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THE EXTRACTION OF METALS FROM ORES USING BACTERIA

D. KEITH EWART* and MARTIN N. HUGHES**

* Division of Biosphere Sciences, King's College London, London W8 7AH, England, and
** Department of Chemistry, King's College London, London WC2R 2LS, England

I. Introduction

There is currently much interest in the use of microorganisms to recover toxic or valuable metals from solution and to extract metals from low-grade or refractory ores. In both cases, the availability of this new technology depends upon the exploitation of natural processes in which microorganisms cause the deposition or the solubilization of minerals.

Microorganisms are responsible for the precipitation of a range of metal compounds out of solution, notably oxide, sulfide, and carbonate minerals. Two fundamentally different mechanisms may be envisaged for such mineral formation. The first involves the precipitation of metallic compounds by the action of anions produced by microorganisms. Thus, some metal sulfide minerals are formed by the action of sulfate-reducing bacteria, which produce sulfide and cause the precipitation of metal sulfides. The second pathway is part of the subject of biomineralization (*80*), which is currently attracting much attention. In this case the microbial cell has precise control over the type of mineral that is formed. The metallic compound is probably deposited initially on an organic matrix, leading to controlled crystallization. Examples include the rare manganese dioxide mineral todorokite, formed on the surface of Mn(II)-oxidizing bacteria (*13*), and the controlled intracellular deposition of crystals of magnetite, Fe_3O_4 (*15*), or iron sulfide minerals (*38, 79*) in magnetotactic bacteria.

Also present in certain environments are acidophilic microorganisms whose normal metabolic processes result in the oxidation and solubilization of minerals, usually metal sulfides. This process is responsible for acid runoff from disused mines, spoilage tips, and ore bodies. Such acid runoff may contain substantial amounts of metal ions such as Cu(II), which have been solubilized or "leached" out by the action of the acid. Indeed, it is probable that Roman and earlier Mediterranean civilizations obtained copper from this copper-rich acid runoff.

The subject of biohydrometallurgy has grown out of the exploitation of these two distinct processes. The appropriate biotechnology is now available for the extraction of copper and uranium and for the substantial enhancement of gold recovery from refractory, sulfidic ores. Metal ions and compounds can also be removed from process and effluent streams by living and dead microbial cells, either by surface binding or intracellular uptake. Several products are now commercially available for this purpose. This review will deal only with the use of acidophilic, autotrophic bacteria in the extraction of metals, but a number of books and reviews are available that give accounts of these applications, either for the separate topics of biosorption (*33, 77*) and metal extraction (*16, 19, 76, 117, 118, 120*), or as general reviews on microbiological methods for metal extraction and recovery (*17, 34, 35, 53, 54, 60, 64, 99, 106*).

II. Metal Extraction Using Bacteria: The Basic Principles

The bacteria utilized in metal-extraction processes are classified as autotrophic; that is, they grow in the absence of organic materials, fixing CO_2 from the atmosphere. These bacteria obtain energy from the oxidation of inorganic compounds, notably Fe(II) and reduced sulfur compounds, which may either be available in solution or in mineral form. In some cases they can fix nitrogen. Acid is formed by the action of the bacteria, which are acidophilic, with optimum growth often between pH 1 and 2. Trace metals are obtained from the minerals that are oxidized and solubilized during growth of the bacteria. The acid produced during growth leaches out soluble metallic compounds, a process that is facilitated by the breakup of the mineral structure by oxidation. These bacteria are also described as "chemolithotrophic" or "rock eating," in a graphic reference to their ability to solubilize minerals.

These bacterial reactions have been used commercially in the following three ways to extract metals from ores or concentrates:

