0.525 g/l) to the biomass (0.83 g/l) at pH 4.7–5.5, Cu^{2+} concentration in the solution fell to 0.01 g/l.

In this test, the metal uptake values were 0.323 and 0.223 g metal/g biomass dry weight for copper and cadmium respectively.

Sorption of the different metals occurs at different pH values. Thus, optimum pH's for the sorption of U, Cd and Cu were estimated to be 3.5, 5.5 and 6.5 respectively.

Selective removal of Cu, Cd and U from the solutions containing a mixture thereof is achieved by adjusting pH values. Biomass (final concentration of 0.83 g dry weight/l) was added to the mixture of dissolved metals (0.5 g/l Cd^{2+}, 0.308 g/l Cu^{2+} and 1.28 g/l UO_2^{2+})stirring continuously. The pH is first adjusted to 3.1. After a 15-minute exposure the flocs are separated from the solution by centrifugation. The remaining supernatant is used for repeated flocculation at the same pH. After the fourth flocculation at pH 3.1, uranium concentration in solution dropped to 0.0005 g/l. Uranium uptake reached 0.37 g/g of biomass dry weight.

Copper was removed from the same solution after 4–5 flocculations at pH 4.7 to 5.5. Cadmium was most effectively removed after six flocculations at pH 6.5. Cadmium uptake was 0.171 g/g of biomass dry weight. The total amount of metals removed from solution was 2.06 g after adding a total of 6.13 g biomass (dry weight).

Monovalent sodium and divalent magnesium did not affect the sorption of copper but 10 mM of trivalent aluminum reduced the uptake of copper by biomass from 0.305–0.323 to 0.241 g/g dry weight.

Recycling of biomass. The pellet, consisting of bacterial biomass and adsorbed metal ions, is diluted with an equal volume of distilled water. It is then acidified with HCl to pH 3.0–4.0 and stirred for 15 minutes. The stirring rate must not exceed 100–150 rpm otherwise the flocs break up and polysaccharide dissolves. During this theatment the flocs release the previously bound Cd and Cu ions which are separated therefrom by centrifugation or decantation. The remaining pellet is dissolved in water under intensive stirring (800 rpm) and is further used for flocculation of metals.

Emulsan and Emulsanosol

Emulsan is a polyanion heteropolysaccharide with main components of N-acetyl-D-galactosamine (25 %) and N-acetyl-aminuronic acid (33 %) [37]. Emulsan is able to absorb metals and can be used as a biosorbent in the form of

homogenenous aqueous solutions and hydrocarbon emulsions (emulsanosol) [35], the uranium-binding capacity of the latter surpassing that of emulsan [36].

Emulsan production. Emulsan is produced by **Acinetobacter calcoaceticus** RAG-1 (ATCC 31012). It is grown in ethanol-containing medium and separated by ammonium sulfate precipitation [33].

Emulsan was purified as follows [37]: culture broth was separated from bacterial cells and evaporated; dried cell-free pellet was washed with warm ethanol to remove colored impurities, dissolved in 80 % ethanol and then precipitated by adding a saturated solution of calcium nitrate in 90 % ethanol; the precipitate was dissolved in 80 % ethanol and calcium nitrate precipitation was repeated. Emulsan preparations were deproteinized by the hot phenol method [37] and dialyzed at 4 °C overnight with 0.02 N HCl and then with distilled water. This method of emulsan preparation and purification prevents its polymerization. The preparations contained 1–2 % residual protein and less than 3 % ash.

Uranium binding by emulsan [36]. Aqueous solutions of emulsan (1 mg/ml) and uranyl nitrate (0.2–1.7 μM/ml) were mixed on a reciprocal water bath shaker (100 strokes/min) at 27 °C for 1 hour. Emulsan was then separated from the solution by ultrafiltration using a CF-50A Diaflo membrane. At initial concentrations of UO_2^{2+} of less than 100 μg/ml, over 90 % of the uranium was bound by 1 mg emulsan/ml. Maximum binding capacity of 1 mg emulsan is 0.9 μM UO_2^{2+}.

