MICROBIOLOGICAL PROCESSES FOR THE LEACHING OF METALS FROM ORES

State-of-the-Art Review

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MICROBIOLOGICAL PROCESSES FOR THE LEACHING OF METALS FROM ORES

State-of-the-Art Review

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The role of microorganisms in the oxidation of sulfide minerals and leaching of metals from ores and concentrates, and the factors underlying the kinetics of the above processes, as well as technical, technological, economic, and environmental aspects of the biogeotechnology of metals, are reviewed.

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

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PREFACE

Bacterial leaching deals with the extraction of metals from ores, concentrates and rocks under the influence of bacteria or their metabolites at atmospheric pressure and temperatures ranging from 5 to 80° C. For the most part, the leach solution contains sulphuric acid (which can be of bacterial or chemical origin) as well as organic acids, proteins, polysaccharides and other products of the microbial synthesis. Therefore, it is probably more correct to denote this leach technique as a mixed bacterial and chemical process. There are at least three different microbiological processes which play an important role in hydrometallurgy:

1. Oxidation of sulphide minerals, sulphur and ferrous iron;
2. Production of organic compounds, peroxides and so on, by heterotrophic microorganisms. These products dissolve minerals through complexation and oxidation; and
3. Precipitation of metals in order to purify industrial effluents or to extract non-ferrous and precious metals from solutions.

Recently, a number of reviews have appeared concerning the leaching of non-ferrous and other metals from ores [1—7]. This *Review* considers mostly the microbiological aspects of dump, underground and tank leaching of metals from ores and concentrates, and is related to the International Training Course on Microbiological Leaching of Metals from Ores. The technological problems of bacterial leaching of metals are considered in general since they have been covered in detail by other authors [1—5].

The section on Economic Aspects includes the few data available in the literature. It is not possible to estimate the application of this technology in different countries and geographical zones in economic terms since such information is not available. Obviously, the economic aspects of leaching will be determined by many factors typical of each country.

This *Review* does not consider the role of heterotrophic microorganisms in hydrometallurgy since this problem is still under study.

Chapter I

LITHOTROPHIC BACTERIA IN THE OXIDATION AND LEACHING OF SULPHIDE MINERALS

Thionic bacteria and a number of thermophilic microorganisms belonging to new genera are responsible for the oxidation of sulphide minerals, elemental sulphur and ferrous iron in ore deposits. Oxidation of reduced compounds of sulphur and iron is the only energy source for chemolithoautotrophic bacteria. The atmospheric CO_2 is used as a carbon source in the constructive processes.

1. Mesophilic Bacteria

Thiobacillus ferrooxidans [8, 9] (Fig. 1a) oxidizes virtually all known sulphide minerals and a number of elements with variable valency (Fe^{2+}, Cu^{+}, Sn^{2+}, Se^{2+}, Sb^{3+} (?) and U^{4+}) at temperatures ranging from 2 to 40° C (the optimum from 28 to 35° C) and pH from 1.0 to 4.8 (the optimum from 2.0 to 3.0) [3, 10—19]. Sulphide sulphur is oxidized while in such minerals as pyrite, chalcopyrite, arsenopyrite and so on, all reduced valency sulphur and iron compounds.

Leptospirillium ferrooxidans (Fig. 1b) isolated by Markosyan [20] oxidizes only Fe^{2+}. However, in the binary culture with *Thiobacillus organoparus* [21] (syn. *T. acidophilus* [22]) or *Thiobacillus thiooxidans*, they oxidize pyrite and chalcopyrite [23, 24].

T. thiooxidans (Fig. 1c) oxidizes elemental sulphur, $S_2O_3^{2-}$, SO_3^{2-}, $S_4O_6^{2-}$ and Sb_2S_3 and sphalerite (ZnS) at temperatures from 2 to 40° C (the optimum from 28 to 30° C) and pH from 0.5 to 5.0 [25—27].

T. organoparus oxidizes only elemental sulphur and grows at pH 1.5 to 5.0 (the optimum from 2.5 to 3.0) [21].

T. thioparus oxidizes S^0, $S_2O_3^{2-}$ and a number of sulphide minerals (PbS, Bi_2S_3, Sb_2S_3) at pH 8.0 [26, 28]. Khalid and Ralph [27] showed that *T. thioparus* also oxidizes zinc sulphides.

Abdrashitova et al. [29] proved that *Pseudomonas putida* and *Alcaligenes eutrophus* isolated from aurous arsenite ores oxidize As^{3+} to As^{5+} at pH 6 to 9. Oxidation of As^{3+} is accompanied by a decrease in pH of the medium.

Fig. 1. Cells of microorganisms important for hydrometallurgical processes: (a) — *T. ferrooxidans;* (b) — *L. ferrooxidans;* (c) — *T. thiooxidans;* (d) — *S. thermosulfidooxidans*

2. Thermophilic Bacteria

Sulphide minerals, sulphur and ferrous iron are also oxidized by thermophilic bacteria similar to thionic bacteria, such as isolated by Brierley and Le Roux from thermal springs and ore [30—32]. The oxidation of non-organic substrates occurs at temperatures 50 to 55° C and pH 1.6 to 2.2. Addition of 0.02% yeast extract accelerates the bacterial growth and the oxidation processes.

Sulfobacillus thermosulfidooxidans (Fig. 1 d) was isolated from hot spots in the leach dump of sulphide-bearing copper ores. This organism oxidizes sulphur, Fe^{2+} and sulphide minerals (FeS_2, $CuFeS_2$, FeAsS, PbS, copper-zinc ore) in the presence of 0.02%

yeast extract at temperatures 20 to 60° C and pH 1.9 to 3.0 [33].

Sulfolobus acidocaldarius [34], in the presence of yeast extract, oxidizes sulphur at temperatures 80 to 85° C and pH 0.9 to 5.8.

Furthermore, Brierley and other authors isolated from thermal springs a number of other thermophilic bacteria which are similar to the *Sulfolobus* species [7, 35, 36]. Recently, these organisms were identified and received specific names of *S. brierleyi* and *S. solfataricus*. *S. brierleyi* [37] oxidizes Fe^{2+} and S^0 as well as sulphide minerals in the presence of yeast extract at temperatures 45 to 75° C (the optimum 70° C at pH 1.5 to 2.0).

Thus, biogenic oxidation of sulphur, Fe^{2+} and sulphide minerals occurs within a wide temperature (from 5 to 80° C) and pH (from 0.5 to 8.0) range. At low pH values, the majority of non-ferrous and rare metals are leached from the ores [3].

Chapter II

BACTERIO-CHEMICAL OXIDATION AND LEACHING PROCESSES

1. Oxidation of Ferrous Iron

Oxidation of Fe^{2+} by *T. ferrooxidans* can be described by the following equations:

$$Fe^{2+} \rightarrow Fe^{3+} + \bar{e}; \qquad (1)$$

$$2\bar{e} + {}^1/{}_2O_2 + 2H^+ \rightarrow H_2O; \qquad (2)$$

$$2Fe^{2+} + {}^1/{}_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O; \qquad (3)$$

$$Fe^{3+} + 3H_2O \rightleftharpoons Fe(OH)_3 + 3H^+. \qquad (4)$$

The rate of Fe^{2+} oxidation is determined by the rate of bacterial growth which in its turn depends on the method and conditions of cultivation. The doubling time of bacteria cultured on the media containing Fe^{2+} at temperatures 28 to 35° C varies from 3.6 to 10 hours [38—41].

When *T. ferrooxidans* is cultured in a reactor with electrochemical reduction of Fe^{3+}, one can obtain high concentrations of cells (up to 4.5 $g \cdot l^{-1}$ of dry biomass weight) at low concentrations of total iron (4 to 6 $g \cdot l^{-1}$) [42, 43]. The rate of oxidation of Fe^{2+} in this reactor can be as high as 50 $g \cdot l^{-1} \cdot h^{-1}$. Without bacteria at low pH values, the chemical oxidation of Fe^{2+} progresses relatively slowly. In this reactor, the ferric iron is constantly reduced to ferrous iron whereby favourable conditions for bacterial activity are created. As such, both the rate of bacterial growth and the rate of Fe^{2+} oxidation are enhanced. The ferric iron solutions containing large number of bacteria can be used for intensive leaching of metals from ores and concentrates.

2. Oxidation of Pyrite

Pyrite is a rather widely spread sulphide mineral, and can be found in association with many other ore minerals. Its oxidation produces H_2SO_4 and sulphates of the trivalent iron. Pyrite, like other sulphide minerals, is a semiconductor. It can possess either electron (*n*-type) or hole (*p*-type) conductivity [44]. Differences are observed in the chemistry of pyrite oxidation for samples possessing different conductivities. According to Yakhontova and Nesterovich [45], pyrite of the *n*-type at $pH<4.0$ is oxidized according to the scheme:
Diluted solutions:

$$FeS_2+3O_2+8H_2O \rightarrow Fe(H_2O)_6^{2+}+2HSO_4^-+2H^++2\bar{e}; \qquad (5)$$

Concentrated solutions:

$$FeS_2+3^1/_2O_2+H_2O \rightarrow [FeHSO_4]^{2+}+SO_4^{2-}+H^++\bar{e}. \qquad (6)$$

At $pH>4.0$ FeS_2 is oxidized according to the scheme:

$$FeS_2+2^1/_2O_2+9H_2O \rightarrow Fe(H_2O)_6^{3+}+2H_2SO_4+2H^++4\bar{e}. \qquad (7)$$

Pyrite of the *p*-type is oxidized according to equation (7) within a wide range of pH (2 to 9). In addition, the trivalent iron readily interacts with the pyrite:

$$FeS_2+2Fe^{3+} \rightarrow 3Fe^{2+}+S^0. \qquad (8)$$

Elemental sulphur produced during the chemical oxidation of FeS_2 is further oxidized both by bacteria (*T. ferrooxidans, T. thiooxidans, T. organoparus*) and chemically by Fe^{3+}, according to the following forms:

$$S^0+H_2O+1^1/_2O_2 \rightarrow H_2SO_4; \qquad (9)$$

$$S^0+6Fe^{3+}+4H_2O \rightarrow 6Fe^{2+}+SO_4^{2-}+8H^+. \qquad (10)$$

Calculations by Arkesteyn [46] showed that a large part of the elemental sulphur formed according to equation (8) at low pH values is oxidized not bacterially but chemically according to equation (10). Therefore, indirect chemical oxidation of FeS_2 can be expressed by an overall equation:

$$14Fe^{3+}+FeS_2+8H_2O \rightarrow 15Fe^{2+}+2SO_4^{2-}+16H^+. \qquad (11)$$

Many researchers investigated the kinetics of pyrite oxidation under the influence of *T. ferrooxidans* and found that the rate of oxidation increased 20 to 1000-fold as compared to the purely chemical process. The chemistry of reactions in case of direct bacterial oxidation of FeS_2 is, apparently, different and the end products of oxidation are Fe^{3+} and H_2SO_4.

The extent of bacterio-chemical oxidation of pyrite in natural conditions can be illustrated by the following examples: a) the mine waters of the Degtyarsky copper-pyrite deposit with the daily drainage rate of about 3,000 m^3 had a pH of 2.5; b) according to Davis, the coal mine drainage waters carried daily about 9,000 tons of sulphuric acid into the rivers of the Pittsburg area [in ref. 47].

3. Oxidation of Sulphide Copper Minerals

The most widely spread copper minerals are chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), covellite (CuS) and bornite (Cu_5FeS_4).

Chalcopyrite ($CuFeS_2$) is the most difficult to oxidize sulphide copper mineral. Under the influence of *T. ferrooxidans*, the rate of chalcopyrite oxidation increased 6—12-fold as reported by Duncan, Torma et al. [49—51]. The process of its chemical oxidation, as reported by Yakhontova et al. [48], depends on the pH value and can be described by a number of equations:

at pH 1 to 3:

$$CuFeS_2 + 3^1/_4O_2 + 1^1/_2H_2O \rightarrow [CuHSO_4]^+ + Fe^{2+} + HSO_4^- + H^+ + 3\bar{e}; \qquad (12)$$

at pH 3 to 7:

$$CuFeS_2 + 4O_2 \rightarrow Cu^{2+} + SO_4^{2-} + [FeSO_4]^+ + \bar{e}; \qquad (13)$$

at pH 7 to 9:

$$CuFeS_2 + 3.9O_2 + 0.2H_2O \rightarrow Cu^{2+} + SO_4^{2-} + [FeSO_4]^+ + 0.4H^+ + 1.4\,\bar{e}. \qquad (14)$$

During bacterial oxidation of $CuFeS_2$, Fe^{3+} is formed as an end product. Ferric iron, in its turn, interacts with chalcopyrite [52] according to the formula:

$$CuFeS_2 + Fe^{3+} \rightarrow Cu^{2+} + Fe^{2+} + 2S^0. \qquad (15)$$

Secondary sulphide copper minerals, such as chalcocite (Cu_2S), bornite (Cu_5FeS_4) and covellite (CuS), are more readily oxidized under the impact of bacteria and chemical factors. The chemistry of their oxidation, according to Yakhontova and Grudev [44], may be expressed as follows:

at pH<4:

$$Cu_2S + 1^3/_4O_2 + 6^1/_2H_2O \rightarrow [CuHSO_4]^+ + Cu(H_2O)_6^{2+} + 3\bar{e}; \qquad (16)$$

at pH>4:

$$Cu_2S + 2O_2 + 12H_2O \rightarrow 2Cu(H_2O)_6^{2+} + SO_4^{2-} + 2\bar{e}; \qquad (17)$$

$$Cu_5FeS_4 \xrightarrow{O_2+H_2O} Cu(H_2O)_6^{2+} + 4[CuHSO_4]^+ + Fe(H_2O)_6^{3+} + 9\bar{e}. \qquad (18)$$

The aqua-complex $Cu(H_2O)_6^{2+}$ will be further transformed into $[CuHSO_4]^+$ due to the presence of H^+ ions, which seems to be the main responsible for increased pH of the medium. Therefore, oxidation of Cu_2S and Cu_5FeS_4 is proton-consuming and results in an increase in pH. Both bacterial and chemical oxidations of Cu_2S will stop when pH reaches 4.6—4.7. Thus, unlike pyrite and chalcopyrite oxidation, bacterio-chemical oxidation of Cu_2S and Cu_5FeS_4 consumes sulphuric acid [3, 45, 53]. As shown by Beck [53], *T. ferrooxidans* catalyzes the oxidation of Cu_2S to $CuSO_4$ and CuS:

$$2Cu_2S + O_2 + 2H_2SO_4 \rightarrow 2CuS + 2CuSO_4 + 2H_2O. \qquad (19)$$

The rate of this reaction in Beck's experiments was increased by the bacteria about 40-fold over that of the sterile control.

Another distinctive feature of secondary sulphide minerals of copper is the fact that they are relatively easy to oxidize by trivalent iron:

$$Cu_2S + Fe^{3+} \rightarrow Fe^{2+} + CuS + Cu^{2+}. \qquad (20)$$

According to Razzell and Trussell [54], in the presence of bacteria and Fe^{2+}, which is oxidized to Fe^{3+}, the amount of copper solubilized is about 6 times greater, and about 3 times greater in the presence of bacteria alone, than that in the control without iron and bacteria. In Groudev's [55] experiments, the highest rate of copper extraction from covellite (CuS) in the presence of *T. ferrooxidans* reached 170 $mg \cdot l^{-1} \cdot h^{-1}$. By adding ferrous iron to the leach solution, copper extraction was further accelerated by 15 to 20%.

Thermophilic microorganisms were reported to participate in the oxidation of sulphide minerals of copper. For example, at 60° C and pH 2.5, they were observed to extract up to 50% of Cu from a copper ore with particle size in the range of 1.0 to 0.3 mm and Cu content of 0.32% [56]. Under similar leach conditions, the rate of copper extraction from a chalcopyrite concentrate (with particle size varying from 0.105 to 0.07 mm and containing about 27.6% Cu) was about 10—16 $mg \cdot l^{-1} \cdot day^{-1}$, as contrasted to 1.0—1.8 $mg \cdot l^{-1} \cdot day^{-1}$ without bacteria. Investigations by Chakrabarty and Murr [57] revealed that at 50° C in the presence of thermophilic bacteria only the same amount of copper could be extracted from a low-grade ore as was obtained at 30° C in the presence of *T. ferrooxidans*.

4. Oxidation of Sulphide Minerals of Other Metals and Metalloids

Zinc

Sulphide minerals of zinc can be oxidized by *T. ferrooxidans*, *T. thiooxidans* and *T. thioparus* [27, 58, 59]. Wurtzite is the most difficult to oxidize sulphide mineral of zinc. Marmatite was oxidized by *T. ferrooxidans* and *T. thiooxidans* faster than sphalerite either in the presence or absence of Fe^{3+}. Iron-free synthetic sulphide of zinc was oxidized faster by *T. thiooxidans* than by *T. ferrooxidans* or *T. thioparus*. The oxidation process may be expressed by the formula:

$$ZnS + 2O_2 \rightarrow ZnSO_4. \qquad (21)$$

The rate of oxidation of zinc sulphides in the presence of bacteria in the experiments by Trussell and Duncan increased 4 to 5-fold as compared to control tests without bacteria [59]. The rate of zinc extraction from polymetallic ores in the presence of *T. ferrooxidans* was two to three-fold faster by comparison to experiments without bacteria [60]. The intensity of bacterial leaching of Zn from sulphide minerals was also increased in the presence of pyrite and Fe^{3+}. According to Kulebakin [61], the bacterial oxidation of kleiofan and marmatite was 19.3—20 times faster than the chemical oxidation by trivalent iron.

