Bioleaching: metal solubilization by microorganisms

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Abstract

Bioleaching is a simple and effective technology for metal extraction from low-grade ores and mineral concentrates. Metal recovery from sulfide minerals is based on the activity of chemolithotrophic bacteria, mainly *Thiobacillus ferrooxidans* and *T. thiooxidans*, which convert insoluble metal sulfides into soluble metal sulfates. Non-sulfide ores and minerals can be treated by heterotrophic bacteria and by fungi. In these cases metal extraction is due to the production of organic acids and chelating and complexing compounds excreted into the environment. At present bioleaching is used essentially for the recovery of copper, uranium and gold, and the main techniques employed are heap, dump and in situ leaching. Tank leaching is practised for the treatment of refractory gold ores. Bioleaching has also some potential for metal recovery and detoxification of industrial waste products, sewage sludge and soil contaminated with heavy metals.

Keywords: Bioleaching; Biohydrometallurgy; Metal solubilization; Microorganisms; Bacteria; Fungi; *Thiobacillus*; *Penicillium*; *Aspergillus*

Contents

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1. Introduction

Microbial leaching methods are being increasingly applied for metal recovery from low-grade ores and concentrates that cannot be processed economically by conventional methods. As is the case with many biotechnological processes such methods may have been used since prehistoric times and probably the Greeks and Romans extracted copper from mine water more than 2000 years ago. However, it has been known only for about 50 years that bacteria are mainly responsible for the enrichment of metals in water from ore deposits and mines [1]. The solubilization process is called bioleaching and occurs in nature wherever suitable conditions are found for the growth of the ubiquitous bioleaching microorganisms.

2. Microorganisms

2.1. Thiobacillus

The bacteria most active in bioleaching belong to the genus *Thiobacillus*. These are Gram-negative, non-spore forming rods which grow under aerobic conditions. Most thiobacilli are chemolithoautotrophic species which use the carbon dioxide from the atmosphere as their carbon source for the synthesis of new cell material. The energy derives from the oxidation of reduced or partially reduced sulfur compounds, including sulfides, elemental sulfur and thiosulfate, the final oxidation product being sulfate [2,3].

Bacterial leaching is carried out in an acid environment at pH values between 1.5 and 3 at which most metal ions remain in solution. Therefore the acidophilic species *Thiobacillus ferrooxidans* and *T. thiooxidans* are of particular importance. Other thiobacilli are also able to oxidize sulfur and sulfides but they grow only at higher pH values at which metal ions do not maintain in solution.

T. thiooxidans, isolated in 1922 by Waksman and Joffe [4], is well known for its rapid oxidation of elemental sulfur. Other partially reduced sulfur compounds are also utilized and sulfuric acid is generated, decreasing the pH in the medium to 1.5 to 1 and even lower. The intensive sulfuric acid production leads to a rapid decomposition of rocks so that acid-soluble metal compounds can pass into solution as sulfates.

However, the most important role in bacterial

leaching is played by *T. ferrooxidans*. This bacterium was first isolated in 1947 by Colmer and Hinkle [1] from acid coal mine drainage. Morphologically the cells are identical to *T. thiooxidans*, but they differ from the latter by the much slower course of the oxidation of elemental sulfur. *T. ferrooxidans* differs from all other thiobacilli by the fact that besides deriving energy from the oxidation of reduced sulfur compounds ferrous iron can be used as an electron donor. In the absence of oxygen *T. ferrooxidans* is still able to grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor [5]. An excellent overview of the current knowledge of this species was provided by Leduc and Ferroni [6].

Two new species of acidophilic thiobacilli have been described by Huber and Stetter [7,8]: *T. prosperus* represents a new group of halotolerant metal-mobilizing bacteria [7], *T. cuprinus* is a facultatively chemolithoautotrophic bacterium which oxidizes metal sulfides but does not oxidize ferrous iron. This microorganism is described as preferentially mobilizing copper from chalcopyrite [8]. Because of their physiological peculiarities both strains may have some potential in bioleaching.

2.2. Leptospirillum

Leptospirillum ferrooxidans is another acidophilic obligately chemolithotrophic ferrous iron oxidizing bacterium, which was first isolated by Markosyan from mine waters in Armenia [9]. This microorganism tolerates lower pH values and higher concentrations of uranium, molybdenum and silver than *T. ferrooxidans*, but it is more sensitive to copper and unable to oxidize sulfur or sulfur compounds [10,11]. Therefore, by itself, *L. ferrooxidans* cannot attack mineral sulfides. This can only be done together with *T. ferrooxidans* or *T. thiooxidans*.

T. thiooxidans, *T. ferrooxidans* and *L. ferrooxidans* are mesophilic bacteria which grow best at temperatures of 25–35°C.