Mono and divalent cations Li^+, Na^+, NH_4^+, K^+, Rb^+, Cs^+, Mg^{2+}, Cd^{2+}, Ca^{2+}, Ba^{2+}, Rb^+ do not significantly alter UO_2^{2+} binding capacity of emulsan.

Preparation of emulsanosol [36]. Emulsanosol was prepared by ultrasound treatment (35–40 W for 60–75 s) of a mixture of 1.5 ml hexadecane and 8.5 ml of an aqueous solution containing 5.5–6.0 mg emulsan, 0.02 M Tris buffer (pH 7.2) and 0.01 M $MgSO_4$. After allowing the emulsion to stand for 24 h at room temperature and then centrifuging at 8000 g for 20 min, a creamed upper layer – the emulsanosol – was obtained having the following composition: 1.5–2.0 mg emulsan/ml, 38–45 % water and 55–62 % hexadecane. Hexadecane-in-water emulsions not stabilized by emulsan break up within 1 h. Emulsanosol remains stable for several days.

Uranium binding by emulsanosol. To study uranium-binding capacity of emulsanosol, 1 ml of the freshly prepared emulsion was mixed with an equal volume of a uranyl nitrate solution of given initial concentration. The emulsions were left to stand at 23 °C for 18–20 hours. Emulsanosol was then separated by centrifugation at 8000 g for 20 min, and uranium concentration of the aqueous phase was measured. Emulsanosol activity in binding UO_2^{2+} far surpasses that of emulsan; the former is characterized by the following values: with 50 μl emulsanosol containing 90 μg of emulsan and an initial UO_2^{2+} concentration of 0.33 mM, 3.55 μM uranyl ion were bound per mg of emulsan. A higher concentration of emulsanosol produces a better purification of the solution. With an initial concentration of 16.8 μg uranyl ion/ml, 50 μl/ml of emulsanosol (75 μg/ml emulsan) and 75 μl/ml of emulsanosol (113 μg/ml emulsan) reduced the uranyl ion concentration to 1.9 and 1.2 μg/ml respectively.

Hexadecane-in-water emulsion stabilized by methylcellulose binds 2.5 times less uranium than emulsanosol.

Emulsanosol has a greater uranium-binding capacity than other biosorbents of microbiological origin. Chitin from cellular walls of **R. arrhizus** binds 6 mg/g of UO_2^{2+}, **Rhizopus arrhizus** mycelia bind 180 mg/g as compared to 800 mg of UO_2^{2+} bound by 1 g of emulsan in emulsanosol. Furthermore, emulsanosol can reduce the uranium concentration in water from 15 to 1 μg/ml in a single cycle process. Emulsanosol can be prepared from a variety of natural or synthetic hydrocarbon mixtures which are less expensive than pure hexadecane [35].

Effect of pH. pH has a marked effect on uranium binding by emulsan and emulsanosol. In both cases the amount of bound uranium decreased sharply below pH 4.0. However, with emulsan in solution, even at pH 1.0 more than 50 % of the uranyl ion remained bound, while with emulsanosol more than 70 % of the bound uranium was released at pH 3.0.

Emulsanosol recycling. The used emulsanosol can be recycled at pH 2 when more than 90 % of the bound uranium has been desorbed, while less than 10 % of the emulsan is released into the solution. Emulsan is ecologically safe as it does not contain phosphorus, is biodegradable and nontoxic. Zosim et al. [36] claim that on the basis of these findings, the possible application of emulsanosols for wastewater treatment and uranium recovery should be further explored.

7.2.2. Biosorbents Constituted of Microorganism Biomass

7.2.2.1. Removal of Dissolved Uranium and Nitrates by Denitrifying Bacteria

Denitrifying bacteria immobilized on coal particles can be used for removing uranium [34] and nitrates [15] from industrial wastewaters.