Nickel

Nickel is leached from sulphide minerals (pentlandite and millerite) and from ores in the presence of *T. ferrooxidans* about 2—17 times faster as compared to the chemical process [11, 62—66]. Oxidation of pentlandite follows the scheme:

$$(Ni, Fe)_9S_8 + 17^5/_8O_2 + 3^1/_4H_2SO_4 \rightarrow$$

$$\rightarrow 4^1/_2NiSO_4 + 4^1/_4Fe^{3+} + 6^3/_4SO_4^{2-} + 3^1/_4H_2O. \qquad (22)$$

According to Torma [64], nickel is intensively leached from ores and concentrates in the presence of bacteria. The rate of Ni extraction from concentrates may reach ca. 200 $g \cdot l^{-1} \cdot h^{-1}$. In 66 hours, 73—97% of Ni were extracted from a low-grade ore containing 0.2% nickel. Over 90% of Ni were extracted in 8 days at the temperature of 50° C in the presence of thermophilic bacteria isolated from the Icelandic thermal springs [67]. The same authors found that in the presence of *T. ferrooxidans* at 30° C the same amount of nickel was extracted only in 14 days.

Antimony

T. ferrooxidans was reported to oxidize antimonite (Sb_2S_3) at pH 1.75 and 35° C according to the scheme [6]:

$$Sb_2S_3 + 6O_2 \rightarrow Sb_2(SO_4)_3. \qquad (23)$$

Antimonite is also oxidized by *B. thioparus var. antimoniticus* at pH 8.0 and by *T. thiooxidans* at low pH values [26, 28]. Antimony sulphate is partially hydrolyzed forming an insoluble oxide of trivalent antimony according to the scheme:

$$Sb_2(SO_4)_3 + 2H_2O \rightleftharpoons (SbO)_2SO_4 + 2H_2SO_4. \qquad (24)$$

The trivalent antimony sulphate is partially oxidized to pentavalent antimony according to the scheme:

$$Sb_2(SO_4)_3+O_2+2H_2SO_4 \rightarrow Sb_2(SO_4)_5+2H_2O. \tag{25}$$

Presumably, the reaction occurs under the influence of *T. ferrooxidans* but there is no direct proof for this statement. Sulphate of Sb^{5+} hydrolyzes with the formation of an insoluble oxide-sulphate:

$$Sb_2(SO_4)_5+4H_2O \rightleftharpoons (SbO_2)_2SO_4+4H_2SO_4. \tag{26}$$

It has also been shown that Sb_2S_3 is oxidized by thionic bacteria to Sb_2O_3 [28]. Further oxidation, up to pentoxide, can be achieved with *Stibiobacter senarmontii* [68]. Due to the very low solubility of the hydrolysis products of antimony (V) oxide-sulphate, the amount of antimony dissolved in the medium is, as a rule, negligible.

Lead

T. ferrooxidans was reported to oxidize galenite, lead sulphide (PbS), and Tomizuka [69] proposed the following scheme of this process:

$$PbS+H_2SO_4+{}^1/_2O_2 \rightarrow PbSO_4+H_2O+S^0; \tag{27}$$

$$PbS+H_2SO_4 \rightarrow PbSO_4+H_2S; \tag{28}$$

$$S+1{}^1/_2O_2+H_2O \rightarrow H_2SO_4. \tag{29}$$

T. ferrooxidans is a more effective oxidizer of galenite than Fe^{3+}. However, in the presence of bacteria and ferric iron the PbS oxidation is intensified because Fe^{3+} contributes in the process according to the next equation:

$$PbS+Fe_2(SO_4)_3 \rightarrow PbSO_4+2FeSO_4+S^0. \tag{30}$$

Fe^{2+} produced in reaction (30) will be oxidized to Fe^{3+} by *T. ferrooxidans* as indicated in equation (3). Nevertheless, solubilization of lead virtually does not occur because the lead sulphate $PbSO_4$ is insoluble in the aqueous acid media. This fact can be used for selective separation of a number of elements from lead.

Tin

Data on the oxidation of tin sulphides by *T. ferrooxidans* are scarce. According to Duncan [70], 12% of Sn were extracted from stannite in the presence of bacteria as compared to 4% in the sterile control. Besides, it was stated that Sn^{2+} could be oxidized by *T. ferrooxidans* [16]. The optimum pH for this reaction was reported to be 2.2 at 37 to 40° C.

Molybdenum

T. ferrooxidans is known to oxidize molybdenite (MoS_2) according to the scheme:

$$2MoS_2+9O_2+6H_2O \rightarrow 2H_2MoO_4+4H_2SO_4. \qquad (31)$$

In the presence of bacteria the process of oxidation of MoS_2 occurs faster than in the sterile control sample. However, molybdenum was found to be toxic to bacteria which perish at concentrations as low as 9 to 12 $mg \cdot l^{-1}$ [71, 72].

The thermophilic *Sulfolobus*-like bacteria are more promising for the leaching of molybdenum as indicated by Brierley [37]. A distinctive feature of these bacteria is that they are resistant to 2 $g \cdot l^{-1}$ of Mo and grow at its 750 $mg \cdot l^{-1}$ content in the medium. The rate of Mo extraction from MoS_2 at 60° C reached 26.5 $mg \cdot l^{-1} \cdot day^{-1}$, and exceeded the intensity of molybdenum leaching in the sterile controls about 130-fold [35, 37, 73]. In 30 days, 3.3% of Mo were extracted from a molybdenum concentrate (98.5% Mo) with particle size from 0.012 to 0.062 mm at 60° C and pH 2.5. When adding 0.02% yeast extract or yeast extract and 1% $FeSO_4$, 8.3% and 13.3% of Mo were extracted respectively. Without bacteria only 0.1% of Mo were extracted [7]. Molybdenite (MoS_2) may be oxidized by trivalent iron but the process results in the formation of insoluble products of oxidation.

Arsenic

More complete studies are available about the role of bacteria in the oxidation of arsenopyrite (FeAsS) [10, 74—77]. Like pyrite, arsenopyrite is a semiconductor with either electron (*n*-type) or hole (*p*-type) conductivity. In the electron-type arsenopyrite the conductivity is due to the abundance of sulphur present in the compound, while the conductivity in the hole-type arsenopyrite is due to the abundance of arsenic. According to Yakhontova and Nesterovich [45], arsenopyrite of the *p*-type, within the range of pH 2 to 6, is oxidized as expressed by the following equation:

$$4FeAsS+12^3/_4O_2+6^1/_2H_2O \rightarrow 3Fe^{3+}+Fe^{2+}+$$
$$+2H_3AsO_4+2H_2AsO_4^{-}+H_2SO_4+3SO_4^{2-}+H^{+}+4\bar{e}. \qquad (32)$$

Arsenopyrite of the *n*-type at the same pH values is oxidized according to the following scheme:

$$2FeAsS+4^1/_2O_2+5H_2O \rightarrow 2Fe^{2+}+SO_4^{2-}+$$
$$+HAsO_2+H_3AsO_4+HSO_4+5H^{+}+6\bar{e}. \qquad (33)$$

Arsenopyrite also interacts with trivalent iron, probably, according to the scheme:

$$FeAsS + Fe^{3+} \rightarrow 2Fe^{2+} + As^{3+} + S^0; \qquad (34)$$

$$As^{3+} + 3H_2O \rightarrow H_3AsO_3 + 3H^+. \qquad (35)$$

Arsenic acid, Fe^{2+} and S^0 are unstable in the medium, and are oxidized according to the scheme [78]:

$$H_3AsO_3 + H_2O \rightarrow H_2AsO_4 + 3H^+ + 2\bar{e}, \qquad (36)$$

$$Fe^{2-} \rightarrow Fe^{3+} + \bar{e}; \; S^0 \xrightarrow{+H_2O} SO_4^{2-} + 8H^+ + 6\bar{e}. \qquad (37)$$

In the presence of Fe^{3+}, iron arsenite will be formed:

$$Fe^{3+} + H_2AsO_4^- \rightarrow FeAsO_4 + 2H^+. \qquad (38)$$

Kinetics studies of bacterial oxidation of arsenopyrite in tin- and gold-bearing concentrates with the initial arsenic content up to 15% revealed that extractions as high as 80 to 90% of arsenic can be obtained using direct-flow Pachuca tanks in 70 to 80 hours [78].

Bismuth

Bismuthite is not oxidized by *T. ferrooxidans* [79]. In this process, the bacterial involvement is indirect and can be represented by the following scheme:

$$Bi_2S_3 + 6Fe^{3+} + 6O_2 \rightarrow Bi_2(SO_4)_3 + 6Fe^{2+}. \qquad (39)$$

It was reported that solutions with Fe^{3+} and H_2SO_4 extracted about 80% of bismuth from copper ores containing about 0.4% of Bi [80]. In a second stage of treatment with sulphuric acid of a copper ore containing bismuthite, the bismuth content of the solution reached concentrations as high as 5 $g \cdot l^{-1}$. Furthermore, it was found that bismuthite can be partially oxidized by *T. thioparus* at pH 8.0 [26, 28].

Vanadium

The mechanism of bacterial leaching of vanadium is similar to that of bismuth. In this process, trivalent vanadium is oxidized to its pentavalent state by Fe^{3+}, and the latter is regenerated by *T. ferrooxidans.*

5. Leaching of Rare and Scattered Elements

Rare and scattered elements are present in the crystal lattice of many sulphide or silicate minerals. Minerals of these elements as such are seldom encountered. Rare elements are released as a result of oxidation of sulphide minerals or destruction of silicate minerals under favourable leach conditions [3].

Therefore, the efficiency of leaching of rare and scattered elements is determined by the rate of oxidation or destruction of respective minerals.

Gallium and Cadmium

As reported by Lyalikova and Kulikova [81], *T. ferrooxidans* accelerated the leaching of gallium and cadmium from sphalerite (the main carrier of these elements) respectively by 2 and 5—8 times relative to the sterile controls. In this process, indium can also be solubilized. In the presence of *T. ferrooxidans* up to 90—92% of Cd were extracted from a copper-zinc-bearing concentrate in leach suspensions containing 20% pulp density at pH 2.0—2.5 and 35° C in 72 hours in continuous tank process [82, 83].

Brissette et al. [84] used *T. thiooxidans* leaching cadmium from pure CdS in the presence of elemental sulphur. Under optimum conditions they realized cadmium extractions as high as 72% in six weeks. It was found that the presence of sulphur was beneficial to the extraction. The extraction of cadmium leached by sulphuric acid in the sterile control was only 13%. The above authors believed that in the presence of elemental sulphur, *T. thiooxidans* oxidized cadmium sulphide to sulphate.

Torma reported [85] that *T. ferrooxidans* can oxidize gallium sulphide (Ga_2S_3). He used in his experiments a chalcopyrite concentrate containing gallium sulphide and a pure synthetic gallium sulphide. 40.2 $g \cdot l^{-1}$ of Cu and 2.2 $g \cdot l^{-1}$ of Ga were extracted in 120 hours from the pulp of 25% solids at pH 1.8 and 35° C. The extraction of gallium and copper in the sterile controls amounted to 8 to 15%. The specific rate of O_2 uptake ($\mu l\ O_2 \cdot h^{-1} \cdot mg^{-1}$ of protein) during the oxidation of Ga_2S_3 in the presence or absence of bacteria was 17.8 ± 0.16 and 5.1 ± 0.25, respectively. This divergence demonstrates the influence of bacteria on the oxidation of Ga_2S_3, which is oxidized by *T. ferrooxidans* and by the trivalent iron according to the next equations:

$$Ga_2S_3 + 6O_2 \rightarrow Ga_2(SO_4)_3; \qquad (40)$$

$$Ga_2S_3 + 3Fe_2(SO_4)_3 \rightarrow Ga_2(SO_4)_3 + 6FeSO_4 + 3S^0. \qquad (41)$$

Germanium and Cobalt

Germanium isomorphically substitutes copper, zinc and lead in their respective sulphides, and can be leached within a wide pH range. According to Lyalikova and Kulikova [81], during the oxidation of galenite by *T. thioparus var. antimoniticus*, there is about six times as much germanium in solution, than this is in the sterile controls. Respectively, germanium concentration was 100 and 17 $\mu g \cdot l^{-1}$ in the inoculated and sterile controls. Cobalt is always associated in varying amounts with pyrite, pyrrhotite, arsenopyrite, sphalerite and other minerals, and will be leached during the oxidation. In bacterial oxidation of cobaltite [10], the rate of extraction of Co increased about 75 times as compared to the control. In experiments by Torma [64, 86], 68—76% of Co were leached from a nickel concentrate. The detection of Co, along with other elements, in acid mine drainage waters is the evidence of its leaching under natural conditions [87].

Rhenium

Rhenium is present in deposits of molybdenite, pyrite, chalcopyrite and galenite. Although no data are available on bioleaching of this element, it is known that in the natural oxidation of sulphides, rhenium is largely dissolved. Its content in the mine waters of corresponding deposits varies from 5 to 500 $mg \cdot l^{-1}$.

Selenium and Tellurium

Selenium and tellurium are present in pyrite, chalcopyrite, sphalerite, galenite and elemental sulphur. Selenium is also found in the elemental form as well as in the form of selenides. Tellurium is also found as a constituent of minerals, tellurides. The leaching of these elements is evidenced by their transport with waters and by a decrease in elements concentration in oxide ores. The role of bacteria in the leaching of selenium has been so far poorly studied. Lipman and Waksman [88] pointed out as early as 1922 that some autotrophic bacteria could obtain energy through oxidation of selenium into selenic acid. Torma and Habashi [12] reported that *T. ferrooxidans* could use the energy available from oxidation of copper selenide. As a result of this process, copper is dissolved in the leach medium, and selenium is oxidized to its elemental form:

$$CuSe + 2H^+ + {}^1/_2O_2 \rightarrow Cu^{2+} + Se^0 + H_2O. \qquad (42)$$

Titanium

The role of lithotrophic bacteria in the leaching of titanium has been studied inadequately. In acid media below pH 2.0, titanium is dissolved from ores. This is indicated by its transport from the oxidative zone in copper-pyrite deposits. Presumably, *T. ferrooxidans* takes part in the process.

Uranium

The role of microorganisms in the leaching of uranium has been well studied. The data are summarized in a number of reviews and books [89, 90]. The essence of uranium leaching by

T. ferrooxidans is the oxidation of pyrite or Fe^{2+} with the formation of H_2SO_4 and Fe^{3+}. Ferric iron in the acid medium oxidizes U^{4+} to U^{6+}, which is soluble in sulphuric acid solutions. The leaching of uranium follows the scheme:

$$UO_2+Fe_2(SO_4)_3 \rightarrow UO_2SO_4+2FeSO_4; \tag{43}$$

$$UO_3+H_2SO_4 \rightarrow UO_2SO_4+H_2O. \tag{44}$$

Consequently, the presence of pyrite as a supplier of Fe^{3+} plays an important role in the bacterio-chemical leaching of uranium. When 3% of FeS_2 and 1.5% of Fe^{2+} were added to a uranium ore, uranium extraction exceeded 90% in 18 weeks. In experiments without addition of FeS_2 and Fe^{2+}, there was practically no extraction of uranium achieved despite the presence of thionic bacteria in the leach suspension [91]. *T. ferrooxidans* is known to oxidize also U^{4+} [18]. Tomizuka et al. [92] conducted continuous leaching of uranium in media containing $FeSO_4$ and 10% of ore at pH 2.0 and 30° C. The highest extraction of uranium was reached at the dilution rate about 0.060 $hour^{-1}$, when the highest rates of *T. ferrooxidans* growth and formation of Fe^{3+} ions were observed. However, to obtain 80% uranium extraction, the authors suggested performing the continuous leaching process at a dilution rate of 0.011 $hour^{-1}$. This method of leaching uranium as well as other metals from ores and concentrates seems to be the most progressive one.

Chapter III

INTENSIFICATION OF THE LEACHING OF METALS

The rate of extraction of metals from sulphide-bearing minerals depends largely on physico-chemical factors (medium acidity, redox potential, temperature, etc.), technological conditions (pulp density, mixing, gas transfer phenomena, etc.), and peculiarities of bacteria themselves.

1. Influence of Physico-Chemical Properties of Sulphide Minerals and of the Medium

Type of Conductivity of Sulphide Minerals

The theory of physico-chemical principles of oxidation of sulphide minerals has been summarized by Yakhontova and Grudev [44]. One of the major factors determining the intensity of oxidation processes is the redox potential (Eh) of the «electrolyte» (medium) and electrode potential (EP) of minerals as well as the type of their conductivity and structure of crystals. For example, minerals of *n*- and *p*-type conductivity are oxidized with different efficiencies. According to Yakhontova and Nesterovich [45], pyrite and arsenopyrite of the *n*-type conductivity possess a lower energy of electron discharge, and are characterized by a high initial oxidation rate which rapidly slows down as reaction progresses. Sulphides of the *p*-type conductivity are initially oxidized at a lower rate but the process is more stable.