2.3. Thermophilic bacteria

Thiobacillus-like bacteria, so-called Th-bacteria, are moderately thermophilic bacteria and grow on pyrite, pentlandite and chalcopyrite at temperatures

in the range of 50°C [12]. Ferrous iron is used as the energy source, but growth is observed only in the presence of yeast extract [13]. Extremely thermophilic bacteria growing at temperatures above 60°C were isolated by Brierley, Norris, Karavaiko and their co-workers [14–16]. *Acidianus brierleyi*, formerly associated with the genus *Sulfolobus* [17], is a chemolithoautotrophic, facultatively aerobic, extremely acidophilic *Archaeon* growing on ferrous iron, elemental sulfur and metal sulfides. Under anaerobic conditions elemental sulfur is used as an electron acceptor and is reduced to H_2S. Members of the genus *Sulfolobus* are aerobic, facultatively chemolithotrophic bacteria oxidizing ferrous iron, elemental sulfur and sulfide minerals. The same compounds are used as energy source by *Sulfobacillus thermosulfidooxidans*, a spore-forming facultatively autotrophic bacterium. Growth, however, will only occur in the presence of yeast extract.

2.4. Heterotrophic microorganisms

Heterotrophic bacteria and fungi which require organic supplements for growth and energy supply may contribute to metal leaching. As in the case of manganese leaching, metal solubilization may be due to enzymatic reduction of highly oxidized metal compounds [18] or is effected by the production of organic acids (e.g., lactic acid, oxalic acid, citric acid, gluconic acid) and by compounds with at least two hydrophilic reactive groups (e.g., phenol derivatives) which are excreted into the culture medium and dissolve heavy metals by direct displacement of metal ions from the ore matrix by hydrogen ions and by the formation of soluble metal complexes and chelates [19,20]. The heterotrophic microorganisms do not have any benefit from the metal leaching. Among the bacteria, members of the genus *Bacillus* are most effective in metal solubilization, with regard to the fungi the genera *Aspergillus* and *Penicillium* are the most important ones.
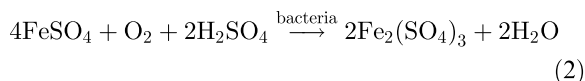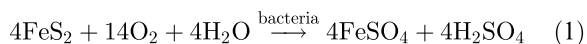
3. Bioleaching mechanisms

At the present time bioleaching processes are based more or less exclusively on the activity of *T. ferrooxidans*, *L. ferrooxidans* and *T. thiooxidans*

which convert heavily soluble metal sulfides via biochemical oxidation reactions into water-soluble metal sulfates. The most important reaction steps are summarized in a simplified form in Eqs. 1–4 given below. In principle metals can be released from sulfide minerals by direct and indirect bacterial leaching [21].

3.1. Direct bacterial leaching

In direct bacterial leaching, there is physical contact between the bacterial cell and the mineral sulfide surface, and the oxidation to sulfate takes place via several enzymatically catalyzed steps.

In this process, pyrite is oxidized to iron(III) sulfate [22] according to the following reactions:

$$4FeS_2 + 14O_2 + 4H_2O \xrightarrow{\text{bacteria}} 4FeSO_4 + 4H_2SO_4 \quad (1)$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{\text{bacteria}} 2Fe_2(SO_4)_3 + 2H_2O \quad (2)$$

The direct bacterial oxidation of pyrite is best summarized by the reaction:

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad (3)$$
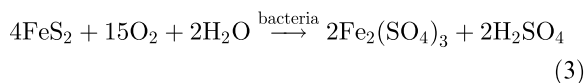
Investigations by Torma [23,36] have shown that the following non-iron metal sulfides can be oxidized by *T. ferrooxidans* in direct interaction: covellite (CuS), chalcocite (Cu$_2$S), sphalerite (ZnS), galena (PbS), molybdenite (MoS$_2$), stibnite (Sb$_2$S$_3$), cobaltite (CoS), millerite (NiS).

Therefore direct bacterial leaching can be described according to the following reaction:

$$MeS + 2O_2 \xrightarrow{\text{bacteria}} MeSO_4 \quad (4)$$

where MeS is the metal sulfide.

There is some evidence that the bacteria have to be in intimate contact with the mineral surface. The mechanism of attachment and the initiation of metal solubilization are not completely understood. Obviously the bacteria do not attach to the whole mineral surface but prefer specific sites of crystal imperfection, and metal solubilization is due to electrochemical interactions [24–26].
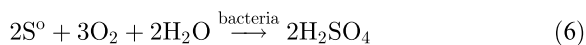
3.2. Indirect bacterial leaching

In indirect bioleaching the bacteria generate a lixiviant which chemically oxidizes the sulfide mineral. In acid solution this lixiviant is ferric iron, and metal solubilization can be described according to the following reaction:

$$MeS + Fe_2(SO_4)_3 \rightarrow MeSO_4 + 2FeSO_4 + S^o \quad (5)$$
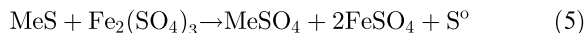
To keep enough iron in solution the chemical oxidation of metal sulfides must occur in an acid environment below pH 5.0. The ferrous iron arising in this reaction can be reoxidized to ferric iron by *T. ferrooxidans* or *L. ferrooxidans* and as such can take part in the oxidation process again. In indirect leaching the bacteria do not need to be in contact with the mineral surface. They only have a catalytic function because they accelerate the reoxidation of ferrous iron which takes place very slowly in the absence of bacteria. As shown by Lacey and Lowson [27], in the range of pH 2–3 bacterial oxidation of ferrous iron is about 10^5–10^6 times faster than the chemical oxidation of ferrous iron.