Sorption of uranium. The immobilized biomass consisted of a mixed culture of denitrifying bacteria obtained from a fluidized pulsed-bed bioreactor [34] on 30–60 mesh (*) anthracite coal particles. Solutions, containing 500–5000 g/m^3 of ethanol as an energy and carbon source and 250-500 g/m^3 sodium nitrate were used as culture media. The temperature of the bioreactor was kept at 25 ^{o}C. Bacteria grew as films on coal particles; the weight of immobilized biomass was controlled within 5–10 % of the dry coal weight by directing the slurry of particles onto a Sweko vibrating screen (30 mesh). (*) Microbial film on the particles passing through the screen was collected either by centrifugation or filtration and used for uranium biosorption.

The adsorbing capacity of the biomass was determined in laboratory shake-flask tests using an incubation shaker or a New Brunswick fermenter (model M19), with a 7-liter mechanically stirred tank equipped with a pH and tempera-

(*) if "mesh" refers to A.S.T.M., 30 mesh corresponds to 0.595 mm and 60 mesh to 0.250 mm.

ture controller, or in a 7-liter cylindrical tank with conical bottom, where agitation was provided by an air-driven propeller. In solutions with a desired concentration or $UO_2(NO_3)_2$ the pH and ionic strength were adjusted to values of 4.0 and 1.0 M respectively, using nitric acid, sodium hydroxide and sodium nitrate. The temperature was maintained at 40 °C. Suspensions of immobilized cells were added to the solution. Samples were withdrawn at specified time intervals, centrifuged at 21,000 g for 10 minutes and the supernatant was assayed for uranium content.

At the same time an aliquot of each sample was analysed to determine the biomass. Coal particles coated with bacterial film were removed from the solution by centrifugation, washed with water to remove the unattached biomass, dried for 24 h at 105 °C, cooled to room temperature and weighed. Immobilized biomass was desorbed by soaking the samples in 4 M of NaOH for 4 hours. Then the coal particles were again washed with water, dried for 24 h at 105 °C and weighed after cooling; the biomass was determined as the difference between the weight of film-coated particles and film-free particles.

Under the experimental conditions, 1 g of biomass (dry weight) adsorbs 0.14 g of uranium in 15 minutes. Temperature variations within the range 25–50 °C had no effect on the distribution of uranium.

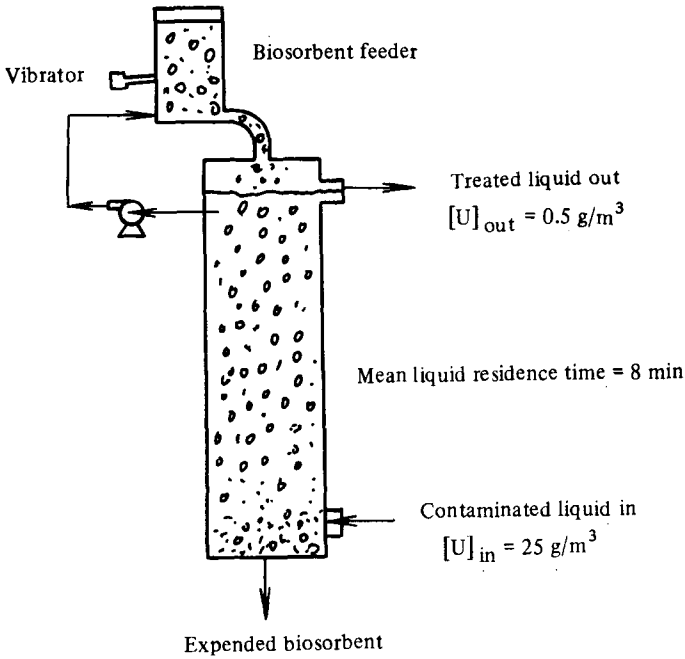

Fig. 7.5. Schematic diagram of countercurrent contactor used for continuous separation of uranium by film of denitrifying microorganisms attached to coal particles