As shown by Silverman and Ehrlich [93], the intensity of oxidation of sulphide minerals is determined by the structure of crystals. Later Berry, Murr et at. [56, 94—96] reported that the distribution of bacteria on sulphide minerals depends on the structure of crystals and presence of dislocations. Obviously, the structure, conductivity and electrochemical properties of crystals are interrelated, and will determine the kinetics of their oxidation by bacteria. The relation between the Eh of the medium, EP and conductivity of a sulphide mineral can be seen from an example of pyrite oxidation in Figs. 2 and 3 [44, 97—99]. Experiments were carried out on pyrite with the electron (FeS_2^-) and hole

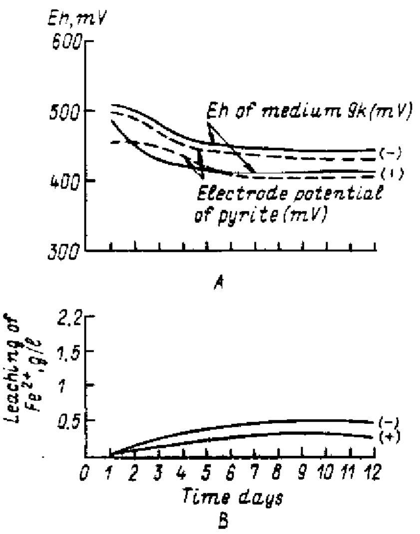

Fig. 2

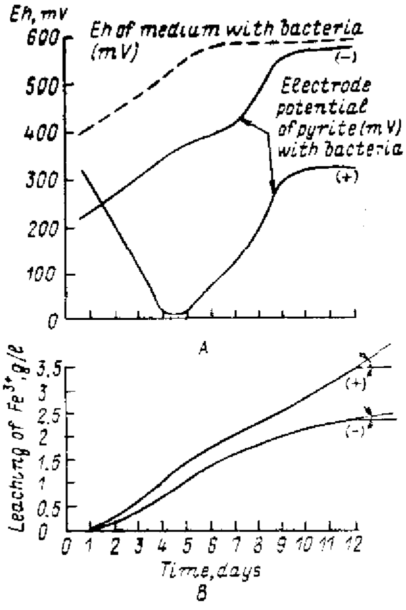

Fig. 3

Fig. 2. Changes in EP of pyrite with positive (+) and negative (—) conductance; in Eh of the 9 K medium without bacteria (pH 2.5) (A); and in Fe^{2+} leaching (B) [97]

Fig. 3. Changes in EP of pyrite with positive (+) and negative (—) conductance; in Eh of the 9 K medium with *T. ferrooxidans* (pH 2.5) (A); and in Fe^{3+} leaching (B) [97]

(FeS_2^+) type conductivity. It is clear from Fig. 2 that without bacteria there is no oxidation of pyrite regardless of its conductivity. This is due to the fact that the electrode potential of both types of pyrite is close to the Eh of the medium. The absence of any difference between EP and Eh indicates that both the medium and the mineral are energetically at equilibrium. Therefore, the latter cannot be oxidized under these conditions. In the presence of *T. ferrooxidans*, pyrite is oxidized intensively and behaviour of sulphides with different types of conductivity is different (Fig. 3). The difference between Eh and EP for $FeS_2(+)$ was about 600 mV and about 200 mV for $FeS_2(—)$. The initial difference between Eh and EP was rather large, but gradually disappeared and the leaching of Fe practically stopped. Thus, these findings suggest that the adsorption of bacteria on the mineral surface sharply decreases the EP and ensures a high oxidative capacity of the system.

Table 1 contains experimental Eh and EP values during the

Table 1

Optimum Eh and EP values for the bacterial oxidation of sulphide minerals [7, 45, 82, 83, 92, 100]

Minerals	Eh of the medium, V	EP of minerals, V
$CuFeS_2$	0.5—0.7	0.4—0.5
Cu_2S, Cu_5FeS_4	0.6—0.5	0.3—0.4
FeAsS	0.77—0.8	0.62—0.64
+ $CuFeS_2$ in concentrate		0.76—0.77
ZnS	0.6—0.7	0.23—0.43 (pH 2.3—1.5)
+ $CuFeS_2$ in concentrate		0.68—0.63 (pH 2.3—1.5)
U^{4+}/U^{6+}	0.75	0.41

oxidation of some sulphide minerals. According to Yakhontcva and Nesterovich [45], the leaching of chalcopyrite stopped when the EP and Eh curves reached the stable level (0.5 and 0.7 V). For Cu_2S and Cu_5FeS_4, the leaching of copper stopped after the following changes: EP from 0.3 to 0.4 and Eh from 0.6 to 0.5 V.

Electrochemical Interaction of Sulphide Minerals

Sulphide minerals in the pulp or in ores interact electrochemically, i. e. there occur galvanic currents between them. In this case, the electrode potentials of some minerals may substantially differ from the stationary potentials measured individually.

The intensity of oxidation of a metal sulphide in the mixture of sulphides depends both on the difference between the electrode potentials of the sulphides and on the difference between the sulphide EP and the medium Eh. The sulphide mineral that has the lowest EP, i. e. the sulphide-anode, is more readily oxidized chemically. To illustrate, the following examples may be cited. When extracting metals from a concentrate containing arsenopyrite and chalcopyrite, it was established that microorganisms most actively oxidize arsenopyrite, i. e., the sulphide-anode possessing the lowest EP (Table 1, Fig. 4). The difference between EPs of FeAsS and CuFeS$_2$ amounts to 0.14—0.15 V. Thus, in the examined case, chalcopyrite favoured the microbiological oxidation of arsenopyrite. Consequently, the microbiological oxidation of sulphide minerals has the same trend as the electrochemical dissolution, but is accelerated to a large extent due to the presence of bacteria [100, 101].

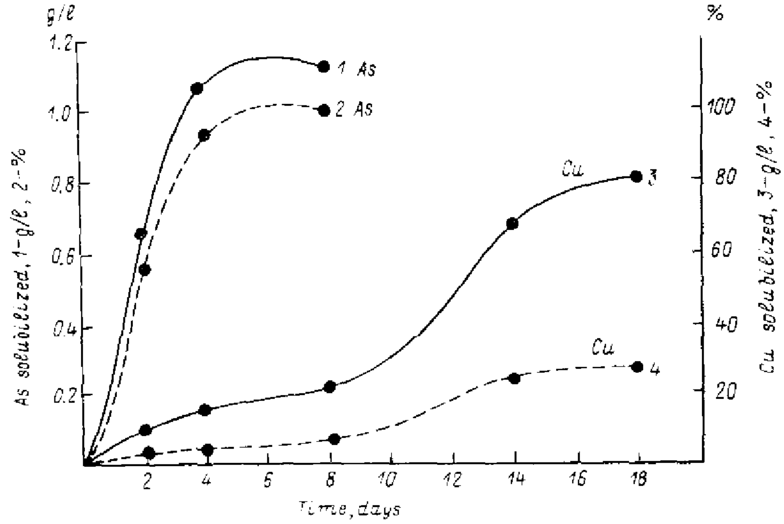

Fig. 4. Oxidation of arsenopyrite in the presence of chalcopyrite in a tin-containing concentrate [100]

Data by Yakhontova and Grudev [44] suggest that in the presence of several sulphide minerals the ore which will be oxidized preferentially by the bacteria depends on the type of its conductivity. For example, it was observed that arsenopyrite of the *p*-type acted as the cathode and favoured the destruction of chalcopyrite. The galvanic couple FeAsS of the *n*-type — FeS$_2$ favours the oxidation of arsenopyrite. However, the use of samples of the mixed composition (at the weight ratio of 1 : 1) proved, according to Yakhontova and Nesterovich [45], that arsenopyrite-pyrite couples in the bacterial solution had a minor difference of EP

(~0.05 V) and only slightly deviated from the Eh of the solution (0.60—0.65 V). The «work» of these galvanic couples was negligible.

According to various authors [in ref. 56], when pyrite (EP~0.6 V) contacts chalcopyrite (EP~0.5 V) the latter corrodes more readily. Oxygen is reduced on the surface of pyrite (cathode):

$$O_2+4H^++4\bar{e}\rightarrow 2H_2O. \qquad (45)$$

Chalcopyrite acts here as the anode:

$$CuFeS_2\rightarrow Cu^{2+}+2S^0+4\bar{e}. \qquad (46)$$

Hence, the galvanic reaction may be expressed by the equation:

$$CuFeS_2+O_2+H^+\rightarrow Cu^{2+}+Fe^{2+}+2S^0+2H_2O. \qquad (47)$$

When the two minerals were treated separately the results were reversed, i. e., pyrite was oxidized faster than chalcopyrite both in the presence and absence of bacteria.

During the oxidation of ZnS and $CuFeS_2$ concentrates the electrode potential of sphalerite varied from 430 mV at pH 1.5 to 230 mV at pH 2.3, while the potential of chalcopyrite amounted to 600 mV at pH 1.5 and 680 mV at pH 2.3. Thus, the electrode potential of $CuFeS_2$ is by 170—450 mV higher than that of ZnS, the lower pH favouring oxidation of chalcopyrite while the higher pH oxidation of sphalerite (Table 1, Fig. 5) [82]. Therefore, sphalerite is less resistant in the described system, and is oxidized more readily. Here 92% of Zn and only 20—25% of Cu passed into solution.

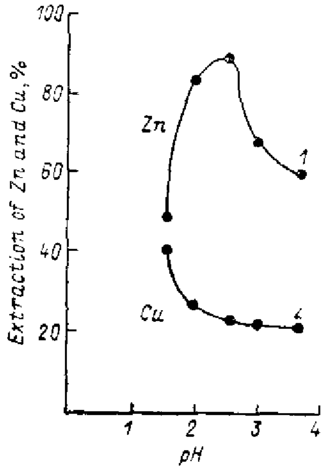

Fig. 5. Extraction of zinc (1) and copper (2) as a function of the pH of the medium in bacterial leaching of a copper-zinc concentrate [5]

The oxidation-reduction potential of U^{4+}/U^{6+} is equal to 0.41 V, and the highest rate of chemical oxidation of U^{4+} is realized at Eh of the medium about 0.75 V in the presence of Fe^{3+}.

Sulphide-cathode cannot be considered as absolutely inert in this system, either. It is partially oxidized, apparently due to the formation of microgalvanic couples and development of bacterial processes isolated on the mineral where a decrease in EP is observed. But the process seems to be marginal.

Existing data suggest that bacterial leaching of metals, at least in the tank process, may be intensified by controlling the oxidation-reduction conditions of the pulp. Furthermore, leaching intensity can be increased through appropriate selection of the concentrate for leaching with respect to its composition in order to achieve a desired interaction between minerals.

Medium Acidity

The maximum rate of oxidation of sulphide minerals, sulphur and Fe^{2+} correlates with the optimum growth of bacteria and ranges within pH 2.0—3.0 (the optimum pH 2.3—2.5). However, depending on other factors of the medium, the optimum pH varies. The following optimum pH values have been reported in the literature for the oxidation of sulphide minerals: $CuFeS_2$ — 2.2—2.5 (the optimum 2.3) [102], Cu_2S — 1.7—2.3; CuS — 2.3 [3, 6, 7, 102, 103]; ZnS — 2.0—3.0 (the optimum 2.5) [82, 83, 104], FeAsS — 2.0—3.0 (the optimum 2.5) [75, 76]. Nickel sulphides are readily oxidized at pH 2.5. Kamalov reported on cultures of *T. ferrooxidans* oxidizing Fe^{2+} and sulphide minerals at pH 1.5. The oxidation of galenite occurs most actively at pH 3.0—3.5 [69].

In dump and underground leaching of metals from ores the pH is controlled by adding sulphuric acid to solutions. In tank leaching of metals from concentrates, pH as a function of time sharply decreases (unless controlled), which results in suppressed life activity of bacteria [82]. In order to avoid this adverse effect, lime or calcite should be added to the leach suspension.

Thus the basic thermodynamic regularities typical of the oxidation of sulphide minerals are not altered by the biochemical activities of microorganisms. Bacteria merely influence the kinetics of these processes.

Role of Ferric and Ferrous Iron in the Bacterial Oxidation of Sulphide Minerals

Fe^{2+} and Fe^{3+} are the constant components of leach media for the biogenic extraction of metals from ores and concentrates. They exert a decisive influence on the oxidation-reduction capacities of the system. These ions are engaged in complex interrelations both between themselves and with sulphide minerals. As shown by Kelly and Jones [105], the rate of oxidation of Fe^{2+} by *T. ferrooxidans* depends on the Fe^{2+}/Fe^{3+} ratio in the medium. At different concentrations in the system, Fe^{3+} either accelerates the oxidation of Fe^{2+} or acts as an inhibitor.

Furthermore, the degree to which the rate of oxidation of Fe^{2+} is inhibited by the trivalent iron, depends on the age of culture, its adaptation to Fe^{3+} and the concentration of the latter in the medium. In experiments by Landesman et al. [106], 2.5—10 μmoles of Fe^{3+} (in 28 ml) decreased the duration of the lag-phase and increased the rate of breathing by 12%. However, addition of over 50 μmoles of Fe^{3+} caused the inhibition of breathing of *T. ferrooxidans* cells. In experiments by Kelly and Jones [105], the concentration of Fe^{3+} up to 50 mM (2.8 $g \cdot l^{-1}$) stimulated the consumption of oxygen by *T. ferrooxidans*. Yet, the Fe^{3+} ion depressed the oxidation of Fe^{2+} in the concentration of 100 mM (over 5.6 $g \cdot l^{-1}$).

Limited data are available on the role of Fe^{3+} and Fe^{2+} in bacterial oxidation of sulphide minerals. For instance, in the presence of *T. ferrooxidans* but without Fe^{3+}, 5.2 $g \cdot l^{-1}$ of arsenic were extracted from arsenopyrite [75, 76], while in the presence of bacteria and Fe^{3+} end concentrations as high as 1.0—3.3 $g \cdot l^{-1}$ of As were realized. In the presence of 10^{-4}—10^{-2} M of Fe^{3+}, the rate of bacterial oxidation of NiS, CoS, Cu_2S and CuS increased more than two-fold; higher concentrations of Fe^{3+}, however, yielded no effect [6]. The optimum concentration of Fe^{3+} for leaching copper from a copper-molybdenum concentrate was 1 $g \cdot l^{-1}$ [107]. Fe^{3+} is known to be a weak oxidizer of chalcopyrite. Nevertheless, from a finely ground sulphide concentrate (~0.5 μm) in the presence of Fe^{3+} alone 90% of copper were extracted in three hours [52]. The optimum concentrations of Fe^{3+} for the oxidation of Cu_2S and CuS are 0.004—0.01 M and 0.004—0.02 M respectively [103]. At Fe^{3+} concentrations above 1 $g \cdot l^{-1}$, the rate of leaching of copper did not increase. However, increased Fe^{3+} concentrations in solutions, up to 8 $g \cdot l^{-1}$, enhanced the extraction of Zn from a copper-zinc concentrate by 27% [108]. Duncan and Drummond [109] showed that pyrite was oxidized only by *T. ferrooxidans* and added Fe^{3+} in the presence of bacteria did not accelerate its oxidation. Addition of Fe^{2+} to the medium may either intensify or depress the bacterial oxidation of sulphide minerals. For instance, the rate of oxidation of $CuFeS_2$ was depressed 1.5-fold in the presence of 2 $g \cdot l^{-1}$ of Fe^{2+}, and the rate of FeAsS oxidation decreased upon addition of 1.0 $g \cdot l^{-1}$ and 2.5 $g \cdot l^{-1}$ of Fe^{2+} by 1.5 and 3 times respectively [3, 76]. The oxidation of PbS by *T. ferrooxidans* occurred most intensively upon addition of 0.0005—0.0025 moles of $FeSO_4$ per 100 ml of the medium. Both higher and lower $FeSO_4$ content decreased the rate of bacterial oxidation of PbS.

The mechanism of the inhibitory effect of Fe^{2+} and Fe^{3+} on bacterial oxidation of sulphide minerals is not yet completely clear. However, it was suggested that the competitive oxidation of sulphides by Fe^{3+} and *T. ferrooxidans*, as well as the prevention of bacterial attack on sulphides were due to the precipitation on the mineral surface of iron hydroxides [110]. As shown by Karavaiko, one of the reasons for the depression of bacterial activity of sulphide oxidation by iron ions is the formation of an unfavourable oxidation-reduction potential of the medium and EP of sulphide minerals.

The existing data demonstrate that a number of sulphide minerals may be oxidized by Fe^{3+}, e. g., cubanite, energite, chalcocite, covellite, tetrahedrite, sphalerite as well as tetravalent uranium and trivalent vanadium. In these cases, sulphuric acid solutions containing Fe^{3+} can be considered as the main leaching agent. Oxidation of other sulphide minerals may be accelerated in the presence of Fe^{3+}. Therefore, one of the conditions to be fulfilled in order to intensify the bacterio-chemical oxidation of sulphide minerals is to establish optimal concentrations of Fe^{3+} and Fe^{2+} in the leach solutions.