The sulfur arising simultaneously (Eq. 5) may be oxidized to sulfuric acid by *T. ferrooxidans*, but oxidation by *T. thiooxidans* which frequently occurs together with *T. ferrooxidans* is much faster.

$$2S^o + 3O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2H_2SO_4 \quad (6)$$

The role of *T. thiooxidans* in bioleaching obviously consists in creating favorable acid conditions for the growth of ferrous iron oxidizing bacteria such as *T. ferrooxidans* and *L. ferrooxidans*.

A well known example of an indirect bioleaching process is the extraction of uranium from ores, when insoluble tetravalent uranium is oxidized to the water-soluble hexavalent stage of uranium:

$$U^{IV}O_2 + Fe_2(SO_4)_3 \rightarrow U^{VI}O_2SO_4 + 2FeSO_4 \quad (7)$$
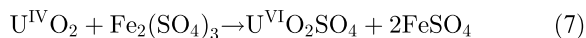
The lixiviant may be generated by *T. ferrooxidans* by the oxidation of pyrite (Eq. 3) that is very often associated with uranium ore. Besides the indirect leaching of uranium there is some evidence that *T. ferrooxidans* can oxidize U^{IV} to U^{VI} enzymatically and uses some of the energy of this reaction for the assimilation of CO_2 [28].

Altogether, in a classical way, bioleaching is based on the interaction of biological and chemical oxidation processes. Particular importance must be attributed to the cycle of ferrous and ferric iron. In nature and in technical application both mechanisms, the direct and the indirect leaching, will undoubtedly occur in concert.

However, referring to newest publications by Sand and co-workers [29–31] there is some doubt whether a direct leaching mechanism does exist at all. The authors have indications that pyrite is degraded to sulfate via thiosulfate in a cyclic mechanism. The degradation is mediated or at least initiated by the ferric iron being complexed in the exopolymeric compounds of *T. ferrooxidans* and *L. ferrooxidans*. In addition, these iron(III) ions enable the bacteria to attach to the pyrite surface by an electrochemical mechanism. The function of leaching bacteria is thought to be in maintaining a high redox potential by keeping the ferric iron in the oxidized state to optimize the indirect attack on the metal sulfide.

4. Factors influencing bioleaching

The leaching effectiveness depends largely on the efficiency of the microorganisms and on the chemical and mineralogical composition of the ore to be leached. The maximum yields of metal extraction can be achieved only when the leaching conditions correspond to the optimum growth conditions of the bacteria.

4.1. Nutrients

Microorganisms used for metal extraction from sulfide materials are chemolithoautotrophic bacteria and therefore only inorganic compounds are required for growth. In general the mineral nutrients are obtained from the environment and from the material to be leached. For optimum growth iron and sulfur compounds may be supplemented together with ammonium, phosphate and magnesium salts.

4.2. O_2 and CO_2

An adequate supply of oxygen is a prerequisite for good growth and high activity of the leaching bacteria. In the laboratory this can be achieved by aeration, stirring, or shaking. On a technical scale, particularly in the case of dump or heap leaching, sufficient supply with oxygen may cause some difficulties. Carbon dioxide is the only carbon source required, but there is no need for addition of CO_2.

4.3. pH

The adjustment of the correct pH value is a necessary condition for the growth of the leaching bacteria and is decisive for the solubilization of metals. pH values in the range of 2.0–2.5 are optimum for the bacterial oxidation of ferrous iron and sulfide. At pH values below 2.0, a considerable inhibition of *T. ferrooxidans* will occur but *T. ferrooxidans* may be adapted to even lower pH values by increasing addition of acid [32].

4.4. Temperature

The optimum temperature for ferrous iron and sulfide oxidation by *T. ferrooxidans* is between 28 and 30°C [33,34]. At lower temperatures a decrease in metal extraction will occur, but even at 4°C bacterial solubilization of copper, cobalt, nickel and zinc was observed [35]. At higher temperatures (50–80°C) thermophilic bacteria can be used for leaching purposes [12–16].