1. The metal is extracted from the ore by the solubilizing action of the acid produced during bacterial growth. At the same time, insoluble metal sulfides are oxidized to soluble metal sulfates, either directly by the bacteria or indirectly by the action of the Fe(III) produced by bacterial oxidation of pyrite. Low-grade ores that cannot be economically extracted by conventional processes can be treated by this method, as described in Section IV. This process, which is usually described as bacterial leaching, has been carried out on an enormous scale for the extraction of copper.
2. A more recent development involves the use of chemolithotrophic bacteria to solubilize sulfidic material which interferes with the normal chemical extraction process for a particular metal. A striking example is that of gold in refractory sulfidic ores, such as pyrite or arsenopyrite, where gold recovery on cyanidation may be as low as 10–15% of the total gold present. Bacterial pretreatment of the concentrate to oxidize and solubilize the sulfide leads to enhanced recoveries of gold on cyanidation, often close to 100% of the total (Section V).
3. The growth of autotrophic bacteria on metal sulfides produces acidic solutions of Fe(III). Such solutions can effectively oxidize and solubilize metallic species. The best known example is that of uranium, where insoluble UO_2 is oxidized by Fe(III) to give UO_2^{2+}, which dissolves in the acidic solution and subsequently can be recovered chemically. The same result can be achieved using chemically produced Fe(III), but the bacterial method results in the regeneration of the Fe(III) and is economically more attractive.

All three processes have a number of advantages over conventional extraction procedures, particularly with respect to energy conservation and environmental issues.

III. The Mineral Sulfide-Oxidizing Acidophilic Bacteria

A wide variety of bacteria participate in the oxidation of mineral sulfides. These bacteria catalyze the oxidation of Fe(II) and reduced sulfur species by dioxygen. Equations (1)–(5) describe the typical reactions that occur during the bacterially catalyzed oxidation of pyrite. Reactions (1), (2), and (4) are catalyzed by bacteria, the overall catalytic effect being about 10^6. Some classes of bacteria are only able to oxidize Fe(II), whereas others only oxidize reduced sulfur species. Such organisms may be less effective in solubilizing minerals in pure cultures than are iron- and sulfur-oxidizing bacteria. However, these same organisms play an important role in commercial operations, wherein mixed cultures of bacteria are likely to be of overriding importance.

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4 \qquad (1)$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \qquad (2)$$

$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2[S] \qquad (3)$$

$$2[S] + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \qquad (4)$$

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

The bacteria used in the extraction of metals are usually discussed in terms of the temperature range over which they grow. The exothermic character of some of the chemical reactions described above implies that temperature is an important factor in assessing the suitability of an organism for use in a particular commercial operation. Bacteria are divided into three groups, mesophiles, moderate thermophiles, and extreme thermophiles, but the distinctions between these groups are not sharp. The optimal temperatures for growth in these classes are around 30, 45–50, and 70°C, respectively. It should be noted that some extreme thermophiles grow at temperatures around 100°C (*113*).

A. TYPES OF BACTERIA

1. Mesophiles

a. Thiobacillus ferrooxidans. This organism is widely associated with the oxidation of mineral sulfides at temperatures below 40°C. It was first described in the late 1940s. Other early isolates, such as

Ferrobacillus ferrooxidans and *Ferrobacillus sulfooxidans,* are all now considered to be *T. ferrooxidans.*

Thiobacillus ferrooxidans is a motile, non-spore-forming, gram-negative, rod-shaped (0.5 × 1.5 μm) obligate acidophile, occurring singly or in pairs. It may sometimes possess a polar flagellum and/or pili. *Thiobacillus ferrooxidans* obtains energy by oxidation of both Fe(II) and reduced sulfur compounds, using the energy to fix carbon dioxide by the Calvin cycle (*55*) and, if required, nitrogen (*78*). The optimum temperature for most strains of *T. ferooxidans* lies in the range 30–40°C, although this can vary with pH, and some strains grow at temperatures as low as 2°C (*1, 2*). The pH optima for various isolates usually lies in the range 1.6–2.8.

It appears that different strains of *T. ferrooxidans* show reproducible variations in behavior with respect to temperature and pH for optimal growth, although it is possible that this may reflect some incorrect classifications. Harrison (*44*) has divided 23 strains of *T. ferrooxidans* into groups on the basis of their DNA homology, one of which included all the strains able to grow at 40°C. Table I includes the G + C content of the DNA from several *Thiobacillus* species.