Effect of Temperature

The optimum temperature of oxidizing Fe^{2+}, S^0 and sulphide minerals by mesophilic bacteria ranges within 28—35° C. When the temperature deviates from the optimum, the rate of growth and oxidation decreases. Experiments by Karavaiko et al. [3] showed that the rate of ferrous iron oxidation in mine waters by mesophilic microflora decreased about 2—4-fold and the rate of CO_2 fixation 4—5-fold when the temperature decreased by 10° C within 12—16° C to 22—26° C. When other factors, such as pH, deviated from the optimum value, the intensity of CO_2 fixation and oxidation of Fe^{2+} by autochthonous microflora decreased 9- and 7-fold respectively at a drop of temperature by 10° C.

Obviously, such a sharp decrease in the rate of *T. ferrooxidans* growth and of Fe^{2+} oxidation is due not only to the temperature (Q_{10} usually equals 2) but also to other unfavourable factors. According to the impact of temperature on *T. ferrooxidans* in the oxidation of Fe^{2+}, Kovalenko and Karavaiko [111] noted two temperature ranges, 26—15° C and 15—5° C. As the temperature drops to 15° C, regardless of the initial Fe^{2+} concentration, the specific rate of bacterial growth and the rate of oxidation of Fe^{2+} decrease 1.5—2.5 and 1.1—2.3-fold, respectively. However, as the temperature drops from 15 to 5.5° C, the specific rate depends on the initial Fe^{2+} concentration, and will be depressed 4.2—16.3-fold in respect of bacterial growth and only 2.4—5.2-fold in respect of Fe^{2+} oxidation. At 15° C, the range of optimum Fe^{2+} concentrations is wider than this is at 26° C and 5.5° C, which are 1.2 to

12.5 $g \cdot l^{-1}$, 1.6 to 8—9 $g \cdot l^{-1}$ and 3—4 to 12 $g \cdot l^{-1}$, respectively. At 5.5° C, one can observe a significant growth of the saturation constant (R_s) (2.9 $g \cdot l^{-1}$ of Fe^{2+}) as compared to the temperatures 15 and 26° C (0.71 and 0.42 $g \cdot l^{-1}$, respectively). The low Fe^{2+} content of 1.6 $g \cdot l^{-1}$ at 5.5° C does not provide enough energy for the growth of *T. ferrooxidans* which needs a large amount of energy at low temperatures in order to support the life of bacteria. The effects of temperature and of pH of the medium on bacterial growth and Fe^{2+} oxidation are also interrelated [112, 113]. At 28° C, the range of pH values that do not affect bacteria, is wider and varying from 2.5 to 2.8 than that at 12° C varying from 2.3 to 2.4. Thus, the effect of temperature on the activity of *T. ferrooxidans* depends on a number of factors (pH, substrate concentration, ion concentrations, and so on).

It is also evident that the growth of bacteria at lower temperatures is depressed to a larger extent than the oxidation of Fe^{2+}. Hence to accelerate the oxidation processes at lower temperatures one should, first and foremost, increase the number of bacterial cells at least to 10^7—10^8 per 1 cm^3 [114].

A number of facultative thermophilic and thermophilic bacteria actively oxidize sulphide minerals within temperature range of 40 to 90° C [7, 32, 33]. In natural conditions, the facultative thermophilic bacteria, e. g. *S. thermosulfidooxidans*, are found at 20—25° C in quantities of 10^7 $cell \cdot cm^{-3}$ and take an active part in the oxidation of sulphides in ores at 20—50° C (the optimum 45—50° C).

Effect of Light

The effect of light on *T. ferrooxidans* was investigated by Le Roux and Marshall [31], and Groudeva et al. [115]. They proved that both visible light and UV light depressed the activity of thionic bacteria regardless of the substrate used. In experiments with visible light using wave lengths 430—560, 480—610 and 600 nm it was found that the degree of inhibition of bacterial activity depended on the wave length. In the visible range, the most inhibitory is the blue light, while the red light does not affect bacteria. Irradiation within the range of 300—400 nm during 1 to 8 minutes decreased the oxidation activity of bacteria by 4 to 100%. More bactericidal for *T. ferrooxidans* was found to be UV light with wave length of 200—300 nm. Short-wave light during 30 and 45 seconds depressed bacterial oxidation of Fe^{2+} by 14 and 100% respectively. Light resistance of *T. ferrooxidans* increases with the increase in the cell concentration of the medium. Trivalent iron also possesses protective properties in respect of bacterial activity.

Effect of Oxygen and Carbon Dioxide

Intensive oxidation of Fe^{2+} and of sulphide minerals may take place only with the optimum supply of oxygen and carbon dioxide. The O_2 consumption depends on the type of the substrate used and its concentration. Tuovinen and Kelly [116] calculated O_2 and CO_2 consumption by *T. ferrooxidans* in the medium containing Fe^{2+}, and demonstrated that O_2 and CO_2 consumption normally was about 183 and 81 times greater than the maximum amounts of oxygen and carbon dioxide soluble in the medium. The rate of copper leaching from a low-grade ore was proportional to the amount of oxygen consumed [7].

The growth limitation of *T. ferrooxidans* by CO_2 in direct-flow situation was shown by Egamberdieva et al. [117]. McDonald and Clark [118] proved that the specific rate of bacterial growth was independent of CO_2 concentration within 0.01—10%. However, Beck and Schaffia [119], and Kodama and Mori [120] found that increased CO_2 content of the air used for aeration resulted in an increase in the biomass of thionic bacteria and their activity.

It is known that the amount of CO_2 required will be varied with the modifications of the environment. For instance, the oxidation of a sphalerite concentrate demonstrated the existence of an interrelation between the pulp density, specific surface of particles and CO_2 consumption of *T. ferrooxidans*. According to Torma et al. [50], at the CO_2 content equal to 0.03% (normal air condition) the rate of zinc extraction increased linearly with the increase in the pulp density only up to 12%. At higher pulp densities, the low CO_2 concentration (in normal air) limited the oxidation of sulphides. At the CO_2 content amounting to 0.23—1.03%, the rate of Zn extraction from the concentrate increased linearly until the pulp density reached 24%. The above factors are closely connected with the specific surface of particles. At the CO_2 content amounting to 0.03% and 1%, the extraction of zinc from the concentrate occurred until the specific surface of particles reached 1 $m^2 \cdot g^{-1}$ and 3 $m^2 \cdot g^{-1}$, respectively. In these conditions, the rate of zinc extraction was about 0.4 and 1 $g \cdot l^{-1} \cdot h^{-1}$, respectively [50]. The supply of CO_2 into the pulp may be combined with the pH control through adding chalk according to the formula:

$$CaCO_3 + H_2SO_4 \rightarrow CaSO_4 + H_2CO_3. \qquad (48)$$

It is evident that the intensity of bacterial oxidation of both Fe^{2+} and sulphide minerals depends on the rate of supply and dissolution of O_2 and CO_2 under existing technological conditions.

The highest rate of Zn extraction (96.5 $mg \cdot l^{-1} \cdot h^{-1}$) and Cu extraction (82.4 $mg \cdot l^{-1} \cdot h^{-1}$) from a lead concentrate was obtained at the specific surface of particles exceeding 1.65 $m^2 \cdot g^{-1}$ [121]. Studying Zn leaching from a flotation concentrate, Torma et al. [104] proved that one of the many factors limiting the oxidation process is the total surface of mineral particles per unit volume of leach solution. It was reported that with the increase in the specific surface area of solid particles, the rate of Zn extraction was increased.

2. Influence of Technological Conditions

Size of Particles and Pulp Density

The rate of bacterial oxidation of sulphide minerals was found to increase with the decrease in the size of particles [50, 54, 102, 103, 121—124]. The highest rate of oxidation of sulphides in the pulp is observed when the diameter of particles is from 2.2 to 40 μm. The maximum extraction of Zn (98.1%) and Cu (96.5%) from a lead sulphide concentrate was obtained at particle size less than 5 μm in diameter and the pulp density of 12% and higher [121]. Studying zinc leaching from concentrate, Torma proved that the rate of its extraction under the above conditions exponentially increased with the decrease in the size of particles according to the equation:

$$V = V_m \cdot e^{(cd)},$$

where V_m — maximum rate of leaching of Zn, c — proportionality constant, and d — average diameter of particles.

The size of particles determines their surface area which also affects the rate of oxidation of sulphides. For instance, at the 16% pulp density, pH 2.3, temperature 35° C and 1% CO_2 content, the rate of Zn extraction increased linearly with the increase in the specific surface up to 3 $m^2 \cdot g^{-1}$.

Finally, the rate of leaching of metals from concentrates depends on the pulp density. For example, the extraction of Zn from a zinc concentrate was most intensive until the pulp density reached 16% [104]. The final Zn concentration in the solution was 72 $g \cdot l^{-1}$.

Experiments by Pol'kin et al. [108] proved that the rate of Zn extraction from a copper-zinc concentrate increased proportionally with the pulp density up to 25% concentration. The highest copper concentration (14 $g \cdot l^{-1}$) was obtained at the pulp density of a chalcopyrite concentrate equal to 26.7% [125].

In dump and underground leaching it is impossible to use a finely ground ore as particle size is closely connected with water permeability and aeration of the ore in dumps or in the ore body. In this case, to estimate the effect of particle size on bacterial oxidation of sulphide minerals, Bruynesteyn and Duncan [126] have introduced the notion of «active leaching volume» which is a product of the ore's particles surface and the depth of penetration of bacteria and of the solvent. The rate of extraction of metals in this case is a hyperbolic function of the ore particles.

Solubility of the Final Products of Oxidation of Sulphide Minerals

Torma and Sakaguchi [127] have indicated that the highest rate of oxidation of a sulphide mineral by *T. ferrooxidans* is obtained when the product of oxidation possesses the highest solubility. The relationship between the rate of oxidation of a metal (dM^{2+}/dt) and the solubility of the product of a sulphide mineral (K_{sp}) may be expressed by a formula:

$$V = \frac{dM^{2+}}{dt} = AK_{sp} = A\,[M^{2+}]\,[S^{2-}],$$

where A — proportionality factor.

The direct relationship between the rate of oxidation of a sulphide and solubility of the product indicates that bacteria should remain in a close contact with the surface of substrate where the dissociation depends on the solubility of its oxidation product:

$$MS \rightleftharpoons M^{2+} + S^{2-}. \tag{49}$$

The sulphide portion is quickly oxidized by bacteria to the sulphate:

$$S^{2-} + 2O_2 \rightarrow SO_4^{2-}. \tag{50}$$

When the dissociation equilibrium (in equation 49) is shifted to the right, theoretically this process may last until the complete dissolution of the substrate (MS) takes place. However, upon accumulation of the oxidation products, particularly metals, in the solution, bacterial activity is inhibited. Hence, the process may be completed at a low concentration of the initial substrate, or else the oxidation products should be removed from the system. The following succession of dissolution rates of sulphides has been established:

$$NiS > CoS > ZnS > CdS > CuS > Cu_2S.$$

Effect of Chemical Elements

General principles of metals toxicity for *T. ferrooxidans* have been formulated by Norris and Kelly [128]. Essentially, toxicity of metals depends on the physiological state of the microorganism, chemical form of metals and degree of their interaction in the environment.

Karavaiko et al. [129] reported that the activity of *T. ferrooxidans* in mine waters would be determined by the complexity of metal ions and chemical composition of solutions which may depress or intensify the activity of bacteria. Research data on the influence of a number of chemical elements on *T. ferrooxidans* are rather of a theoretical interest and show the resistance of a given strain only under specific experimental conditions. Qualitative data on the resistance of *T. ferrooxidans* to heavy metals can be found in a number of publications. Various strains of *T. ferrooxidans* are resistant to 0.37 *M* (10 $g \cdot l^{-1}$) of Al, 0.15 *M* (10 $g \cdot l^{-1}$) of Zn, 0.17 *M* (10 $g \cdot l^{-1}$) of Co, 0.17 *M* (10 $g \cdot l^{-1}$) of Ni, 0.18 *M* (4 $g \cdot l^{-1}$) of Mn and 0.16 *M* (10 $g \cdot l^{-1}$) of Cu. The maximum inhibition by Au ions was at 10^{-7} *M*. Silver and anions of selenium, tellurium and arsenic depressed bacterial activity in a concentration range varying from 0.2 to 0.9 mM (from 50 to 100 $mg \cdot l^{-1}$) and molybdenum in a concentration over 0.03 mM (5 $mg \cdot l^{-1}$) [7, 78, 116, 130, 131]. However, thermophilic bacteria close to *Sulfolobus* develop at the content of Mo in the medium up to 2 $g \cdot l^{-1}$ [35, 73].

Minimal concentrations of As^{3+} and As^{5+} ions depressing the oxidation of Fe^{2+} by *T. ferrooxidans* were found to be 1.0 and 2.0 $g \cdot l^{-1}$, respectively [78].

Anions of Se, Te, As and Mo are more toxic for *T. ferrooxidans* than most cations of metals. However, the most toxic metals for these bacteria are Cd, Ag, Hg and U.

Mercury ions inhibit *T. ferrooxidans* in a concentration of $5 \cdot 10^{-4}$ mM [Le Roux, in ref. 7] and uranium ions in a concentration of 10^{-3}—$5 \cdot 10^{-4}$ M. The activity of *T. ferrooxidans* in the medium containing Fe^{2+} is depressed upon addition of 0.1—10.0 $mg \cdot l^{-1}$ of Ag^+ [132, 133]. However, quantitative values of metal toxicity are affected by physico-chemical factors of the medium and bacterial culture condition. The following examples were cited by Norris and Kelly [24]: $AgNO_3$ in a concentration of 10^{-9}—10^{-8} *M* depressed the growth of *T. ferrooxidans* which was evidenced by an increase in the lag-phase. After addition of $AgNO_3$ in a concentration of 10^{-7} *M* there was no growth of bacteria. However, after addition of $AgNO_3$ in the same concentration (10^{-7} *M*) to the growing culture, its growth was at first somewhat depressed but resumed after 100 hours in the presence of $5 \cdot 10^{-2}$ *M* of K^-. Toxicity of $AgNO_3$ added in a concentration of $5 \cdot 10^{-8}$ *M* was also reduced upon pH increase from 1.7 to 2.0 which was indicated by the lag-phase dropping from 15 to 1 hour. When *T. ferrooxidans* was grown on sulphur and sulphides its resistance to Ag^+ considerably increased. After addition of 10^{-5} *M* of $AgNO_3$ to the culture growing on pyrite, the extraction of iron from FeS_2 was not depressed. This is due to a close affinity of Ag^+ to sulphur leading to a partial binding of the silver ion in the form of sulphide Ag_2S. Toxicity of other heavy metals (Zn, Ni, Co) was lower than that of Ag when bacteria were cultured on sulphur, and increased when thiosulphate was the substrate [38]. There exist other mechanisms of interaction between Ag^+ and sulphide minerals which is indicated by enhanced activity of *T. ferrooxidans*. Bacterial activity was enhanced upon addition of Ag^+ ($AgNO_3$) during the oxidation of sulphide minerals of copper and zinc, according to McElroy and Duncan [134], McElroy and Bruynesteyn [135]. Austrian scientists suggested intensifying the leaching of copper from ore by adding 0.2% sodium lignosulphonate and 0.1% $AgNO_3$ or Ag_2SO_4. This resulted in copper extractions up to 93% (Patent No. 319616). Recently, Snell and Morgan [136] reported that silver catalyzed the oxidation of copper sulphide minerals. This indicates that silver interacts with sulphide minerals favouring both their bacterial and chemical oxidation.

As pointed out above, uranium is toxic to *T. ferrooxidans* in concentrations above 10^{-3}—$5 \cdot 10^{-4}$ M, depressing the fixation of CO_2 to a greater extent than the oxidation of Fe^{2+}. Addition of 100—200 mM of Zn^{2+} and Ni^{2+}, Mg^{2+} or Mn^{2+} to the medium partially reduced the toxicity of 2 mM of UO_2. Nevertheless, the cation concentrations of 2—20 mM did not reduce the toxicity of uranyl sulphate.

Inhibition of the growth of *T. ferrooxidans* in the presence of 0.7 $g \cdot l^{-1}$ of uranyl sulphate was partially reduced in the presence of 200 mM of potassium, sodium, lithium or NH_4^+ in the form of sulphates [130]. Zinc in the system containing copper reduced its toxicity for *T. ferrooxidans* [137]. Toxicity of Tl was also reduced in the medium with a high K^+ concentration. Thallium and rubidium in a concentration of 10^{-4} M inhibited the growth of *T. ferrooxidans* in a phosphate-free medium and were non-toxic in a concentration of 10^{-3} M in the normal medium [130]. Chlorides of sodium or potassium, according to the same authors, inhibited bacterial development in the absence of sulphates and their inhibitory concentration was dependent on the content of phosphorus in the medium.