4.5. Mineral substrate

The mineralogical composition of the leaching substrate is of primary importance. At high carbonate content of the ore or gangue material the pH in the leaching liquid will increase and inhibition or complete suppression of bacterial activity occurs. Low pH values, necessary for the growth of the leaching bacteria, can be achieved by external addition of acid, but this may not only cause the formation and precipitation of gypsum but will also affect the cost of the process. The rate of leaching also depends on the total surface of the substrate. A decrease in the particle size means an increase in the total particle surface area so that higher yields of metal can be obtained without a change in the total mass of the particles. A particle size of about 42 μm is regarded as the optimum [36].

An enlargement of the total mineral surface area can be obtained also by an increase in pulp density. An increase in the pulp density may result in an increase in metal extraction but the dissolution of certain compounds which have an inhibitory or even toxic effect on the growth of leaching bacteria will increase as well.

4.6. Heavy metals

The leaching of metal sulfides is accompanied by an increase in metal concentration in the leachate. In general the leaching organisms, especially the thiobacilli, have a high tolerance to heavy metals and various strains may even tolerate 50 g/l Ni, 55 g/l Cu or 112 g/l Zn. Different strains of the some species may show completely different sensitivities to heavy metals. Very often it is possible to adapt individual strains to higher concentrations of metals or to specific substrates by gradually increasing the concentration of metals or substrates [37].

4.7. Surfactants and organic extractants

Surfactants and organic compounds used in solvent extraction generally have an inhibitory effect on the leaching bacteria, mainly because of a decrease in the surface tension and reduction of the mass transfer of oxygen [38–40]. Solvent extraction is currently preferred for the concentration and recovery of metals from pregnant solution. When bacterial leaching and solvent extraction are coupled the solvents become enriched in the aqueous phase and have to be removed before the barren solution is recirculated to the leaching operation.

5. Leaching techniques

The bioleaching of minerals is a simple and effective technology for the processing of sulfide ores and is used on a technical scale mainly for the recovery of copper and uranium. The effectiveness and economics of microbial leaching processes depend highly on the activity of the bacteria and on the chemical and mineralogical composition of the ore. Therefore, processes tested on individual types of ores cannot be transferred to other ones. Before a

technical application is possible the optimum leaching conditions have to be elaborated for each type of ore.

5.1. Laboratory investigations

5.1.1. Percolator leaching

Details of laboratory test methods are described by Bosecker [41] and Rossi [42]. The first experiments on bacterial leaching were carried out in air-lift percolators. In the simplest case, the percolator consists of a glass tube provided in its bottom part with a sieveplate and filled with ore particles (Fig. 1). The ore packing is irrigated or flooded with a nutrient inoculated with bacteria. The leach liquor trickling through the column is pumped up by compressed sterile air to the top of the column for recirculation. Simultaneously the stream of air takes care of the aeration of the system. To monitor the course

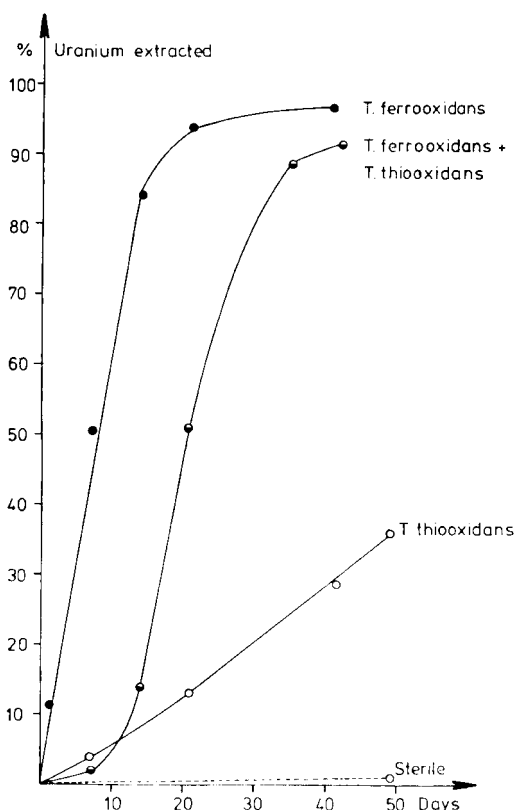Fig. 1. Leaching of low-grade copper ore in air-lift percolators.

Fig. 2. Bacterial leaching of uranium ore in shaking flasks. Extraction of uranium during leaching with *T. ferrooxidans*, *T. thiooxidans*, and a mixed culture of both strains. (Particle size < 600 µm, pulp density 5% (w/v))

of the leaching process liquid samples are taken at intervals and the state of the leaching process is determined on the basis of pH measurements, microbiological investigations and chemical analysis of the metals that have passed into solution.