TABLE I

ISOLATION AND PROPERTIES OF SOME MESOPHILES AND MODERATE THERMOPHILES

Organism	Source	Temp. (°C)	GC/DNA (%)
TH1	Steam vents, mud springs	—	49.5
TH2	Copper mine waste	—	—
TH3	Copper leach dump	50	68.5
ALV	Coal spoil heap	50	56.6
BC	Washed coal pile	—	50.0
S.t.[a]	Cu/Zn/FeS_2 deposits	50	47.2
S.t.[b]	Pb/Zn ore dumps	37–42	49.3
S.t.[c]	Drainage water, sulfide ore deposits	50	45.5
T. ferrooxidans	—	30	57.5
L. ferrooxidans	—	35	51.8
T. thiooxidans	—	30	50–53
T. prosperus	—	37–40	64
T. cuprinus	—	20–50	66–69
T. acidophilus	—	—	63.5

[a] *Sulfobacillus thermosulfidooxidans.*

[b] *Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans.*

[c] *Sulfobacillus thermosulfidooxidans* subsp. *asporogenes.*

If inorganic substrates are replaced by organic substrates in the culture medium, then contamination may result from the growth of heterotrophic *Acidiphilium* species or facultatively autotrophic *Thiobacillus acidophilus* (see Section II,A,5).

b. Thiobacillus thiooxidans. The gram-negative, rod-shaped bacteria are usually 0.5 × 1–2 μm. Cells occur singly, and only rarely in pairs. They are motile by means of a single flagellum. *Thiobacillus thiooxidans* cannot catalyze the oxidation of Fe(II), but oxidizes elemental sulfur and thiosulfate to sulfate at pH values between 3 and 1.0. It has an optimum temperature for growth of about 30°C. It cannot oxidize pyrite but is able to solubilize some minerals such as zinc sulfide (*73*). It can be an important component of mixed cultures, as it can oxidize sulfur [formed chemically by Eq. (3)], which would otherwise protect the surface of the mineral from bacterial attack.

c. Leptospirillum ferrooxidans. This gram-negative, acidophilic, highly motile, obligately chemolithotrophic autotroph is readily distinguishable from other iron-oxidizing acidophilic bacteria in view of its spiral form. It cannot catalyze the oxidation of sulfur compounds, but uses pyrite as a substrate (*47*). The most commonly studied strain of *L. ferrooxidans* grows in batch culture, using Fe(II) as substrate, at about half the rate of *T. ferrooxidans*. The growth of slower growing strains of *L. ferrooxidans* is accelerated by the addition of Zn^{2+} (*100*). *Leptospirillum ferrooxidans* is less widespread than *T. ferrooxidans*, but this may reflect an inability to compete under the usual conditions of isolation.

d. Mixed Cultures. Natural habitats appropriate for the growth of acidophilic, autotrophic organisms usually contain a range of bacteria. This is a major factor in determining the stability of the natural mixed culture. However, isolation procedures usually involve the use of conditions that select for one particular organism, so that the other components are lost. Though pure cultures are required for study of the organism, it is probable that a natural mixed culture would be more effective in catalyzing oxidation of minerals. Much work has been carried out on artificial mixed cultures. In general, such a mixed culture solubilizes pyrite more effectively than do the individual pure components. A mixed culture can adapt better to changing conditions during growth: the organism best fitted for growth under the new conditions (for example, pH or temperature) will then predominate. Factors other than temperature may also dictate the nature of the dominant organism. Thus, *L. ferrooxidans* will dominate over *T. ferrooxidans* in a mixed culture growing on Fe(II), partly because of its higher affinity for the substrate (K_m = 0.25 m*M* compared to 1.34 m*M* for *T. ferrooxidans*) and its much lower sensitivity to the Fe(III) product, as well as its increased tolerance to acid. A similar result was found for growth on pyrite due to the much higher affinity of *L. ferrooxidans* for the pyrite (*98*). The presence of a toxic metal may affect the components of a mixed culture differently. Thus, *T. ferrooxidans* will predomiante over *L. ferrooxidans* in a mixed culture of these two organisms growing on chalcopyrite because it is more tolerant to Cu(II) (*98*).

The presence of heterotrophic organisms in a mixed culture may enhance or inhibit biooxidation. Reaction could be accelerated if the heterotroph fixed nitrogen, thereby supplying nitrogen nutrients, or if it removed autoinhibitory organic secretions released by the autotrophs, such as pyruvate (*32*). Heterotrophic organisms could retard the leaching process by blocking the flow of the leaching liquors or by competing for sites on the mineral surface.