Kovalenko and Karavaiko [111] demonstrated that the inhibitory effect of copper ions on the rate of Fe^{2+} oxidation by *T. ferrooxidans* depended on the stage of bacterial culture development, substrate concentration and temperature. The level of inhibition of Fe^{2+} oxidation in the presence of 4.4 $g \cdot l^{-1}$ of Cu^{2+} was lowered with the increase in substrate concentration regardless of the temperature variations within 6—28° C. The constants of Cu^{2+} inhibitor are as follows: at 28° C: from 0.5 to 0.6 $g \cdot l^{-1}$; at 12° C: from 2.5 to 2.8 $g \cdot l^{-1}$; at 6° C: from 4.0 to 4.4 $g \cdot l^{-1}$ of Cu^{2+}. Therefore, toxicity of the inhibitor decreases with the decrease in the temperature.

Toxicity of metals may be reduced in the presence of metal-complexing agents. For example, ethylenediaminetetraacetic acid in a concentration of 20 mM reduced the inhibition of Fe^{2+} oxidation in the presence of 1.5—2.0 mM of UO_2^{2+} [131]. Addition of cysteine (10^{-4} M) protected *T. ferrooxidans* against the inhibitory action of $AgNO_3$ (10^{-5} M) on the Fe^{2+}-containing medium [128]. Yeast extract seems to have a similar effect on the media for thermophilic bacteria. The high resistance of thermophilic bacteria to metals is attributed to the presence of yeast extract [128].

According to Lazaroff [138], some anions, such as TeO_4^{2-}, WO_4^{2-}, AsO_4^{3-}, and PO_4^{3-}, increased the rate of Fe^{2+} oxidation by *T. ferrooxidans* in the presence of SO_4^{2-} or SeO_4^{2-} and produced no effect when the latter were absent. Under these conditions, low concentrations of Cl^-, $B_4O_7^{2-}$ and ClO_3^- produced no significant effect while Br^-, NO_3^- and MoO_4^{2-} inhibited the oxidation of Fe^{2+}.

The mechanism of cation and anion effect on *T. ferrooxidans* has so far been studied inadequately. The inhibitory effect of metals is attributed to their accumulation in the cell. They block the enzymes and disrupt cation transport through the membranes [128]. Kelly and Jones [105], and Kovalenko and Karavaiko [111] proved the competitive mechanism of inhibition of bacterial oxidation of Fe^{2+} by Fe^{3+} and Cu^{2+} ions, respectively. Apparently, this mechanism of inhibition of bacterial oxidation of Fe^{2+} and hence, of metal sulphides, is dominant in the presence of other metals as well.

When estimating the technological aspects of the problem, it is obvious that the influence of chemical elements on bacteria in dump, underground and tank leaching should be determined taking into consideration all physical factors as well, which are created during the technological process.

Sources of Nutrients

Nitrogen and phosphorus are present in mine waters mostly in minor quantities which often causes the depression of bacterial oxidation. Apatite-containing ores provide an exception. *T. ferrooxidans* uses practically only ammonium salts as a source of nitrogen. When added to the leach solutions, they, as well as phosphorus, accelerate the growth of *T. ferrooxidans* and the rate of oxidation of Fe^{2+} and of sulphide minerals. For instance, adding $(NH_4)_3PO_4$ to the solutions up to final concentrations of 17 $mg \cdot l^{-1}$ of NH_4^+ and 50 $mg \cdot l^{-1}$ of PO_4^{3-} increased the number of cells 10-fold according to Tuovinen et al. [139]. Optimum concentrations of KH_2PO_4 and $(NH_4)_2SO_4$ in mine waters of the Degtyarsky deposit according to Karavaiko et al. [3] were 400 and 300 $mg \cdot l^{-1}$, respectively, and in some cases, as high as 2 $g \cdot l^{-1}$ of $(NH_4)_2SO_4$ [140]. Optimum concentrations of nitrogen and phosphorus in metal leaching should be established for each particular case. According to MacIntosh [141], *T. ferrooxidans* is able to fix N_2. However, Tuovinen et al. [139] proved that the acid medium absorbs ammonium from the atmosphere enabling

T. ferrooxidans to grow in a nitrogen-free medium. Thus, the ability of these bacteria to fix atmospheric nitrogen has not been proved. Chakrabarty [142] also noted the importance of introducing the RP_4 plasmids of *Klebsiella* which contain the nitrogen-fixing genes into *T. ferrooxidans* cells. As pointed out above, the oxidation rate of sulphide minerals of copper and zinc on a nitrogen-free medium increased in the presence of N_2-fixing *Beijerinckia lacticogenes* [143].

Sulphates are used by thionic bacteria for biosynthesis and certain fermentative functions. Schnaitman et al. [144] proved that addition of SO_4^{2-} in a concentration from 3.6 to 50 mM increased the oxidation of Fe^{2+} by *T. ferrooxidans* two-fold. Sulphates are also important as complexing agents in the consumption of some elements and in the oxidation of ferrous iron. Hence, large amounts of SO_4^{2-} are required for an intensive oxidation of Fe^{2+} by *T. ferrooxidans* [138, 145].

Magnesium and other elements are important for bacterial development as microelements. They are present in sufficient quantities in mine waters and leaching solutions.

Influence of Organic Solvents

Microbiological leaching of metals from low-grade ores is a cyclic process. When leaching copper from ores, copper may be recovered from the solution by solvent extraction and electrowinning. However, the organic solvents used may inhibit the growth of *T. ferrooxidans*. As reported by Torma and Itzkovitch [146], reagents may be arranged in the following sequence according to the degree of inhibitory effect: Lix 70 $<$ Lix 73 $<$ Lix 71 $<$ Lix 64N $<$ Lix 65N $<$ TBP $\sim$ isodecanol $\sim$ nonylphenol $<$ Lix 63 $\lll$ D_2EHPA $\sim$ Kelex 100 $<$ Kelex 120 $^\wedge\ll$ alamin 336 $\sim$ alamin 308 $\sim$ alamin 310 $<$ alamin 304 $\sim$ adogen 381 $\sim$ aliquat 336 $<$ adogen 364. To offset the toxic effect of extractants, solutions should be treated with activated carbon.

3. Influence of Microbiological Factors

Use of Active Bacterial Cultures

In close relation to the practical use of microorganisms for the leaching of metals from ores is the problem of obtaining highly active bacterial strains, which are resistant to extreme conditions. Resistance of «wild» strains of *T. ferrooxidans* to metals varies depending on their habitats and adaptation under natural conditions [47].

From the zones of sulphide mineral oxidation, various authors have isolated strains of *T. ferrooxidans* which are resistant to high metal concentrations in solutions: Cu — 0.5—10 $g \cdot l^{-1}$; Zn — 9—

30 $g \cdot l^{-1}$; Al — up to 6 $g \cdot l^{-1}$; Ni — up to 20 $g \cdot l^{-1}$; Co — up to 5 $g \cdot l^{-1}$; Cl — up to 0.25 $g \cdot l^{-1}$; Ag — 0.05—0.1 $g \cdot l^{-1}$; and As — up to 1 $g \cdot l^{-1}$.

Kuznetsova and Kuligina [147] reported that the introduction of exogenous bacteria in uranium leach suspension was less effective than the addition of nutrients in activating the autochthonous microflora. Further investigations revealed that in the processes of dump and underground leaching of metals the most urgent problem was how to optimize the activity of autochthonous microflora adapted to the specific conditions of leaching of minerals. Another urgent problem is how to increase the number of bacterial cells in solutions and ores up to 10^7—10^8 $cell \cdot cm^{-3}$ ($cell \cdot g^{-1}$), especially at low temperatures [3, 111—114].

Experimental isolation of highly active cultures of bacteria including the thermophilic ones promises well for tank leaching of minerals. In this process, stable and adequate conditions can be created for bacterial growth. For this purpose both adaptation and classical selection methods may be applied. Methods of gene engineering seem to be promising as well. It is known that only those cultures of *T. ferrooxidans* are kinetically stable which have been obtained in the presence of high concentrations of heavy metals and sulphide concentrates [100]. These bacteria also possess a higher ability to adsorb the oxidized substrates [83]. According to the same authors, the leaching of metals from concentrates in a dense pulp occured readily at the content of 10^9—10^{11} cells per 1 ml.

Bacterial cultures were derived under conditions of a gradually increasing concentration of a number of elements. For example, information is available about *T. ferrooxidans* strains resistant to the following concentrations of various elements and compounds: Cu — 50 $g \cdot l^{-1}$; Zn — 120 $g \cdot l^{-1}$; Co — 30 $g \cdot l^{-1}$; As — 6—10 $g \cdot l^{-1}$; Cl — 10 $g \cdot l^{-1}$; Fe — 160 $g \cdot l^{-1}$; Mo — 200 $mg \cdot l^{-1}$; Al — 20 $g \cdot l^{-1}$; Ni — 72 $g \cdot l^{-1}$; Ag — 1.0—10 $mg \cdot l^{-1}$; F — 100 $mg \cdot l^{-1}$; Cd — 120 $mg \cdot l^{-1}$ and U_3O_8 — 12 $g \cdot l^{-1}$ [6, 50, 100, 102, 128, 132, 148, 149—151].

Dave et al. [137] provided data on the oxidation of Fe^{2+} by *T. ferrooxidans* in the presence of 160 $g \cdot l^{-1}$ of $CuSO_4$ and 640 $g \cdot l^{-1}$ of $ZnSO_4$. The mechanism of *T. ferrooxidans* resistance to metals has not yet been studied adequately. There exist probably two mechanisms, i. e., physiological adaptation and development of mutants resistant to the extreme conditions. According to Tuovinen and Kelly [131], the frequency of mutants resistant to 1.0 and 1.5 mM UO_2^{2-} is about unit per $1.3 \cdot 10^6$ or $9.0 \cdot 10^8$ of cells, respectively. The frequency of mutants was increased by adding 15—150 mM of Ni, Zn and Mn.

It has of late been reported that plasmids are important for the resistance of bacteria to metals, and suggested that metal-resistant

T. ferrooxidans strains can be obtained by introducing appropriate plasmids into them [142].

Finally, another important technological problem to be solved is the culturing of a large biomass of active bacteria to be added to the leach solutions. This will have a beneficial effect in dump, underground and tank leaching of minerals. A possible method is the separation of bacteria from pregnant solutions. This will produce a biomass of bacteria adapted to the leach conditions. The culturing of *T. ferrooxidans* according to Kovrov et al. [42, 43] also seems to deserve attention. This method is based on the bacterial oxidation of Fe^{2+} in the conditions of continuous electrochemical reduction of Fe^{3+}.

Role of Mixed Bacterial Cultures in the Leaching of Minerals

In natural or tank leaching of ores associations of microorganisms may be beneficial. This can be illustrated by the leaching of Cu and Ni from a flotation copper-zinc concentrate on a nitrogen-free medium by mixed culture of *T. ferrooxidans* and *Beijerinckia lacticogenes* [143, 152]. In the presence of mixed culture 50% Cu and 100% Ni were extracted in 150 days, while in the presence of *T. ferrooxidans* alone only 18% Cu and 58% Ni were solubilized during the same period.

Typical of the thermophilic lithotrophic bacteria is that they readily oxidize sulphide minerals only in the presence of yeast extract or some other organic substances, e. g. glucose. It may be assumed that thermophilic bacteria can obtain organic substances that are necessary for their growth from other lithotrophic and organotrophic bacteria. Brierley [153] proved that *T. ferrooxidans* can provide organic compounds for the facultative thermophilic bacteria growing on the medium with Fe^{2+}. Data obtained by Golovacheva [154] also indicate that there is a synergism between *S. thermosulfidooxidans* and *L. ferrooxidans* grown on a copper-zinc sulphide ore. In this case, intensive oxidation of sulphide minerals occurs in the absence of yeast extract. Another example of the combined effect of bacteria is the oxidation of sulphide minerals by mixed cultures of *L. ferrooxidans* and *T. organoparus* or *L. ferrooxidans* and *T. thiooxidans* [23, 24]. Pure cultures of these bacteria do not oxidize sulphide minerals. A mixed culture oxidizes sulphide minerals at the same rate as *T. ferrooxidans*.

Chapter IV

TECHNOLOGICAL, ECONOMIC AND ECOLOGICAL ASPECTS OF BACTERIO-CHEMICAL LEACHING OF METALS

1. Technological Aspects

Dump and Underground Leaching

The leaching of copper from ores has been known for a long time. For example, as far back as 1497 copper was leached and recovered by the cementation method in Northern Hungary [155]. A complete leaching cycle is known to have been in operation there in 1566. Around 1750 some 200 tons of cement copper were produced annually in this way by employing the irrigation technique. In Germany, leaching of copper from dumps was practised also in the sixteenth century [156]. At the Rio Tinto mine in Spain, dump leaching of copper ores was started in 1725, while the accumulation of copper from mine waters dates back to 1670 [59]. In Kedabek (the Azerbaijan SSR), dump leaching was in use at the close of the last century. In 1939, practically simultaneously, underground leaching of copper was begun in the Novo-Levinsky and Belorechensky mines in the Urals [3].

At present, dump leaching of copper is used in the USSR, USA, Mexico, Australia, Peru, India, Yugoslavia, Bulgaria, and elsewhere [3, 6, 7, 157—159]. Roughly 5% of the world output and 11.5% of the U. S. copper production are obtained by leaching of low-grade ores [Wadsworth 1975; in ref. 160]. In a number of countries large quantities of uranium are being extracted by this method [7, 90, 161]. Leaching of uranium from low-grade ores in dumps was started in Canada in 1960.

The first research reports on bacterial leaching of metals from sulphide ores were published in 1922 by Rudolfs and Helbronner [162] who used unidentified bacteria. They put forward the notion that microorganisms might be used for leaching metals from low-grade sulphide ores. Somewhat later, in 1947, Colmer and Hinkle [8] isolated *T. ferrooxidans*, a microorganism with unprecedented physiological properties, which was capable to oxidize sulphur, ferrous iron and sulphide-containing minerals at low pH values.

The first patent for the use of *T. ferrooxidans* in the leaching of metals from ores was issued in the USA in 1958 [163]. In the USSR, the physiology and geochemical activity of microbes in ore deposites were first studied by S. I. Kuznetsov at the Institute of Microbiology of the ASSU, and the first work on bacterial leaching of metals dates back to the late fifties and was done by V. I. Ivanov and B. A. Stepanov at the URALMEKHANOBR Institute. At present, a number of other genera and species of bacteria and their associations are known to take part in oxidation of sulphide-containing minerals and in leaching of minerals. In the USSR, first field tests of bacterio-chemical underground leaching of copper were carried out in 1964 [3].

The entire research cycle on underground and dump bacterio-chemical leaching of metals involves normally four consecutive steps: laboratory exploratory experiments, scaled-up laboratory experiments, tests on a semi-industrial plant, and investigations

Table 2

Research steps involved in developing bacterio-chemical dump and underground leaching of metals from ores

Research step	Experimental conditions	Research objectives
I	Weight of ore samples from 5 to 15 kg. Rigid-vinyl or acrylic plastic percolators: D-100 to 150 mm H-400 to 700 mm	Analysis of the phase and chemical composition of the ore and of its mineralogy and petrography; measurements of the specific water and acid retention of the ore, of its porosity, density and bulk density, etc.. Trying different spraying densities and different time intervals between spraying.
II	Same conditions.	By designing a full factorial experiment, find optimal parameter values for leaching of the metal from the ore in question.
III	Total weight of ore samples up to several tons. Ore size up to 400 mm Metal columns: D-0.7 to 1.2 m H-6 to 7 m	Improvements on the optimal leaching conditions found in the preceding step. Economic evaluation of the method.
IV	Ore dumps or ore bodies in deposits	Testing various irrigation techniques and ways of recovering the metal from solutions. Measuring the permeability to solutions of the soil or specially prepared dump sites. Testing different ways of increasing the rate of leaching in field conditions. Studying hydrodynamic characteristics of the material undergoing leaching. Solving the environment protection problems involved and commercial assesment of the entire system.

on a pilot industrial facility. One example is the research on leaching of copper from ores of the Kounrad deposit which was carried in four steps listed in Table 2 [in ref. 164].

A schematic diagram of dump leaching of copper, showing equipment requirements of the facility, is presented in Fig. 6 [164]. Such technical arrangement of bacterio-chemical dump and underground leaching of metals is, in fact, similar to that involving the sulphuric acid process and is used in many countries [3, 7, 165].

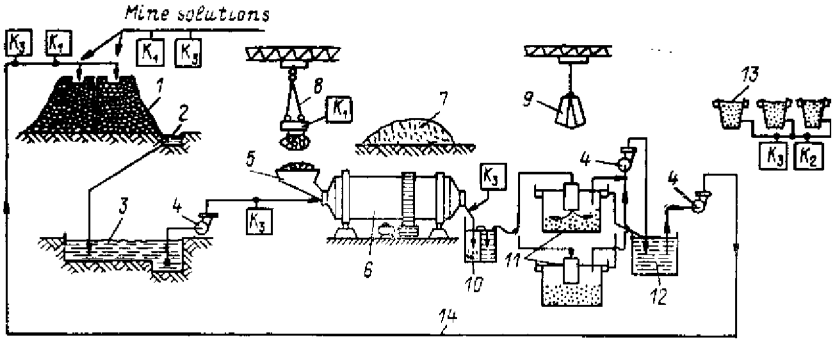

Fig. 6. A schematic diagram of a plant for leaching and cementation of copper:
1 — an ore dump leached; 2 — flumes for solutions; 3 — a settling pond for inflow solutions; 4 — pumps; 5 — an inlet for loading iron scrap; 6 — a cementation drum; 7 — a scrap pile; 8 — a travelling bridge magnetic crane with a telpher; 9 — a grab bucket for discharging cement copper; 10 — a trap for small-sized iron scrap; 11 — a settling basin for cement copper; 12 — a sump for recycle solutions; 13 — containers with output cement copper; 14 — pipe-lines

The flowsheet provides for a cyclic leaching process. Sulphuric acid solutions of ferrous and ferric iron containing *T. ferrooxidans* and other bacteria are normally used as leaching liquor (see Chapter I). Sulphide minerals contained in the ore are oxidized by bacteria and ferric iron according to the reactions considered in Chapter II.