5.1.2. Submerged leaching

Because the oxygen supply is often inadequate and the surface ratio unfavorable, percolator leaching is not very efficient, fairly slow and series of experiments lasting 100–300 days are not unusual. Therefore, percolator leaching has been substantially displaced by submerged leaching using fine-grained material (particle size < 100 µm) which is suspended in the leaching liquid and kept in motion by shaking or stirring. Higher rates of aeration and a more accurate monitoring and control of the various param-

eters favor the growth and the activity of the bacteria so that the reaction times are considerably shortened and the metal extraction substantially increases (Fig. 2). Suspension leaching can be carried out in Erlenmeyer flasks or, in a more sophisticated manner, in a bioreactor. Besides mechanically stirred systems an air-lift reactor has been proved suitable for the treatment of ore concentrates, industrial waste products and for the bio-desulfurization of coal [43–46].

5.1.3. Column leaching

Column leaching operates on the principle of percolator leaching and is used as a model for heap or dump leaching processes. Depending on their size, the columns may be made of glass, plastic, lined concrete, or steel (Fig. 3). Their capacities range from several kilograms to a few tons. At various distances most column systems have devices for taking samples or for installing special instruments for measuring temperature, pH, humidity, oxygen or carbon dioxide. This gives information about what has to be expected in heap or dump leaching and how the leaching conditions can be optimized.

5.2. Industrial leaching processes

Currently bioleaching is used on an industrial scale for the treatment of low-grade ores which generally contain metal concentrations below 0.5% (w/w). The simplest way of conducting microbial leaching is to pile the material in heaps, allow water to trickle through the heap and collect the seepage

Fig. 3. Column leaching facilities.

water (leachate). Since the bacterial oxidation of sulfides is much slower than other biotechnical processes the leachate is recirculated. There are three main procedures in use: dump leaching, heap leaching and underground leaching (Fig. 4).

5.2.1. Dump leaching

Dump leaching is the oldest process. The size of the dumps varies considerably and the amount of ore may be in the range of several hundred thousand tons of ore. The top of the dump is sprinkled continuously or flooded temporarily (Fig. 5). Depending on the ore the lixiviant may be water, acidified water or acid ferric sulfate solution from other leaching operations on the same mining property [26,42]. Before recirculation, the leachate may pass through an oxidation basin, in which the bacteria and ferric iron are regenerated.

5.2.2. Heap leaching

This procedure is mainly used for fine-grained ores that cannot be concentrated by flotation. The leaching is practised in large basins containing up to 12 000 tons of ore. The procedure is similar to that of dump leaching. In some heap leaching operations, pipes are placed in strategic positions within the heaps during its construction to provide the deeper portions of the heap with sufficient amounts of oxygen.

5.2.3. Underground leaching

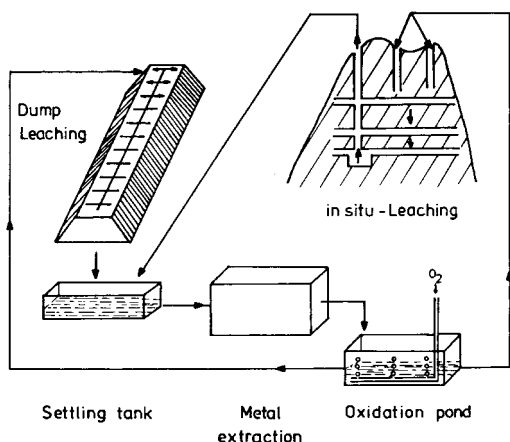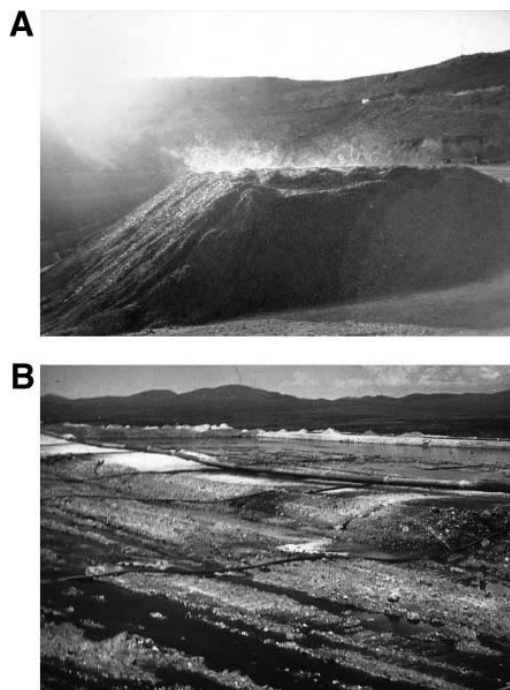Underground leaching is usually done in aban-

Fig. 5. Irrigation of leaching dumps (A) by sprinklers; (B) by flooding.

doned mines. Galleries are flooded or unmined ore or mine waste in side tunnels are sprinkled or washed under pressure. The water collects in deeper galleries and shafts and is then pumped to a processing plant at the surface. The best known application of this procedure is at the Stanrock uranium mine at Elliot Lake in Ontario, Canada [47].