2. *Moderate Thermophiles*

Moderately thermophilic bacteria, those with an optimum temperature for growth of between 45 and 50°C, have been isolated from a variety of sites, such as mud springs, coal piles, various mineral dumps, and drainage waters from sulfide ore deposits, dumps, and mines (*22*). Some examples are listed in Table I. Mixed bacterial cultures isolated from such environments have been purified by serial dilution and plating on thiosulfate or tetrathionate agar (*83*). Some moderate thermophiles are active over a wide temperature range. A mixed culture oxidized pyrite at 30°C as rapidly as *T. ferrooxidans* alone and carried out the same reactions at temperatures over 50°C (*6*). These properties are of considerable value in commercial operations (Section V).

Moderate thermophiles are heterogeneous in genotype, with the G + C content of their DNA spanning the range 47.2–68.5 mol% (Table I), showing that they must be classified in different genera. Moderately thermophilic cultures examined by Harrison (*46*) contained appreciable amounts of 4-methyl and 5-methylcytidine derivatives in their DNA, which may aid in their identification.

Table II shows that moderately thermophilic bacteria display considerable variation in size. For comparison, Table II also includes details of the mesophile *Thiobacillus ferrooxidans*. The gram-negative moderate thermophiles designated TH vary in size (1–6 μm long by 0.5–0.8 μm wide). Strain TH1 grown on Fe(II) and yeast extract has cell dimensions of about 6 by 0.8 μm, but are generally smaller when grown

TABLE II

SIZES OF MODERATELY THERMOPHILIC BACTERIA AND *Thiobacillus ferrooxidans*[a]

Organism[b]	Length (μm)	Range of length (μm)	Mean width (μm)
TH1	2.5	1.6–3.2	0.8
TH2	2.5	1.6–4.2	0.8
TH3	1.2	1.1–1.6	0.5
T. ferrooxidans	1.2	0.8–2.0	0.5

[a] Data on sizes taken from Brierley (*21*) represent the mean values of 30 cells stained with crystal violet. Cell lengths depend upon growth conditions: strain TH1 grown on Fe(II) and yeast extract is about 6 μm long.

[b] TH1, TH2, and TH3 are various thermophiles.

autotrophically on Fe(II) in the absence of yeast extract. Elongated forms have been observed when the TH strains grow on pyrite. Strain TH3 forms very long chains of cells, particularly when growing on iron (*21*).

Moderate thermophiles were originally thought to require yeast extract for growth, but repeated subculturing in autotrophic media has shown that many of these moderate thermophiles are able to grow autotrophically under high levels of CO_2, oxidizing iron or sulfur, providing that reduced sulfur was present (*83, 96*). Williams and Hoare (*125*) isolated a gram-negative, thermophilic thiobacillus that could oxidize reduced sulfur compounds, with optimum growth at pH 5.6 and 50°C.

Sulfobacillus thermosulfidooxidans, an organism isolated from several locations in eastern Europe (*59*), is nonmotile and spore forming and shows a number of morphological variations. These bacteria need a source of reduced sulfur in the growth medium, as they cannot utilize sulfate as a source of sulfur. There are similarities between *S. thermosulfidooxidans* and TH1 (*93*).

3. *Extreme Thermophiles: Sulfolobus*

Sulfolobus species are archaebacteria, distinct from other leaching bacteria, which are eubacteria. They are usually found in acidic environments with temperatures in the range 50–80°C. Archaebacteria lack muramic acid, a constituent of the eubacterial cell wall. *Sulfolobus* cells are typically small and spherical (0.7–1.0 μm in diameter). They lack true cell walls, being surrounded by a plasma membrane

(*95*). All extremely thermophilic species possess a cell envelope composed of a single S-layer. The S-layer of *Sulfolobus acidocaldarius* is composed of a glycoprotein (*66*).

The genus *Sulfolobus* was first described by Brock *et al.* (*24*). *Sulfolobus*-type cells have since been isolated from hot, acid springs and soils in locations around the world (*18, 23*). Recently *Sulfolobus*-type cells, probably *S. acidocaldarius,* have been isolated from a coal pile (Birch Coppice) which, although not the classic metal-leaching habitat, does suggest that the organism could be present in metal-rich habitats (*83*).