Next, the solution coming from the ore dump is fed to a cementation unit where copper is precipitated as a result of the following reaction with the iron scrap:

$$CuSO_4+Fe \rightarrow Cu+FeSO_4.$$

The cement copper is then transported to a copper smeltery.

Prior to further irrigating the ore, the solution containing Fe^{2+} is to be partially or entirely regenerated in a special pond. This involves the oxidation of ferrous iron by microorganisms and a partial removal of solutions from the cycle with their replacement by fresh water. If necessary, sulphuric acid could be added, as well as nitrogen and phosphorus salts. Thereafter the cycle is repeated.

The purpose of regenerating the leach liquor is to increase the content of bacteria and of the oxidizing agent, Fe^{3+}, as well as to reduce to an appropriate level the content of other elements that may accumulate in the solution during the process. In the leaching of Ni, it is recovered from the solution by electrolysis and the sulphuric acid formed in the process is used as the recycle solution.

Also under study is metal recovery from solutions using various sorption processes.

The expediency of leaching of copper and other metals from sulphide ores depends on the type of the ore, on technical factors, (such as preparation of dumps or ore body, supply of oxygen to the ore, maintaining optimal pH values, hydrodynamic characteristics of objects affected, etc.), and also on the number and the activity level of bacteria [1, 3, 4, 126, 166]. The main factors affecting biological oxidation of sulphide minerals in field conditions are summarized below [4].

1. *Physical characteristics of the rock body containing sulphide minerals*
   Geometry of the rock body
   Particle-size distribution
   Permeability to water and gases
   Thermal conductivity and heat capacity
Location as related to influent water, drainage and the water table
2. *Characteristics of sulphidic material*
   (a) Relating to sulphidic component of the ore
      Presence or absence of iron pyrite
      Diversity and types of other metallic sulphides
      Particle size of individual sulphides
      Distribution of individual sulphides
   (b) Relating to the gangue
      Permeability to water and gases
      Capacity for acid neutralization
      Stability to acid and heat degradation and nature of degradation products
      Sorptive capacity for water, H^+, metal ions, and other chemical compounds (including organic matter)
      Ion-exchange properties
      Redox characteristics
3. *Composition of influent water*
   Solutes present (oxygen, carbon dioxide, carbon compounds, nitrogen compounds, other nutrients for bacteria)
   Redox status (largely governed by oxygen level and the ferrous/ferric ion ratio)
   Microbial populations (diversity and magnitude)
   Presence of natural toxic materials (e. g. mercury and molybdenum compounds)

4. *Climatic influences*
Rainfall and run-off patterns influencing the availability of water, water-logging, and the erosion of the rock body
Ambient temperature variations (seasonal and daily)

Studies on *T. ferrooxidans* distribution in dumps and in ore deposites have shown that the number of cells, within the oxidation region, varies from 10^5 to 10^8 per 1 g of ore or 1 ml of the solution depending on the season, technical arrangement of the leaching process, and on the temperature [3, 47, 87, 167—169]. The activity level of bacteria depends on the temperature, pH value, and on chemical composition of leach solutions [165].

During the leaching of copper from industrial dumps of sulphide ores their temperature has been observed to increase to 40—60° C [32, 166, 169—175]. The same process in columns takes it up to as high as 50 to 60° C [172]. Laboratory studies have shown that regeneration of Fe^{3+} and oxidation of sulphide minerals by bacteria are not impeded by high temperatures [30, 32, 35, 57, 67, 73]. It is evident that thermophilic bacteria can play an important role in industrial leaching of ores.

The distribution of thermophilic bacteria oxidizing Fe^{2+} and reduced sulphur compounds (S^0, sulphides) has not as yet been adequately studied. As mentioned above, *S. thermosulfidooxidans* [33] and some thermophilic bacteria close to thionic bacteria [30, 169] were isolated from leach dumps. As reported by Golovacheva and Karavaiko [33], the number of cells of *S. thermosulfidooxidans* in ores and leach solutions amounts to cially from high-grade sulphide ores and at lower temperatures, (20 to 25° C).

The results of ecological and laboratoty studies can indicate a general solution to the technological problems involved in increasing the efficiency of the whole cenosis of microorganisms in dump and underground leaching of metals. It should be emphasized, however, that an active process of metal leaching, especially from high-grade sulphide ores and at lower temperatures, would require concentration of bacteria of the order of 10^9 cells per 1 ml of solution. This can be achieved by incorporating into the system particular operation stages such as solution regeneration or growing biomass either in electrochemical cultivators or by means of the Bacterial Film Oxidation method [42, 43, 173, 174].

Tank Leaching

Developing an industrial process of tank bacterio-chemical leaching of metals from concentrates normally involves several research steps listed in Table 3.

A prototype of tank leaching is the extraction of metals from concentrates in fermenters or Pachuca tanks [17, 78, 135, 151,

Table 3

Research steps involved in developing a tank bacterio-chemical leaching system [175]

Research step	Experimental conditions	Research objectives
I Exploratory laboratory studies	Sample weight up to 1 kg; batch cultivation	Understanding the participation of microorganisms in oxidation of sulphide minerals in concentrates. Identifying underlying conditions, e. g., activity level of bacteria, their tolerance to the concentrate and metals, their adaptation, the pH range, the pulp density, etc..
II Scaled-up laboratory tests	Samples of a few kilograms in weight; continuous culture	Finding optimal conditions (parameter values) for the bacterial leaching process with recycling of regenerated solutions. Obtaining a working culture adapted to the entirety of extreme environment conditions of the leaching system. Economic evaluation of the process
III Pilot industrial tests	Production output exceeding 60 kg·day^{-1}; solid to liquid phase ratio=1 : 4—5	Obtaining and refining data for feasibility evaluation and for designing a pilot commercial system. Stages: 1. Obtaining an active biomass; 2. Putting the plant in the projected operation mode, 3. Leaching operation with complete recycling of biomass and solutions

175, 176]. In the USSR, a direct-flow scheme is used with solid and liquid phases passing simultaneously through a successive series of Pachuca tanks (Fig. 7) [151]. Table 4 lists the parts of a pilot system of this type made from acid resistant materials. Bacterial oxidation of sulphide minerals takes place in the 120-liter Puchuca tanks (items 1—3 in Fig. 7). Next the liquid and solid phases are separated and valuable components are extracted from the solution. The solid sediment (cake) is dumped or is to undergo further processing and the solution is regenerated and recycled to the first Pachuca tank. Thus, the treatment of concentrate in Pachuca tanks is also a closed-circuit process. The active culture of *T. ferrooxidans* adapted to the concentrate in question is grown in the zero Pachuca tank.

A distinctive feature of the tank bacterio-chemical metal leaching from sulphide concentrates is that high-density pulps provide for high rates of oxidation of sulphide minerals and for selective element recovery. The highest rate of oxidation

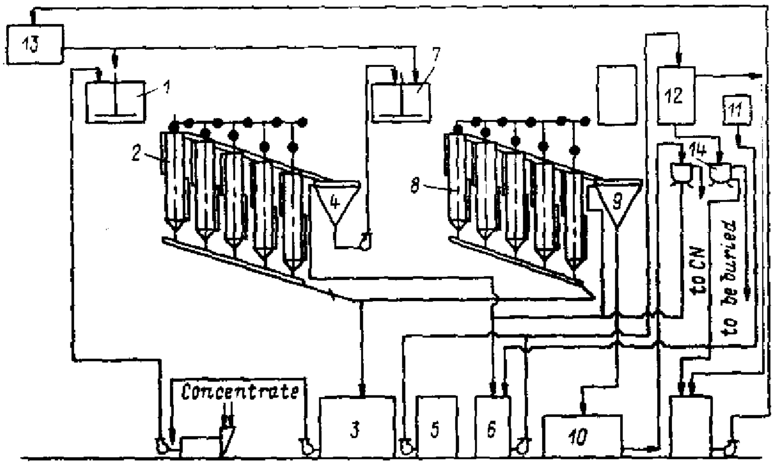

Fig. 7. A schematic diagram of metal leaching in Pachuca tanks [151]:
1 — contact tank No. 1; 2, 8 — Pachuca tanks; 3 — a tank for bacteria-containing solutions; 4, 9 — dehydrating cones; 5, 6 — vats for dehydrating cones underflow following the first and the second leaching stages; 7 — contact tank No. 2; 10 — a collector vat for output metal; 11 — a lime milk feeder; 12 — a settling vat; 13 — a collector vat for recycle solutions; 14 — a Nutch filter

Table 4

A parts list of a pilot leaching system [175]

Units	Capacity, liter	Number of units	Material
Ball mill 400×400 mm	—	1	—
Contact tank	900	1	X18H10T
Pachuca tank for leaching	200	5	—»—
Dehydrating cone	300	1	—»—
Contact tank	900	1	—»—
Pachuca tank for leaching	200	4	—»—
Dehydrating cone	300	1	—»—
Leach liquor vat	500	1	X18H10T
Liquid separator OST-3	—	1	—
Pachuca tank for regeneration of bacterial solutions	200	1	X18H10T
Vat for liquor treatment	500	1	—»—
Stand-by vat	500	1	—»—
Settler vat	500	2	—»—
Nutch filter	60	1	ceramics
Nutch filter	40	1	—»—
Vat for recycle liquor	500	1	X18H10T
Vat for recycle liquor	900	1	—»—
Agitator	30	1	St. 3
Vat for Pachuca tank discharge	900	1	X18H10T
Compressor RMK-2	—	2	—
Receiver	—	1	—

of sulphide minerals is obtained at 15 to 20% pulp density, pH 2.3, at temperatures within 30 to 35° C, with strong aeration in the presence of CO_2 (0.2 to 1.0%), fine grinding of minerals and with biomass content from 2.0 to 10 g (wet wt) per 1 ml of pulp. In tank bacterial leaching, the leach solution accumulates metals at concentrations high enough to be extracted by the electrolysis (see Table 5).

Table 5

Kinetics of bacterial extraction of metals

Concentrates	Pulp density, %	CO_2, %	Maximum rate of leaching		Tank capacity, l	Metal content of solution, $g\cdot l^{-1}$
			$mg\cdot l^{-1}\cdot h^{-1}$	metal		
Chalcopyrite (27.3%	20	0.1	725	Cu	50	50
23.8 %) [124, 176]	to 40 final density	0.1	500—700		50	20—50
Zinc [50, 104, 124]	40	0.1	1300	Zn	10	98
	16	1.0	517	Zn	0.25	70—120
			635		30	
			631		12	
Nickel [86]	25	0.2	175—222	Ni	8	72
	15	0.2	155		8	
Tin-copper-arsenic [78]	20	aeration	95.7 % in 96 hours	As	3	up to 10—15
Gold-bearing [78]	20	—»—	80—90 % in 70—80 hours	As	3	up to 10—15
Pyrite-bearing uranium ore [178]	30—40	—»—	up to 91.1—95 % in 6—9 days	U	0.5—2	—

Apart from those listed in Table 5, the following concentrates may be suitable for bacterial leaching: copper-nickel [11, 64, 86], lead [17, 121], and copper-bismuth [177].

Several flow charts, tested recently in scaled-up experiments, can be effectively implemented for tank extraction of metals from concentrates [78, 101, 121]. For example, tests run on a pilot industrial plant have shown that arsenic content as a toxic ingredient in tin-containing concentrates, which can occasionally reach 15%, can be reduced to 0.17—0.20% in 96 hours (Fig. 8). During a two-stage treatment of gold-arsenite concentrates (10% arsenic) with an intermediate removal of arsenic from the solution and with the solid to liquid phase ratio averaging 1 : 5, about 80 to 90% of FeAsS and 40% of FeS_2 is oxidized in 78 hours. With low arsenic content of the concentrate, the process in completed in a single stage and as much as 93 to 95% of arsenic is extracted in 72 hours. Gold recovery from the residue by the cyanidation method is as high as 92.5% (Fig. 9).

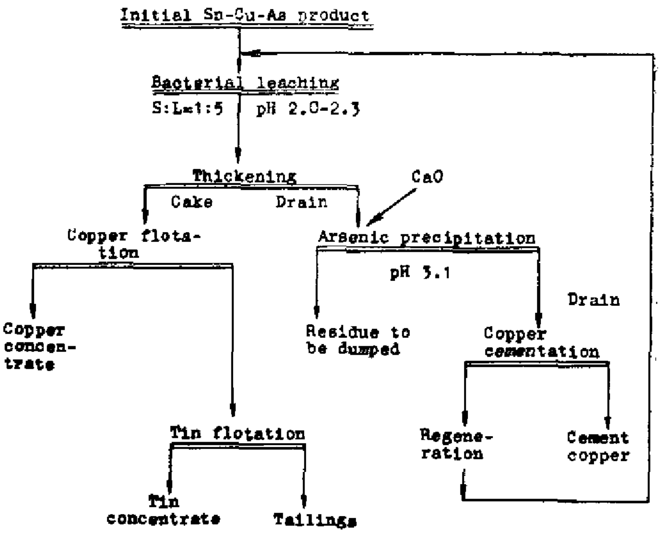

Fig. 8. A flowsheet for bacterial leaching of difficult-to-dress tin-copper-arsenic materials [78]

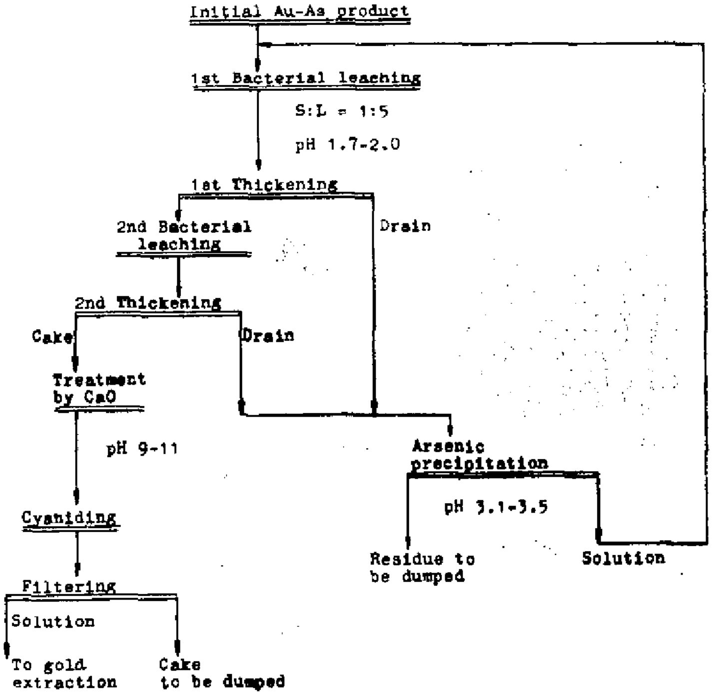

Fig. 9. A flowsheet for bacterial leaching of a gold-arsenic concentrate [78]

To recover copper and zinc from metacolloid composite concentrates, a combined approach has been used. The treatment includes selective bacterial leaching of zinc, cadmium and partially copper, recovery of metals from solutions, and cake flotation after the bacterial leaching, producing high-grade copper concentrate (Fig. 10). The total extraction was 92% of copper, 90% of zinc and 89% of cadmium [82, 83]. According to Torma and Subramanian [121], and Torma [17], a virtually full extraction of copper, zinc and cadmium from lead concentrates can be realized in the presence of *T. ferrooxidans*. Lead remains in the solid phase in the form of $PbSO_4$ and partially in the form of