Ore deposits that cannot be mined by conventional methods because they are too low-grade or because they are too small can be leached in situ. Solutions containing the appropriate bacteria are injected into boreholes in the fractured orebody. After a sufficient time for reaction, the leachate is pumped from neighboring wells or collected in drifts. The procedure requires sufficient permeability of the orebody and impermeability of the gangue rock so that any seepage of the pregnant leaching solution is prevented.

5.2.4. Tank leaching

Considering the high yields in metal extraction by submerged leaching the change from shake flasks to bioreactors was tested very early [48]. Tank leaching

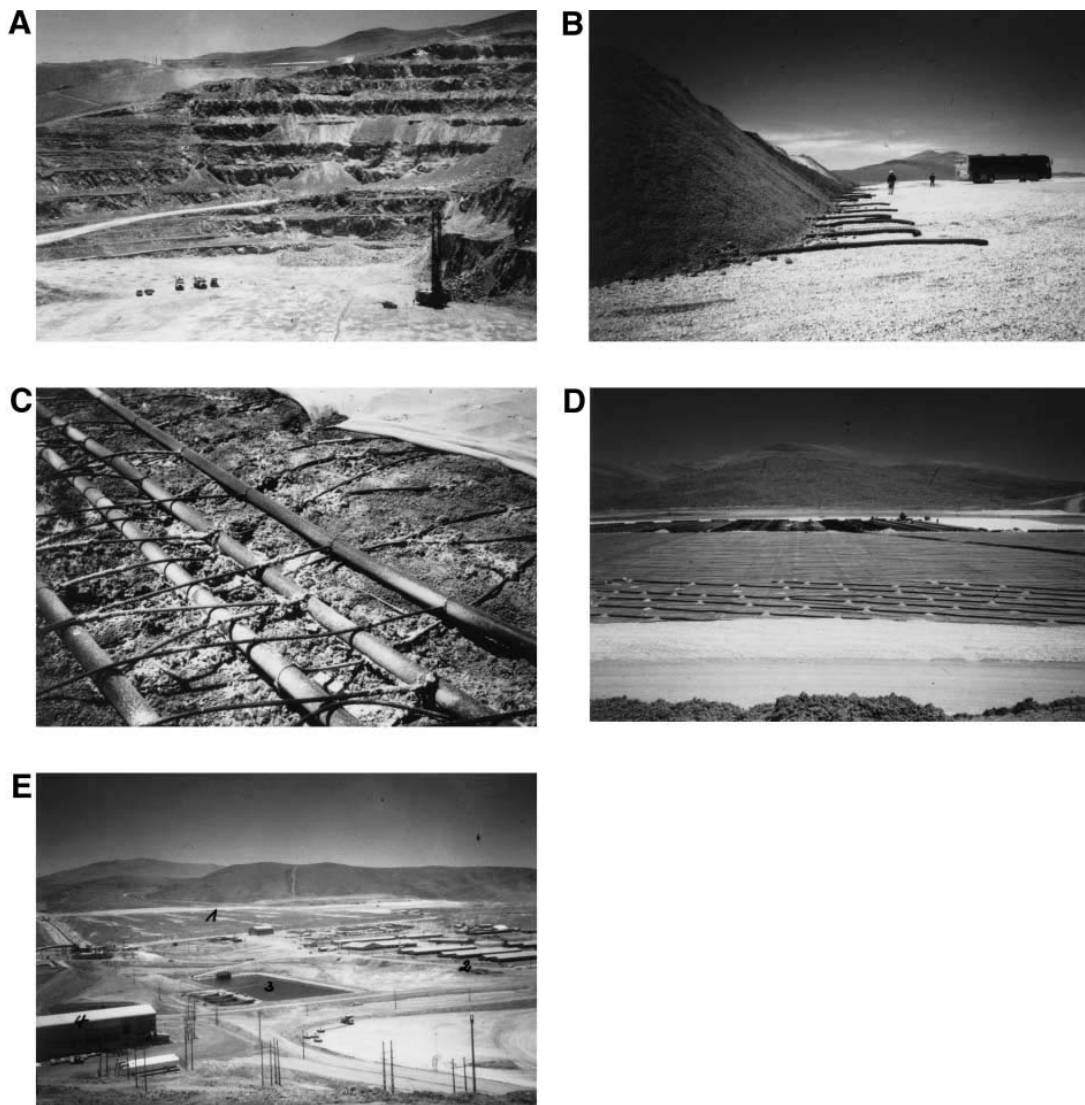Fig. 4. Flow sheet of a dump and in situ leaching process [41].