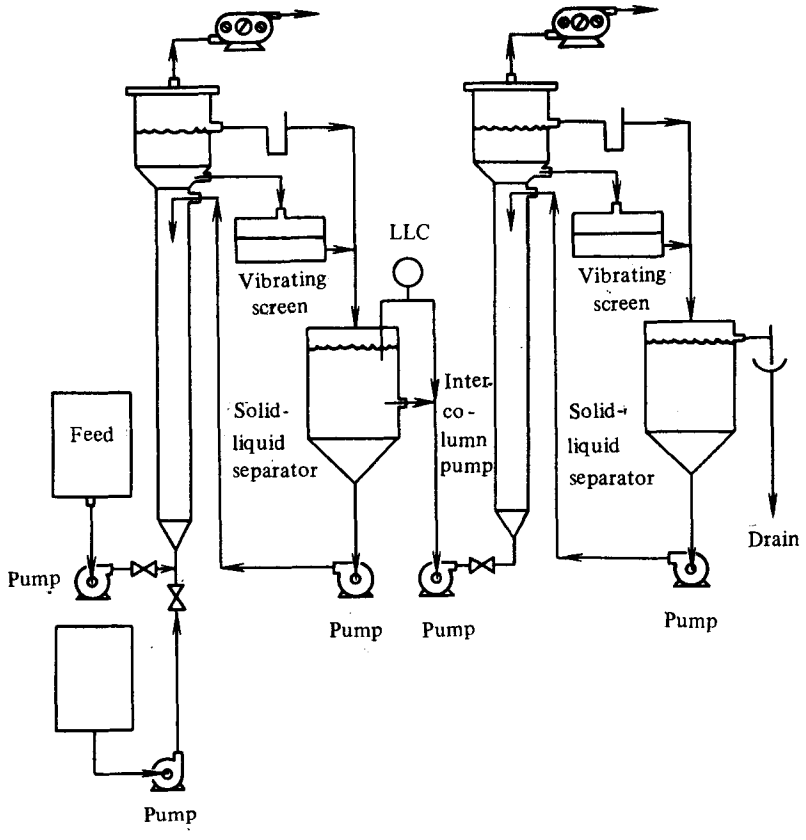

Fig. 7.6. Dual fluidized-bed bioreactor system

Continuous biosorption of uranium was performed in a column contactor (Fig. 7.5) which consisted of a 1 m high glass pipe 5 cm in diameter. A solution containing soluble uranium at a concentration of 25 g/m^3 was pumped into the lower section of the contactor. The biosorbent particles with bound uranium settled at the bottom of the contactor and were periodically removed. Purified solution containing 0.5 g/m^3 uranium was withdrawn from the upper part of the contactor. The liquid temperature was within 22–24 °C. Solution pH in the range 3–4 was found to be optimum for sorption of uranium. In the time interval corresponding to the residence time (8 min), up to 98 % of uranium is adsorbed.

Denitrification of wastewaters [15]. Industrial wastewaters, including the effluents coming from nuclear power stations, need to be denitrified. This can be done by using denitrifying bacteria capable of reducing nitrates to gaseous nitrogen. Hancher et al [15] used mixed cultures of denitrifying bacteria, immobilized on anthracite particles.

The denitrification process was performed in Pyrex glass column reactors from 5 to 10 cm in diameter and up to 6 m high. In Fig. 7.6 the flowsheet of a plant comprising two column reactors with funnel-shaped bottom is shown. The reactors are composed of sections 60 cm in length, with outlet for the solid phase, outlet for the removal of the gases formed. Wastewaters enriched in ethanol (with concentration corresponding to a C/N ratio equal to 1.5), phosphorus and microelements reach the reactor bottom at a flowrate of 800–1000 $cm^3 \cdot min^{-1}$ in 5-cm diameter reactors and of 4000 $cm^3 \cdot min^{-1}$ in 10 cm reactors. The carriers of immobilized cells are located in the upper part of the reactor. The fossil coal particles with attached bacterial cells do not lie on the bottom but are kept in suspension by the up-flowing solution. As a consequence of cell growth, the coal particles become completely covered with bacterial cells and this hinders the transfer of N_2 produced to the medium.

The coal particles loaded with gas bubbles rise and float on the upper layer of the medium in the reactor, wherefrom they are periodically removed and are passed on a vibrating screen with 0.5 mm openings. Thereafter the coal particles are reintroduced into the cycle.