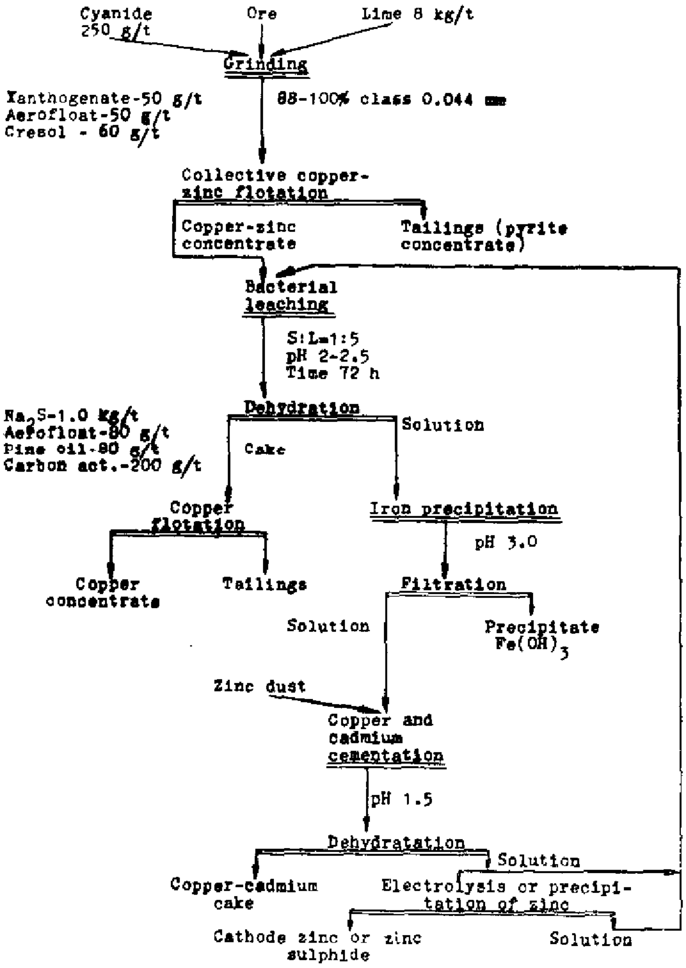

Fig. 10. A flow chart for combined processing of metacolloid copper-zinc ores [83]

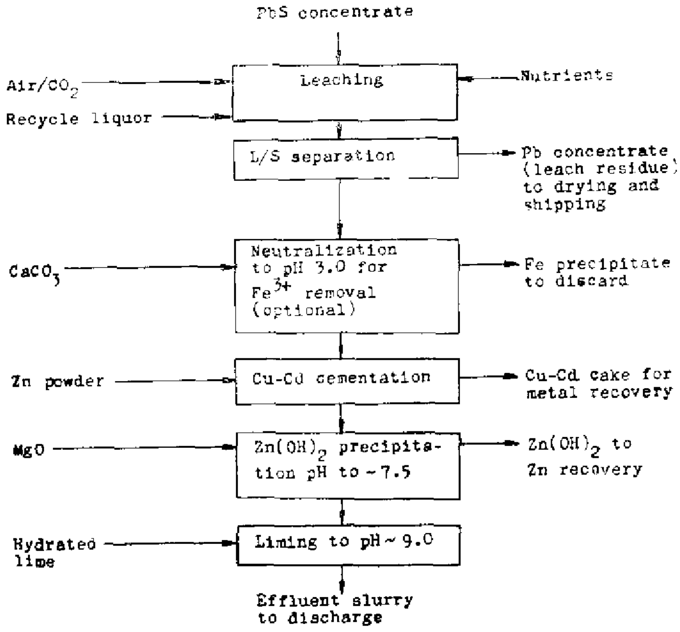

Fig. 11. A suggested flowsheet for leaching and extraction of copper, cadmium and zinc from a PbS concentrate [121]

non-oxidized PbS (Fig. 11). Both iron hydrate and lead sulphate can deposit on sulphide surfaces inhibiting bacterial activity [17]. As reported by Ilyaletdinov et al. [179], treatment of a copper-zinc concentrate with Fe^{3+}-containing bacterial solutions (20 to 25 $g \cdot l^{-1}$) at temperatures within 60 to 65° C resulted in 96 to 98% extraction of lead and 70% extraction of zinc in 3 to 4 hours. Thus, lead can also be extracted hydrometallurgically from concentrates.

Regeneration of Fe^{3+} can be carried out by using thermophilic bacteria *S. thermosulfidooxidans* which oxidize Fe^{2+} at 50° C.

Bismuthite, Bi_2S_3, is known not to be oxidized by *T. ferrooxidans*. For this reason, only a selective extraction of copper occurs during the treatment of copper-bismuth concentrates with a culture of *T. ferrooxidans*. According to Batyrbekova et al. [177], up to 68% of copper is extracted in a 3 to 4 stage treatment of concentrate (in 9 to 10 hours). The residual copper and bismuth can be recovered from the cake by addition of HCl and $CaCl_2$. The total copper extraction amounts to 92 to 95%. *T. ferrooxidans* can also be used to leach nickel and cobalt from ores and concentrates.

Studies conducted on various facilities have shown a high efficiency of *T. ferrooxidans* in uranium leaching. During leaching of uranium from pyrite-containing ores in Pachuca tanks at pH 1.5 to 1.6 for 6 days, 91.1% of uranium was extracted [178]. Under semi-continuous cultivation conditions, 100% of uranium was extracted at 20% pulp density in 5 days [180, 181]. In experiments reported by Derry et al. [182], extraction of uranium from an ore containing 0.12% U_3O_8 was as high as 95%. Their technique makes use of *T. ferrooxidans* to regenerate Fe^{3+}. The process is run at 30° C.

Studies are under way to develop techniques for recovery of non-ferrous metals from various industrial wastes, copper carbonate shales, oil-containing shales, bitumenous sands [180, 183, 184] as well as for coal desulphurization. According to Ebner [185], up to 70% of copper and 95% of zinc can be recovered from industrial waste materials.

In experiments by Dugan and Apel [186], about 97% of py-

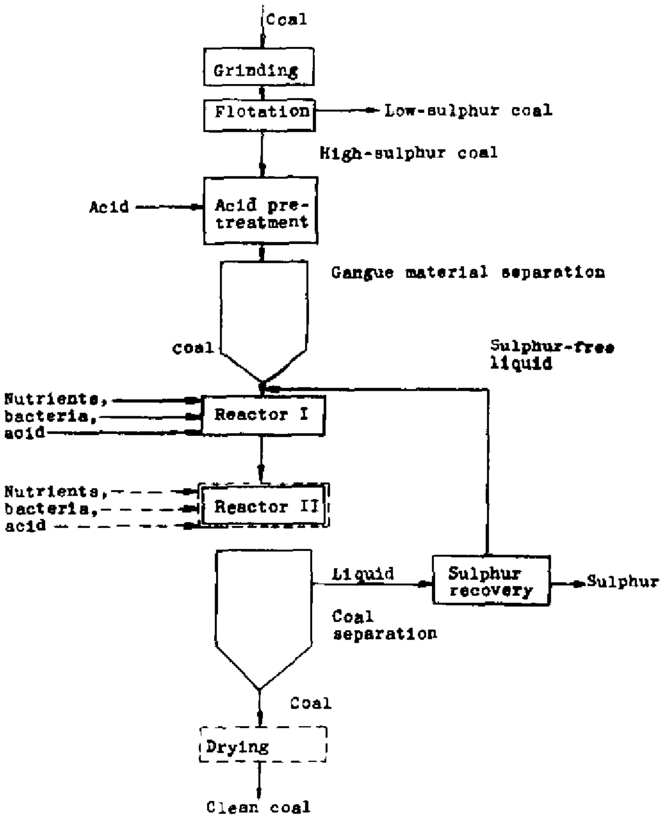

Fig. 12. A possible flowsheet for bacterial desulphurization of coals. Broken lines indicate optional steps [187]

ritic sulphur was removed from coal in 5 days by using *T. ferrooxidans*.

A schematic diagram of bacterial desulphurization of coals using *T. ferrooxidans* is shown in Fig 12. [187]. A two-stage treatment procedure is employed for coal containing not only pyrite but also organic sulphur compounds. At the first stage, FeS_2 is oxidized by autotrophic bacteria; at the second one, a mixed population of heterotrophic bacteria is used to oxidize organic sulphur compounds.

As seen from the above, such metals as copper, uranium, lead, bismuth, zinc, arsenic, nickel and cobalt can be extracted from composite concentrates by means of bacterio-chemical tank leaching.

Bacterial Biomass Production Methods

As pointed out above, the kinetics of bioleaching of metals from ores and concentrates depends on the bacterial biomass density in the pulp or in the ore body. A number of methods of biomass production have been proposed to date.

One such technique is based on cultivation of *T. ferrooxidans* on a Fe^{2+}-containing medium with continuous electrochemical reduction of Fe^{3+}. A schematic diagram of a system for continuous cultivation of *T. ferrooxidans* is shown in Fig. 13 [188]. The system comprises a reactor, being, in fact, an electrochemical cell of the pressure filter type where bacteria are grown, and also a number of equipment units designed to maintain optimum cultivation conditions (Fig. 14). All parts of the reactor casing are acrylic plastic while the cathode and anode are made of platinum and platinum-plated titanium respectively (items 3 and 4 in Fig. 14). The cathode and anode spaces are separated by a cation-exchange membrane of the MK-40 type.

Denisov et al. [188] report the daily biomass output of such a system to be 100 g dry weight per 1 m^2 of cathode area. From this, given an output level, the appropriate dimensions of the reactor can be found. The temperature is maintained by a water jacket and aeration is provided by a membrane type compressor (see Fig. 13). In the reactor, the rates of electrochemical reduction of Fe^{3+} to Fe^{2+} and of bacterial oxidation of Fe^{2+} to Fe^{3+} are balanced by regulating automatically the cathode voltage.

In this method, the major cost of biomass production is that of electric power. In laboratory conditions, 150 kWh of electric power was needed to produce 1 kg of dry cell biomass [189]. A theoretical estimate shows that the required electric power can be reduced by a factor of 2 to 3. This method of biomass production has not, however, been tested in industrial conditions.

In our experiments performed on a pilot tank leaching system it was shown that biomass of *T. ferrooxidans* can be obtained

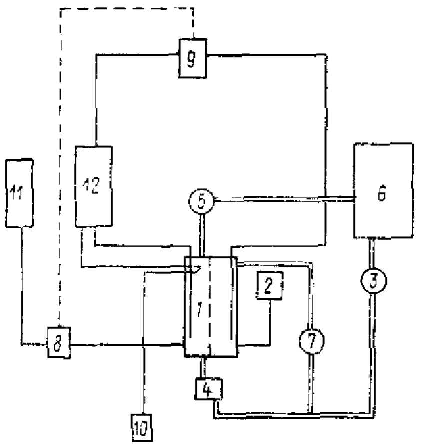

Fig. 13

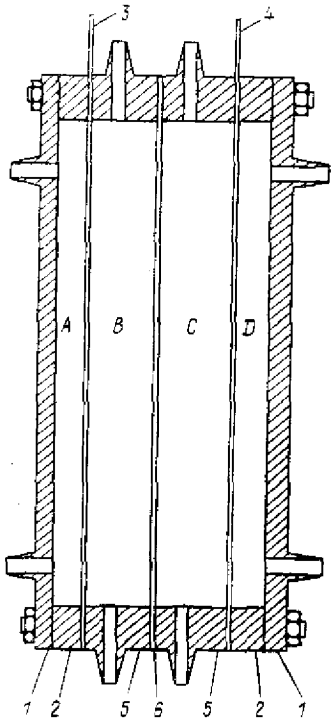

Fig. 14

Fig. 13. A schematic diagram of a system for continuous cultivation of *T. ferrooxidans* with electrochemical reduction of ferric iron [188]:

1 — a reactor; 2 — a storage tank for distilled water; 3 — compressor; 4 — a flow governor; 5 — a cooler; 6 — receiver; 7 — gas flowmeter; 8 — feed meter; 9 — a current integrator; 10 — a tank for output biomass; 11 — a storage tank for nutrient medium; 12 — an adjustable direct voltage source

Fig. 14. The schematics of the reactor from Fig. 13 [188]

A and D are a water jacket; B is the cathode space; C is the anode space; 1 — an outer wall of the water jacket; 2 — the water jacket frame; 3 — the cathode; 4 — the anode; 5 — frames of the cathode and anode spaces; 6 — an ion-exchange membrane

by separating bacterial cells from pregnant solutions. This technique seems to have a somewhat higher potential for industrial implementation than the one discussed above because it has the advantage of recycling into the process the biomass of active bacteria already adapted to leaching conditions. No cost estimate of this biomass production method has yet been carried out.

An innovating technique, termed Bacterial Film Oxidation (BACFOX Process), to increase bacterial content of solutions intended for dump and underground leaching has been recently developed [173, 174]. Basically, the method is as follows. A surface coated with *T. ferrooxidans* bacteria is to be submerged in a solution containing Fe^{2+} through which fresh air is sparged. Oxidation of Fe^{2+} takes place while the inflow solution passes through the tank. A schematic diagram of a plant implementing this technique is presented in Fig. 15. Under such conditions, bacteria have been shown to bind with precipitated jarosite with a capacity to form a bacterial-jarosite film on surfaces of different materials (e. g., glass, plastics). In experiments by Livesey-Goldblatt et al. [173], best results were obtained when a bacterial film coated a corrugated plastic surface. In this case, the specific rate of Fe^{2+} to Fe^{3+} oxidation was as high as 7.5 $g \cdot h^{-1}$ per 1 m^2 of bacterial film area.

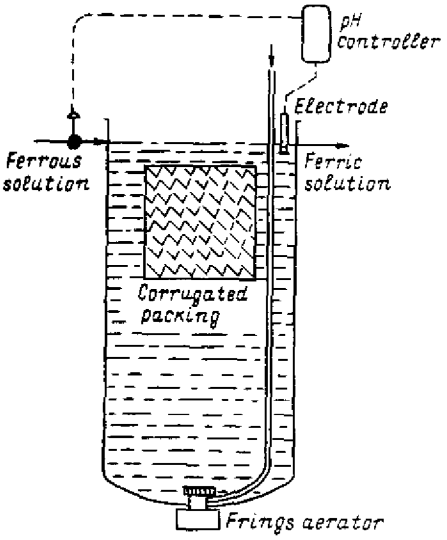

Fig. 15. Submerged corrugated pack unit — forced aeration [173]

Biosorption of Metals by Microorganisms

A major task in hydrometallurgy is the recovery of metals from solutions and treatment of wastewater from different industries. Today, only a few metals are being recovered from solutions, particularly those with relatively high content. However, it is in metal extraction from diluted solutions that the major problems are encountered. A new approach to dealing with these problems has recently evolved. There are many microorganisms with capacity either to sorb metal ions or to precipitate them. Table 6 lists several mechanisms of metal ion precipitation by means of bacterial activity. These include:

1. Biosorption;

2. Precipitation of metals in the form of sulphides;
3. Reduction of Cr^{6+} and of some other elements.

Precipitation of metals in the form of sulphides has been known for a long time. The method consists in using sulphate reducing bacteria to produce H_2S that has a capacity to precipitate practically the total metal contained in a solution. In one study, 98.5% of copper was recovered in this way, its initial content being 8.6 $g \cdot l^{-1}$ [209]. This technique has also been successfully tested on industrial scale, in mine conditions in the USSR [206].

The process of Cr^{6+} to Cr^{3+} reduction in solutions has also found industrial applications in the USSR [208]. The method is based on the ability of *Bacterium dechromaticans* to reduce Cr^{6+} to Cr^{3+} in anaerobic conditions; Cr^{3+} then precipitates. The process occurs at pH values between 8 and 9, and municipal sewage water is used as organic nutrient.

A novel approach to the problem is to employ biosorption of metals from solutions.

The results of experimental studies indicate that by using microorganisms up to 100% of lead, mercury, zinc, copper, nickel, cobalt, manganese, chromium, uranium, etc. can be reclaimed from diluted solutions (Table 6).

Table 6

Metal sorption and precipitation by microorganisms [190—212]

Microorganisms	Metal precipitation process
Fungi (biomass), yeasts, bacteria, algae	1. Biosorption of radioactive (U, Ra) and other elements: Al, Mo, Ag, Cu, Cd, Cr, Mn, Co, Ni, Zn, Hg, Pb, Au, Pt, Pd
Chitin and chitosan	Sorption of Ce, Zr, Hf, Ru from water circulating in a cooling system of a nuclear reactor
Sulphate reducing bacteria	2. Precipitation of metals from solutions: $C_{org.} + SO_4^{2-} \xrightarrow{\text{bacteria}} S^{2-} + CO_2$ $S^{2-} + Me \rightarrow \downarrow MeS$
Chromium reducing bacteria	3. Metal reduction: $Cr^{6+} \rightarrow \downarrow Cr^{3+}$

Using fungi makes it possible to recover 96 to 98% of gold and silver, up to 84% of platinum and 92% of palladium from dilute residue solutions used in the process of gold and silver refining [205].

A mixed bacterial culture under anaerobic conditions enabled the extraction of as much as 81% of uranium and 93% of selenium from effluent solutions [211].

Algae have also proved to be very helpful in biosorption of metals from solutions [211].

Bacterial polysaccharides can also be effectively used in removing from solutions radioactive elements, copper and cadmium [212].

Sorption of metals from solutions leads to their accumulation in the biomass. It can be seen from Table 7 that the content of various metals in the biomass can become quite considerable.

Table 7

Metal accumulation in microorganisms

Solutions	Microorganisms	Metal content of cells	Reference
Solutions containing radio-active elements	Denitrifying	Uranium: 140 mg per 1 g of biomass dry weight	214
»	*Rhizopus arrhizus*	Uranium and thorium: more than 180 mg per 1 g of biomass dry weight	215, 216
»	*Saccharomyces cerevisiae, Pseudomonas aeruginosa*	Uranium: within 10 to 15% of cell dry weight	194, 213
»	M-biosorbent (*Penic. chrysogenum*)	Uranium: 80 to 120 g per 1 kg of biosorbent dry weight at uranium density of 1 $g \cdot l^{-1}$. Upon treatment uranium density below 0.05 $mg \cdot l^{-1}$	217
Silver-containing solutions	Consortia of bacteria: *P. maltophila, Staphylococcus aureus* and unidentified cultures	Silver: up to 30% of the biomass dry weight	218

The mechanism of sorption of metals from solutions by microorganisms is for the most part well understood. Basically, it is due to the involvement of the cell wall. In fungi metal sorption, a very important role is known to be played by chitin and chitosan. For this reason, they should be used as such for metal extraction. One limitation to this is that an ever increasing catch of crustacea threatens to disrupt an ecological balance of the world ocean. A different approach to obtaining chitin and chitosan is their production from fungi.