Fig. 6. Bioleaching of copper ore in Chile. A: Open-cast mining of copper ore. B: Preparation of dumps for leaching. C: Irrigation by perforated tubes. D: Dump leaching area, covered for insulation. E: Overall view of the leaching operation plant (1: dump leaching area; 2: solvent extraction for copper concentration; 3: collecting pond for the concentrated leaching solution; 4: electrowinning).

was found to be most effective for the treatment of ore concentrates and more than 80% of the total zinc was extracted from a zinc sulfide concentrate [49]. Tank leaching is more expensive to construct and to operate than dump, heap, or in situ leaching processes. But the rate of metal extraction is much higher and currently this technique is successfully used for bioleaching of refractory gold ores.

6. Industrial applications

During the last 25 years bioleaching of minerals has opened up new opportunities for extractive metallurgy and biohydrometallurgy is now practised in the copper and uranium industries, especially for the treatment of low-grade ores [50].

6.1. Copper

In the 1970s the largest plant for microbial leaching was that for dump leaching of the Kennecott Copper Corporation at Bingham, UT, USA. The contents of the dumps stored there were estimated at more than 3.6×10^9 tons and about 200 tons of copper were recovered every day by bioleaching. Torma suggested that at that time up to 25% of the U.S. copper production was recovered by bacterial leaching [51]. Meanwhile Chile is the world's major copper producing country and even at 4200 m above sea level (Quebrada Blanca) bioleaching is being operated and will produce 75 000 tons of copper annually (Fig. 6). It is expected that within the next years several industrial applications of bacterial leaching will be operated, yielding 250 000 tons of cathodic copper per year which will be equal to about 16% of the present total copper production in Chile [52].

6.2. Uranium

Commercial application of bioleaching of uranium from low-grade ores has been practised since the 1960s [53]. Best known are the in situ leaching operations in the underground uranium mines in the Elliot Lake district of Canada including the Stanrock, Milliken and Denison mines. At that time the annual production of uranium from the Stanrock Mine was about 50 000 kg U_3O_8 whereas 60 000 kg U_3O_8 was produced in the Milliken Mine after improvement of the leaching conditions. At the beginning of the 1980s a distinct drop in uranium production occurred. In 1984 Denison Mines started new activities and in 1988 90 flood leaching stopes were in various stages of operation or in preparation for flood leaching and 347 tons of uranium with a value of over US$ 25 million were produced from the leaching operation [53,54]. With the present reduction in world demand for uranium the prices are at a low level and Denison Mines have stopped production.

6.3. Gold

During the past 10 years biotreatment of refractory gold ores, which contain finely disseminated gold particles associated with sulfide minerals including arsenopyrite, pyrite and pyrrhotite, has been developed to an industrial application and several tank leaching operations are being run in South Africa, Brazil and Australia [50,54–56]. Refractory gold ores are recalcitrant to direct cyanidation processes and decomposition of the mineral sulfide matrix is required before the gold can be extracted. There are various traditional methods for the treatment of refractory ores but bioleaching was found to be a new, low-energy alternative. Without pretreatment usually less than 50% of the gold is recovered by cyanidation. After bioleaching more than 95% of the gold is extracted depending on the mineral composition of the ore and on the extent of pretreatment. The first industrial plant started at Fairview, South Africa, in 1986. The plant capacity is reported to be 300 tons/month of a pyrite concentrate containing 100–150 g Au/ton [56]. A bio-oxidation plant in Ghana, constructed during 1994, has a capacity of 720 tons of gold-bearing concentrate per day. Because the price of gold has risen many mineral companies now take a second look at deposits that were once considered uneconomical. Many of these deposits are refractory and tend to resist cyanidation. Bioleaching offers a new low-cost alternative for oxidizing these refractory ores.

7. Future aspects

At present, bioleaching is being used commercially only for the recovery of copper, uranium and gold. In the future, however, these processes will become important for zinc, nickel, cobalt and molybdenum recovery. Investment and operating costs are much lower than for conventional pyrometallurgical and hydrometallurgical processes. The processing plant can be built in the immediate vicinity of the ore deposit, saving transport costs. The procedures are not complicated and are easy to control, extensive technical knowledge is not required. This technology should be of great interest for developing countries [52].

Besides the metals recovered in the leachate, there is increasing interest in the insoluble metals left in the residues, e.g., lead. Inferior lead sulfide concentrates can be transformed into high-value concentrates by leaching of metals (e.g., zinc, cadmium,

copper) that interfere with conventional processes for the recovery of the lead [57]. Similar procedures are being investigated for the extraction of silver and other precious metals that are finely disseminated in iron, arsenic, copper and zinc sulfides. The metal sulfides are first removed by microbial leaching and the precious metals are then recovered from the residue.