The process performs best if the concentration of immobilized cells does not exceed from 5 to 10 % of the total weight of carrier particles. The cells which are not attached to coal particles flow out of the reactor (the upper part) with the decontaminated water. The process is carried out at 22 °C.

The denitrification rate increased with the increase of nitrate feed concentrations from 200 to 7500 g/m^3, with all other process variables held constant. The concentration of Na^+ and NH_4^+ below 1 M did not inhibit denitrification.

The authors of this technique [15] propose a mobile pilot plant that will contain two 20-cm-i.d. by 7-m-high column bioreactors. For near-zero NO_3^- effluent (5 to 10 g/m^3), the feed will have a maximum concentration of 4000 g/m^3 and will be introduced at a flow rate of 16 liter/min. The temperature will be regulated between 22 and 30 °C.

7.2.2.2. Biosorbent M

Biosorbent M is obtained by processing the mycelia of **Penicillium chrysogenum** (a waste product of antibiotics production) with ureaformaldehyde polycondensate [2, 22]. The solid product obtained comprises mycelium and its components, polycondensate and the reaction products of urea and formaldehyde with mycelium components. The product is then crushed to 0.3–0.8 mm. The resulting material is hydrophilic, its grains swelling in water and in electrolyte solutions, mechanically strong with stable physical and chemical parameters. The mechanism of cation binding, including radioactive elements, by biosorbent M consists in the following main types of interaction:

1. ion substitution reaction;
2. complex formation;
3. mechanism of sorption and precipitation.

The first and second types of interaction are similar to the processes taking place in ion-exchange resins.

The third type of interaction involves both the binding of cations and anions, and the simultaneous precipitation of insoluble compounds on the surface of and inside the particles of biosorbent M.

The ability of biosorbent to remove radioactive elements from industrial wastewaters has been demonstrated in a semi-industrial plant consisting of two columns in series filled with biosorbent M. Radioactive mine effluents were fed to the plant at a predetermined rate. Under similar conditions commercial ion-exchange resins were unable to reduce uranium concentration down to the 0.05 mg/l and Ra concentration down to the 3–30 picocurie/l achieved with biosorbent M. One volume of this product can treat 800 to 1200 volumes of solution in one cycle. In this series of experiments uptake capacity of biosorbent M was found to be 5 g U/kg dry weight. Maximum uptake capacity was within 80 to 120 g U/kg dry weight with initial uranium concentration of 1 g/l.

At average initial Radium concentration of 396 picocurie/l one volume of biosorbent purified 1000 to 1500 volumes of solution. Recycling of biosorbent M requires elution of bound uranium by a solution containing 10 % NaCl and 0.5 % Na_2CO_3. Radium is eluted with HCl solution of 0.1 N.

7.2.2.3. AMT-bioclaim Technology [3]

AMT-bioclaim is a wastewater treatment and metal recovery technique developed by Advanced Mineral Technologies, Inc. (USA). The essence of the technique is a granulated product derived from biomass. The specifically treated biomass is then dried, ground and segregated to provide granules of given sizes (–30+65 mesh) and used for biosorption of non-ferrous and precious metals.

Laboratory testing. Glass columns (20 cm high, 16 mm in internal diameter) were packed with 6.5 g of granulated biosorbent. Metal-containing solutions were pumped at a rate of 10 ml/min upward through the column. Tests were

Table 7.3

Variation in metal uptake capacity of different types of biomass (100 mg metal/l)

Metals	Type of biomass			
	1	2	3	4
	uptake (mg of metal/g of biomass)			
Ag	86	65	129	–
Cd	101	135	112	–
Cu	152	76	146	–
Pb	601	373	269	62
Zn	137	98	78	–

run until up to 90 % of metal had been removed from solution. Not all biomass converted to granulated sorbent have the same capacity for uptaking metals. Four different types of biomass were examined for metal recovery (Table 7.3):

1. biomass produced by AMT-bioclaim technique;
2. biomass produced from wastes of industrial fermentation;
3. biomass obtained from laboratory-grown microbes;
4. algal cell biomass.