Sulfolobus-type thermophiles are chemolithotrophs (*90, 94*), obtaining energy from the oxidation of Fe(II), reduced sulfur compounds, and metal sulfides. On the basis of DNA composition, homology patterns of DNA-dependent RNA polymerases, and immunochemical cross-reactivity, the existence of three *Sulfolobus* species has been proposed: *S. acidocaldarius, Sulfolobus brierleyi* and *Sulfolobus solfataricus* (*127*). A fourth species, *Sulfolubus ambivalens,* can grow on sulfur either aerobically or anaerobically (*108*). During anaerobic growth, sulfur is reduced to hydrogen sulfide, a property correlated with the presence of a plasmid (*128*).

Sulfolobus can leach molybdenum from molybdenite, MoS_2 (*19, 20*). Chalcopyrite, pentlandite, pyrite, and nickel-containing pyrrhotite were also degraded during the autotrophic growth of two *Sulfolobus* strains, BC and LM (*97*). Because almost complete solubilization of chalcopyrite occurs, *Sulfolobus* could compete with *T. ferrooxidans* as the organism of choice in leaching processes, but complications arise from the fragility of the cell.

4. *Other Organisms*

Although the organisms discussed above tend to be the dominant species in leaching environments, other acidophilic chemolithotrophs, heterotrophic bacteria, fungi, yeasts, algae, and protozoa are also involved (*10*). Many thiobacilli found in acid mine waters are not *T. ferrooxidans.* These may oxidize sulfur or iron, lowering the pH of the surrounding milieu, which then enables other chemolithotrophic organisms to dominate. A motile, sulfur-oxidizing gram-negative bacterium, *Thiobacillus capsulatus,* was isolated from soil adjacent to a sulfur block pad (*67*). Another autotrophic acidophile, *Thiobacillus kabobis,* was isolated from acidic soil (*104*). Huber *et al.* (*50*) isolated some mesophilic and thermophilic bacteria from geothermal areas and ore deposits. These were later described as new species. *Thiobacillus prosperus* is a strictly chemolithoautotrophic, marine gram-negative rod. It is capable of growth on pyrite, arsenopyrite, and chalcopyrite and reduced sulphur compounds, though growth on elemental sulfur and ferrous iron is poor (*51*). The G + C content of *T. prosperus* DNA is about 64 mol% (Table I), showing it to be a new group within the genus *Thiobacillus*. Its marine origin is expressed in its ability to tolerate high levels of chloride (up to 6% NaCl w/v for one isolate), which may be useful property for commercial recovery of metals in salt-containing environments or in regions where only water with high salt content is available. *Thiobacillus cuprinus* is a facultatively chemolithoautotrophic gram-negative rod, capable of growth on sulfur, pyrite, and arsenopyrite but not ferrous iron; these cells were also capable of growth on a range of organic substrates, such as yeast extract or peptone (*52*). Again the difference from *T. ferrooxidans* is confirmed by the G + C content of the DNA (Table I).

5. *Isolation of Pure Cultures*

The study of autotrophic bacteria has been hindered by difficulties in obtaining pure cultures of the organisms. These are needed for studies on their genetics, molecular biology, and biochemistry and for the isolation of mutants that may be of commercial value (for example, in their resistance to the toxic effects of metals). Purification of microorganisms is usually achieved by isolating single colonies from a culture growing on a solid medium. Furthermore, the ability to isolate colonies is necessary for many experiments involving mutagenesis in bacteria. Growth on solid medium has been extremely hard to achieve for *T. ferrooxidans* and other iron and sulfur oxidizers. Accordingly, it is notoriously difficult to obtain pure cultures of *T. ferrooxidans*. Supposedly pure strains obtained from culture collections have been contaminated with acidophilic, facultative, or obligate heterotrophs (*57, 75*). The obligate heterotrophs are able to grow with *T. ferrooxidans* and survive alongside it in the inorganic medium of the latter organism for remarkably long times by using the debris from the *T. ferrooxidans* cells. This probably accounts for various claims that *T. ferrooxidans* is able to grow heterotrophically. Heterotrophs may be eliminated from cultures of *T. ferrooxidans* by growth in the presence of Cu(II) at concentrations that are toxic to the heterotrophs but not to *T. ferrooxidans* (*57*).