7.1. Industrial waste products

The industrial application of microbial leaching is related mainly to the chemolithotrophic iron-oxidizing bacteria *T. ferrooxidans* and *L. ferrooxidans*. Mineral industrial waste products which often contain high amounts of valuable metals cannot be treated in that way because most metals, for example in fly ash and slag, are present mainly as oxides rather than as sulfides. Experiments have shown that the metal oxides in such residues can be leached by acid produced by *T. thiooxidans*. Depending on the metal compounds in the residues, vanadium, chromium, copper and zinc can be almost completely recovered [58]. In some cases, chemical leaching is easier. Bioleaching using *T. thiooxidans* is advantageous if inexpensive sulfur is available so that transportation costs for shipping the acid needed for chemical leaching can be avoided. Another advantage consists in the fact that as a consequence of the sulfuric acid production during growth of *T. thiooxidans* the pH falls only gradually so that the metals pass into solution at different rates corresponding to their solubilities and can be separated from the leaching suspension selectively.

Thiobacilli have also some potential for the detoxification of sewage sludge, soil and sediment contaminated with heavy metals and may contribute to diminishing some of our environmental problems [59–61].

7.2. Heterotrophic leaching

In the case of oxide, carbonate and silicate ores limits are set for the use of thiobacilli. For such ores, research is being done on the use of heterotrophic bacteria and fungi. In this case metals are dissolved by organic acids or complexing or chelating agents produced by the bacteria or fungi [62].

Studies on silicate nickel ores have shown that nickel is dissolved by organic acids produced by microorganisms. The most effective was citric acid. With nickel-tolerant strains of *Penicillium*, up to 80% of the nickel was extracted, depending on the mineralization [19,63]. Various other valuable metals, e.g., gold, titanium, aluminium, chromium, copper, manganese and uranium, can also be leached by heterotrophic microorganisms, however much development remains to be done.

7.2.1. Biobeneficiation
Beside the recovery of valuable metals from non-sulfide minerals, heterotrophic microorganisms can also be used for upgrading mineral raw materials by removal of impurities. Quartz sands, kaolins and clays often contain iron oxides which lower the quality of these mineral raw materials. The impurities can be removed by chemical as well as by microbiological methods, the latter being based on bacterial and fungal production of organic acids and other chelating metabolic agents. Most of the bacteria active in iron removal are related to the genera *Bacillus* and *Pseudomonas*. Among the fungi *Aspergillus* and *Penicillium* were found to be the most effective ones. Best results were achieved when oxalic acid and citric acid were the main components in the leach suspension [64].

7.2.2. Iron
Removal of iron may be practised in the presence of the microorganisms or in a two-step process as already described by Groudev et al. for the treatment of aluminosilicates [65]. Using the two-step procedure, acidification to pH 0.5 and thermal treatment at 90°C, the iron content of some sands was lowered to less than 0.012% Fe_2O_3 and the residual sand was suitable for the production of high quality glass. The iron content in kaolins was reduced from 1.49% Fe_2O_3 to 0.75% Fe_2O_3. As a consequence the whiteness of the kaolins increased. The final product was suitable for the production of high-quality porcelain and could be used in the paper industry.

7.2.3. Bauxite dressing
So-called silicate bacteria are known which can solubilize silicon from silicates and silicate bearing minerals and rocks. The 'silicate' bacteria are related

to different genera of heterotrophic bacteria and do not represent a taxonomic unit. Some of them belong to the species *Bacillus circulans*, others are related to *Bacillus mucilaginosus* [66]. Silicate dissolving bacteria have successfully been used for upgrading low-grade bauxite ores which contained silica to such an extent that conventional methods for bauxite dressing were considered not to be suitable. The removal of silicon was more effective in the presence of growing cells than with resting cells. After leaching the residue was characterized by a higher $Al_2O_3:SiO_2$ ratio and was suitable for conventional treatment (Bayer process) for recovering aluminum.

Microbial leaching of non-sulfide ores which contain no energy source for the microorganisms to grow on represents a new challenge which needs to be answered. Bioleaching of non-sulfide ores and minerals is feasible and may be used for the recovery of valuable metals from ores and minerals as well as to the benefit of mineral raw materials. However, processes which are technically feasible will not always be considered to be economically attractive, at least not at present. For example, refined sugars which are used in the laboratory as substrates for the growth of the heterotrophic microorganisms are too expensive as carbon sources for technical leaching purposes. Therefore special interest should be focused on less expensive alternatives such as organic waste products from wood processing, food and beverage industries. Disposal of these waste products causes environmental problems which are reduced if such products can be used as organic substrates in the heterotrophic leaching. The commercial application will be decided finally by the supply and demand of raw materials and their costs.

8. Conclusion

Bioleaching as far as the recovery of valuable metals is concerned is not being considered today only with respect to its ability to recover valuable metals. There is a demand for less expensive and more environmentally friendly processes. Further development is necessary with respect to both technical and biological aspects. The latter includes increasing the rate of leaching and the tolerance of the microorganisms

to heavy metals. Genetic improvement of bioleaching bacteria, whether by mutation and selection or by genetic engineering, will bring results more quickly than conventional procedures like screening and adaptation, and in the mean time, considerable progress has been made on the development of a genetic system for *T. ferrooxidans* [54,67].

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