AMT-bioclaim biosorbent exhibited the highest uptake capacity for the metals examined. It effectively purified solutions containing 9 mg/l Cu^{2+} (at pH 5.4) and 100 mg/l Pb^{2+} (at pH 3.87). Variations in Cu, Cd, Zn and Pb content in the initial solution of between 10 and 100 mg/l do not affect its uptake capacity.

Copper uptake increased with temperature up to 90 °C. In column tests, 6.5 g of AMT-bioclaim, without replacement or regeneration, efficiently removed (99 %) copper from 50 l of solution containing 9 mg/l Cu and lead from 20 l of solution containing 100 mg/l Pb.

AMT-bioclaim can be used for the recovery of precious metals even when they are complexed with cyanide or in the presence of other metals. A column containing 20 g of the agent removed more than 99 % of metals for the first two liters of solution containing (mg/l): Au, 954; Ag, 30; Cu, 291. CN content was 6.5 mg/l and pH was adjusted to 10.4. After treating four liters, 58 % of gold was retained. In the bottom one third of the column up to 390 mg of Au/g, 1.5 mg of Ag/g and 194 mg of Cu/g accumulated.

Pilot plant tests. Having studied several systems suggested for biosorption [26, 32, 34] the authors of AMT-bioclaim proposed their own technique for bringing into contact the biomass with metal-bearing solution using two types of reactors [3].

The AMT-bioclaim canister unit is a small-scale wastewater treatment system designed specifically for industries generating low volumes (up to 4540 l) of wastewater daily. The process comprises the following stages. The wastewater is collected in an equalization tank. When it reaches a certain level a pump is activated and the wastewater is pumped at a controlled rate (up to 4.5 l/min) through the first of two canisters in series. Each canister contains 18 kg of the granular sorbent. After the biosorbent in the first canister becomes loaded with metal, the exhausted canister is removed and replaced by the second one. A fresh canister is then installed as standby unit.

Larger volumes, 4500–36000 l/day, of wastewater can be processed using the AMT-bioclaim fluidized pulsed-bed units, 4.5 m high and 0.45 m in diameter, which treat waste flows of 4.5–36 l/min or 2700–17000 l/8 hour shift. Initially, the fluidized-bed contactor is filled with 80 kg of granular biosorbent, yielding a bed depth of about 1.8–2.1 m. The wastewater enters an equalization tank and is then pumped to the fluidized-bed contactor. As the water flows up through the contactor, the granular biosorbent becomes fluidized. This fluidization causes the larger, heavier granules to settle and the smaller lighter ones to migrate upwards, yielding a relatively stable, segregated bed that ensures optimum removal of metal ions from the wastewater. As the metal recovery agent becomes loaded with metal, it becomes heavier and sinks to the bottom

of the fluidized bed. Thus, the most loaded material accumulates at the bottom of the contactor, from where it is periodically removed (approximately 1/7 to 1/10 of total vessel volume) and replaced with an equal volume of fresh material.

A pilot plant for the removal of lead from an industrial effluent was run for 39 days. The unit contained 100 kg of granular agent, lead-containing wastewater was pumped upward at the rate of 4.5–18 l/min.

The lead content in the effluent varied from 0.01 to 4.30 mg/l; pH ranged from 4.6 to 9.7. The variation in pH did not influence lead removal by the agent.

A stable and efficient removal of lead from the effluent (98–99 %) whether treated with caustic soda or not, was achieved with this unit. The lime sludge caused by caustic precipitation did not impair the efficiency of lead removal.

The cycle is completed by the elution of the bound metal, i.e. regeneration of granules and their recycling.

The tests demonstrated that the AMT-bioclaim system provides an effective purification of industrial effluents despite the variations in pH and metal contents. The industrial-scale units with pulsed bed reactors will be 4.6 m high, with varying diameter depending on the volume of wastewater to be treated.

This system can be used either independently or in combination with other techniques for wastewater treatment.

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Внешторгиздат. Изд. № 7681А
ВТИ. Зак. 6028