The difficulties in growing *T. ferrooxidans* on solid medium are associated with the low pH, which causes the hydrolysis of some commercial gelling agents. Furthermore, growth of bacteria on such media at pH 2.5 or less tends to produce yellow precipitates of Fe(III) compounds that mask the presence of bacterial colonies. In addition, growth of certain strains of *T. ferrooxidans* is inhibited by some of the organic compounds found in agar. Several alternative media have been proposed to overcome these problems. These have included a membrane-filter technique on Fe(II)-containing agar (*119*) and use of various modified media (*31, 58, 81, 123*); also, Harrison (*45*) has proposed a two-layer gel to avoid the deposition of mineral precipitates on the surface. Difficulties arise with these media, again usually due to their failure to allow all organisms to grow. One general approach has been to substitute very pure agarose for the agar. Das *et al.* (*31*) compared five types of agarose and found that two of these gave the most colonies. In some cases the morphology of the cells depended upon the type of agarose used. The method of Harrison appears to have some advantages, but is more time consuming. In the authors' laboratory, some success has been achieved with the method of Johnson *et al.* (*58*). A procedure for plating *Sulfolobus acidocaldarius* using Gelrite as the gelling agent has been reported (*71*).

B. Mechanism of Bacterial Attack upon Minerals

One of the great controversies in bacterial leaching is whether the microorganisms have a *direct* role or an *indirect* role in the leaching of minerals, such as the solubilization of copper as Cu(II) from copper sulfide ores. In the former case, the bacterial cells interact directly with the ore surface, causing oxidation and pitting of the mineral. It is well established that autotrophic iron oxidizers are adsorbed readily by mineral surfaces, a process that is an essential first step in the "direct" mechanism. In the indirect mechanism, attack upon the mineral involves oxidation by the acidic solutions of Fe(III) generated by the action of the bacteria of pyrite. The two processes may, of course, occur alongside each other and there may also be synergistic interaction between them.

1. *The Indirect Mechanism*

There is no doubt that bacterially generated Fe(III) plays an important role in the solubilization of a range of minerals during bacterial leaching. The reduction potential of the Fe(III) ion in acidic solution (0.67 V at pH 1.5) makes it an effective oxidizing agent for solubilizing a range of minerals. Examples include the solubilization of insoluble metal sulfides to soluble metal sulfates [Eqs. (6)–(8)], the oxidation of insoluble UO_2 to the acid-soluble U(VI) species [Eq. (9)], and the oxidation of As(III) to As(V) during the leaching of arsenopyrite. In some cases the same result could be obtained by the use of chemically generated acidic solutions of Fe(III). The chemical leaching of uranium ores by acidic solutions of Fe(III) has been carried out commercially. However, the bacterial method has the advantage that the Fe(III) is continuously regenerated.

$$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S \quad (6)$$

$$Cu_2S + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 4FeSO_4 + S \quad (7)$$

$$ZnS + Fe_2(SO_4)_3 \rightarrow ZnSO_4 + 2FeSO_4 + S \quad (8)$$

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4 \quad (9)$$

2. *The Direct Mechanism*

The existence of these chemical pathways for the oxidation of minerals during bacterial leaching does not exclude a direct role for bacteria. Both pathways may occur simultaneously, the relative importance of each depending upon the rate constants for the reactions and the concentration of Fe(III) present in solution. Experiments carried out on synthetic iron-free cobalt and nickel sulfides, using carefully washed cells of *T. ferrooxidans* to ensure the absence of Fe(III), showed consumption of oxygen and the solubilization of the metal as sulfate. Solubilization rates were appreciably increased on the addition of Fe(III). These results present support for the presence of both direct and indirect pathways (*116*). Recently, attempts have been made to compare the rates of these pathways for the oxidation of pyrite by *T. ferrooxidans* (*74*).

Bacterial attack on the mineral may also enhance reaction with Fe(III) by exposing fresh surfaces. Such rate enhancement will also be achieved through the bacterially catalyzed oxidation and removal of the elemental sulfur produced in Eqs. (6)–(8). This reaction may be mediated by Fe(III) (*25, 65*).