There are a number of ways of biosorbents application.

Firstly, they can be utilized in biological reactors (Fig. 16), which are essentially biological filters using, e. g., coal as biosorbent carrier [214].

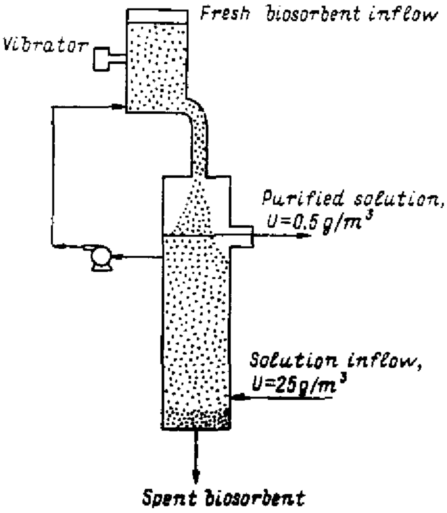

Fig. 16. A counter-flow contactor for continuous extraction of uranium using denitrifying bacteria carried by coal particles (biosorbent is in contact with the solution for about 8 min.) [214]

Another example relates to the use of the M-biosorbent produced in Czechoslovakia [217]. Its composition includes mycelium itself, *Penic. chrysogenum*, and also various reaction products of carbamide and formaldehyde polycondensate, used to fix the biosorbent to its carrier, and of mycelium constituents. The M-biosorbent is produced in the form of ground material with grain size between 0.3 and 0.8 mm. It can be loaded in plants that are designed for and normally run on ion-exchange resins.

Finally, in natural conditions, biosorption of metals by bacteria, algae, etc. occurs in ponds. As reported by Brierley and Brierley [211], pond sediments contained: uranium, from 430 to 1900 ppm; selenium, from 6 to 80 ppm; molybdenum, from 7 to 320 ppm. Thus, it becomes clear that not only plankton but also the sediments should be removed from ponds either to reclaim the metals or simply to bury them in the case the water treatment is our sole objective.

One of the essential merits of metal biosorbents is that they can be widely used in natural environments.

A well operating production of the biomass or, for example, of bacteria-synthesized polysaccharides can become a new source of selective ion-exchange materials. For instance, mixed algae cultures had a cation-exchange capacity greater than 640 μ equivalents per 1 g of dry weight [219].

The capacity of fungi cells exceeded by 2.5 times the capacity of common anionic exchange resins (IRA-400) used by uranium production companies for selective separation of uranium from other ions in solution [215, 216].

The capacity of the M-biosorbent was as high as approximately 5 g uranium per 1 kg of its dry weight (the maximum capacity being in the range 80 to 120 g per 1 kg dry weight at uranium concentration in solution of 1 $g \cdot l^{-1}$).

A few other advantages of biosorbents can be pointed out. These include their availability, ease of their production, etc.

2. Economic Aspects

Commercial viability of dump, underground and tank leaching is closely related to the expenditure pattern. There are reasons to believe that this pattern will remain basically unchanged from place to place although the expenses themselves may to some extent vary.

Below we consider several examples of the metal leaching economics.

Dump and Underground Leaching

As a first example, we consider an estimate of the cost of dump leaching in Miami, USA, under the following conditions [220]:

— acid soluble copper	—0.5—1.0%
— stripping ratio	—1:1
— ultimate copper recovery	—60%
— copper to be recovered by cementation using scrap iron at $ per ton	—50—52.

The capital cost of dump leaching can be relatively low. The major expenses are usually those covering ore mining, consumption of sulphuric acid, and the cost of copper cementation process. The leaching business will be commercially more attractive if the costs of mining of low-grade ores and of their dumping are already included in the overall cost of mining operations. The expenses involved in production of roughly 30 tons of copper per day at a Miami mine are as follows (in $):

Supervision and salaried	140.00
Labor — 10 shifts per day at $40.00	400.00
Power — 4000 kW4 at $0.015	60.00
Iron for cementation — 30×1.5 at $52 per ton	2,340.00
Water	100.00
Miscellaneous supplies	50.00
Total	3,090.00

The production cost of 1 kg of copper was 10.3 cents. Adding here freight to the smelter and smelter charges (15.0 cents$\cdot$$\cdot$kg^{-1}) gives the net production cost of 1 kg copper by dump leaching at 25.2 cents, i. e. $252 per ton.

The expenses involved in producing 1 ton of copper by dump leaching with mining and smelter charges included are as follows (in $):

Mining	110—260
Acid	90—320
Smelting	140
Scrap iron for cementation	76
Labor, power, water and miscellaneous supplies	26
Total	450—850

The expenses involved in copper production in India are listed in Table 8 [221].

Annual production of 2,000 tons of copper is projected, with 96% recovery.

Table 8

Biohydrometallurgical copper powders: indicative cost profile

Operation Major Items	Cost	
	Rs/ton Cu	$/ton Cu
Mining cost (overall)	100.00*	10
Heap leaching and cementation:		
Supervision and labour	200.00	
Power	150.00	
Water	40.00	
Maintenance, etc.	110.00	
Miscellaneous supplies	100.00	
Sulphuric acid**	—	
Scrap iron	2500.00	
Estimated cement copper costs	3200.00	300
Hydrorefining costs (overall)	4000.00	
	7200.00	
Capitalized costs:		
Depreciation Interest Insurance	5000.00	
Total powder production costs	12 200.00	1200

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* Actuals will depend on geochemistry, topography, etc.

** External input not normally required for pyrites deposits.

As can be seen from Table 8, for this type of ores no sulphuric acid is needed, which is usually the case with pyrite-containing sulphidic ores, while in the former example it constituted one of the major cost items. According to the authors, such a net cost of dump copper production is commercially acceptable. This cost, however, tends to vary, specifically, it increases as the metal content of the ore falls down. For example, the net cost of 1 ton of copper produced in the initial years of operation of the dump leaching facility at the Nikolaev deposit amounted to 232.9 roubles, in 1972, and ten years later it exceeded 400 roubles [164].

The net cost of underground leaching of copper was $75—85 per ton in Bore [158] and approximately $80 per ton at the Bingham mine [222].

It was shown [3, 90, 161] that bacterial dump and underground leaching of uranium from ores can also be commercially profitable.

Tank Leaching

The economic aspects of tank leaching of metals are under consideration in the following series of papers [17, 85, 124, 135, 158, 165, 179, 221, 223].

The chalcopyrite concentrate. Capital and operating costs estimates for a flow-through plant to treat 200 tons/day of chalcopyrite concentrate, assuming 350 operating days a year and 96% copper recovery [135], are summarized in Table 9.

Table 9

Summary of capital cost estimates

Facility	Cost ($)	$/Annual net ton Cu
Leach plant	9.4×10^6	560
Electrowinning plant	4.2×10^6	250
Total	13.6×10^6	810

Direct operating costs involved in copper leaching are summarized in Table 10 [135].

Let us assume that the total investment (fixed+working capital) for a 200 tpd plant is to be repaid by annuity at 10% interest over a 15 year period. On this basis, financing cost per pound of copper produced is 5.95 cents. Adding the estimated direct operating cost (7.31 cents, see Table 10) produces a total treatment cost 13.26 cents per pound of copper ($292 per 1 ton).

According to McElroy and Bruynesteyn, in 1973 smelter charges per pound of copper in concentrate were in the range of 8.5 to 14.5 cents ($187 to $319 a ton), i. e. in rough correspondence

Table 10

Summary of estimated direct operating costs

Operation	Costs		% of Total	Cumulative % of Total
	$/net t Cu	cents of pound Cu		
Labour	41.83	2.09	28.6	28.6
Power (10 mills/kWh)	41.20	2.06	28.2	56.8
Maintenance (5% of fixed capital)	40.47	2.02	27.7	84.5
Limestone ($5.00/t)	15.00	0.75	10.2	94.7
Flocculants	4.17	0.21	2.8	97.5
Nutrients	2.47	0.12	1.7	99.3
Grinding balls (cast steel)	1.20	0.06	0.7	100
Total	146.34	7.31	100	

with costs of bacterial leaching under different obtaining conditions. McElroy and Bruynesteyn [135] stress the following virtues of bacterial leaching for processing of chalcopyrite concentrates:

— negligible atmospheric emissions;

— production of refined copper (i. e. potentially lower transport cost and increased market flexibility);

— feasibility for small scale operations (minimum ~40 tpd of concentrate);

— potentially increased returns for silver, gold and (possibly) molybdenite in concentrates;

— production of dilute sulphuric acid suitable for leaching of oxide ore and/or acid consuming waste dumps.

Estimates made earlier by Bruynesteyn and Duncan [124] for a plant to process 100 tons of chalcopyrite concentrate per day and produce annually 21,900,000 pounds of copper (i. e. 9932.6 tons) showed that the cost of solubilization of 1 pound of copper from concentrates could be as low as 2.89 cents. Taking into account electrowinning expenses (2.75 cents) establishes the total cost of copper production at 5.72 cents a pound ($126 a ton). By expanding the business to process 1000 tons of concentrate per day, the total cost of copper production can be brought down to 3.9 cents a pound ($86 a ton).

The lead concentrates. Another example is economic evaluation of bacterial processing of low-grade lead sulphide concentrates containing 42.9% lead, 29.6% sulphur, 16.7% iron, 7.7% zinc, 2.4% copper and 0.02% cadmium [17].

It is projected that 100 tons of the concentrate would be leached for five days at temperature 35° C, pH 2.3, and with pulp density 20%. Minimum extraction of zinc, copper and cadmium is projected to be 95%. Lead in the form of $PbSO_4$ is to remain in the solid phase. The capital cost of this process is estimated to be $7,300,000. This includes the costs of leach tanks, pumps, aeration, regrinding equipment, thickeners, filters, dryer, electrolysis equipment, their installations, and the building.

The yearly metal production is shown in Table 11.

Table 11

Yearly metal production

Metal	Concentrate treated, kg	Metal content, %	Recovery, %	d/Y	Yearly metal production, kg
Zinc	100,000	7.7	95	365	2,700,000
Copper	100,000	2.4	95	365	830,000
Cadmium	100,000	0.02	95	365	7,000

The total market value of zinc, copper, and cadmium produced is estimated at $3,460,000. The yearly expenses are as follows (in $):

Direct Costs:	
Administration	
Labour	
Reactives (oxygen, limestone, hydrated lime, magnesia, H_2SO_4, K_2HPO_4, $(NH_4)_2SO_4$, and zinc powder)	
Electricity	800,000
Indirect Costs:	
Maintenance	
Electricity	
Water	
Depreciation at 10%	
Interest at 10%	
Insurance	1,600,000
Total	2,400,000

The gain ($/kg) of metal produced is given in Table 12.

Table 12

The gain of metal produced

	Costs of metal production, $/kg		
	Zn	Cu	Cd
Costs of mining concentration (estimated)	0.11	0.14	0.39
Costs of leaching, regrinding, cementation, electrowinning	0.53	1.13	5.71
Total expenses (A)	0.64	1.27	6.10
Selling price (B)	0.76	1.62	8.36
Gain (B-A)	0.12	0.35	2.26

These estimates do not take into account any expenses for lead which make up the major part of the costs.

The projected yearly profit of leaching copper, zinc and cadmium amounts to \$630,000 (see Table 11). In other words, an investment of $\$7.3\times10^6$ produces the yearly return of revenue of about $\$6.3\times10^5$. This revenue could be further increased by selling the lead concentrate produced by the leaching process.

Gold-containing arsenopyrite concentrates. An economic analysis of leaching treatment of concentrates of this type has been reported by Livesey-Goldblatt et al. [223]. The analysis has been based on an experiment with a continuous culture grown in 20-liter vessels at temperature 30° C, pH of the medium in the range 1.7 to 1.9, and with the solid to liquid phase ratio of 1 : 8. The arsenic content of the concentrate amounted to 6.31% while the contents of gold and silver were 161.0 and 5.8 $g\cdot t^{-1}$ respectively. The economic estimates summarized

Table 13

Capital and operating costs in gold recovery

Gold extraction parameters	Treatment of 630 tons of concentrate	
	Bacterial leaching	Roasting
	(%)	(%)
Available gold	100.0	100.0
Loss of gold	8.2	8.2
Loss of gold in roasting	—	1.0
Loss of gold in cyanidation process	2.0	4.8
Gold recovery	89.8	86.0
Running expenses	(rand/t)	(rand/t)
Consumption of reagents	6.1	1.0
Power and water	4.3	1.6
Total	10.4	2.6
Capital costs	(thousand rands)	
Flotation	1410	1410
Roasting	—	2400
Bacterial leaching	1455	—
Cyanidation and smelting	2836	2786
Maintenance, etc.	2006	2229
Total	7707	8825

Note: In 1981, \$=0.9667 R. S. A. rands.

in Table 13 relate to a plant treating 650 tons of concentrate per month. As can be seen from the Table, bacterial processing leads to a net cost of production that is lower than the one of conventional roasting technique and also to a higher percenage of gold recovery.

Leaching of other raw materials. According to estimates by Derry et al. [182], the cost of 1 kg of uranium (U_3O_8) produced by tank bacterial leaching would be about $2.75. For the conventional production method it is known to be as high as $4.85.

The microbiological process of coal desulphurization is also to be preferred on economic grounds to the chemical treatment. In desulphurization of coals containing 2% sulphur, the net cost of the biochemical process for a plant with a daily capacity of 8,000 tons is $14 a ton, while that of the chemical leaching is as high as $20 a ton. Expenditures for grinding excluded, the cost of coal desulphurization was $10.5 a ton in the presence of *T. ferrooxidans* and reduced to less than $9 when thermophilic bacteria were used [224].

A comparison examination of data on the dump, underground, and tank leaching economics reveals a common pattern of expenditures required for production of different metals. In fact, the following parameters can be identified as important from the economic viewpoint: the metal content of the ore or concentrate, percentage of metal recovery, the production scale, the leaching period, the cost of mining, the cost of copper cementation, the amount of capital to be invested in installing and operating the tank leaching facility.

As shown above, the rate of a metal leaching process is closely related to the biomass density.

In dump and underground leaching of metals, as pointed out above, wild strains of microorganisms are «at work». Their content in solutions and in ores is often too low to increase markedly the rates of oxidation processes, particularly so at low temperatures.

The economic analysis of biomass production has not yet been performed. Moreover, it is not clear which of the previously discussed processes of biomass production will prove to be the most profitable. Neither has been solved the problem of combined recovery of different metals from solutions in dump and underground leaching. Extraction of accompanying metals will undoubtly affect favourably the commercial viability of the entire production process.

3. Ecological Aspects

In discussing ecological impact of hydrometallurgy, the attention is to be primarily focussed on technical characteristics of this method of metal production. Since all the flowsheets provide for closed-circuit operation, no major biospheric emissions of solutions should occur. Atmospheric emissions of noxious gases are also excluded.

In underground leaching, no large areas of land are to be used as sites for mining plants. This preserves natural landscape.

Nevertheless, each type of biological leaching process does give rise to a certain amount of particular environmentally contaminating waste materials. In hydrometallurgical industries, these are primarily solutions containing heavy metals. Typically, effluent solutions, no longer in the process, are neutralized and discharged into special ponds for settling and natural purification, upon which they are drained into rivers. Although this treatment procedure would normally provide for the removal from effluents of a major fraction of metals contained, the ultimate objective is still the removal of their entire metal content.

As pointed out above, microbiological sorption and precipitation of metals can apparently be very instrumental in solving this problem. Of equal importance is the problem of decontamination of solid waste materials from hydrometallurgical industries. According to Bruynesteyn and Hackl [225], the greatest potential danger to the environment is presented by dumps of leached ores and by rock spoil heaps.

Finally, there are a few problems in utilization and/or burying of certain waste materials, e. g. arsenic compounds (iron and calcium arsenates), produced in the tank leaching process. Not all of these problems have as yet been satisfactorily solved. Fortunately, the microorganisms important for biogeotechnology of metal production are not pathogenic and as such are no danger to the environment.

CONCLUSION

Application of microbiological and other hydrometallurgical methods for the extraction of metal values from low-grade ores introduces considerable changes into the existing practice of processing raw materials.

First and foremost, vast reserves of refractory and lost ores as well as wastes of enriching factories and composite sulphide concentrates will become eligible for processing. The bacterial leaching technology may present a solution for the utilization of refractory deposits of rich ores and large deposits in remote regions. This new method of metal extraction seems economically feasible. It ensures a higher standard of production technology, and provides for an integrated and more comprehensive utilization of mineral raw materials as compared to the classical methods of metal extraction. It also eliminates to a large extent the necessity for a large number of people working underground and the discharge of noxious gases into the atmosphere.

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