Evidence for direct bacterial attack upon the mineral surface comes from microscopy. The use of this technique has shown the attachment of bacteria to solid substrates, consistent with a direct role for bacteria in leaching. The adsorption of cells on suspended materials occurs within a few minutes. Detailed studies, illustrated by the following examples, have shown the attack of the bacteria to be selective with respect to the surface of the mineral, in notable contrast to attack by acidic solutions of Fe(III).

Scanning electron micrographs of biooxidized pyrite showed the formation of deep pits in crystal surfaces. The pores appear to be hexagonal in cross-section, consistent with screw dislocations in a cubic crystal lattice (*43*), and suggesting that the bacteria have attacked structurally disturbed regions of the crystal. Scanning electron micrographs of pyrite crystals, which had been exposed to the action of stationary cultures of *T. ferrooxidans* for 2 years, showed pit arrangements in pairs, clusters, and pearl-string-like chains (*11*). The distribution of bacteria appeared to be dependent on the crystal structure and on defects in the crystal.

Acidic solutions of Fe(III) and a mixed culture of thiobacilli and leptospirilli produced characteristically different pits on a pyrite/arsenopyrite flotation concentrate, suggesting direct bacterial attack on the surface. In both cases, the arsenopyrite was selectively leached (*92*). Using X-ray spectrometry and X-ray mapping on thin sections of chalcopyrite attacked by a variety of chemolithotrophic bacteria, it was shown that attachment of the bacteria was specific to exposed pyrite and chalcopyrite regions (*12*). Different corrosion patterns were seen in the presence and absence of bacteria.

Thiobacillus ferrooxidans was cultivated on synthetic pyrite crystals by Rodriguez-Leiva and Tributsch (*105*). Corrosion patterns of bacterial size, seen by scanning electron microscopy, were correlated with bacterial activity. Bacterial attack appeared to start with the secretion of an organic substance around the contact area between the cell and the sulfide energy source. This may be either part of a "pseudocapsule" that shields the contact area or may form a sulfur-adsorbing and -transporting layer. Degradation of the sulfide occurred in the contact area below the bacterial cell, leading to a corrosion pit.

Bacterial activity on synthetic pyrite films deposited on glass resulted in the formation of corrosion areas around the cells, which could be seen as halos under a light microscope (*9*). Immunofluorescence techniques showed the bacteria to be preferentially accumulated along defect sites and regions of structural imperfections in the sulfide film. Bacteria were seen to be attached to the rim of craters. Acidic solutions of Fe(III) did not form pits but interacted with the entire exposed pyrite surface.

C. Laboratory Studies on the Bacterial Leaching of Minerals

Bench-scale testing of the biooxidation of minerals is a major exercise in view of the number of parameters that have to be evaluated, including pH, temperature, nutrient and oxygen supply, particle size of the ore, and pulp density (percentage solids). It is complicated further because of the heterogeneity of the ore sample. Samples from the same ore body can display considerable variation in properties. It is also important to check the ion-exchange properties of the mineral, which may lead to a rise in pH on addition to the medium, and the possibility that toxic metals may be released on oxidation of the mineral.

1. Laboratory Reactors

Shake flasks are of greatest value for the preliminary testing of materials at low pulp densities. As the pulp density is increased, it is necessary to provide better aeration and mixing. This is often carried out by bubbling a mixture of air and CO_2 (0.5%) through the flasks. However, it is not possible to control essential variables such as pH and oxygen supply, and the main disadvantage of shake flasks is that growth conditions are changing continuously. The absence of a steady state means that it is not possible properly to test the effect of changing conditions on the growth of the organism and the oxidation of the mineral. Results obtained using shake flasks cannot be extrapolated to larger scale operations.

The air-life fermentor consists of two concentric columns. The outer column has a conical bottom section with a perforated plate acting as a gas distributor. The inner column is positioned over this plate. Compressed air, enriched with CO_2, is used to lift a suspension of mineral and bacterial culture in medium through this inner column. The suspension then falls to the reservoir and is air-lifted up the column again. The air-lift fermentor provides a good supply of oxygen and is able to keep high pulp densities (25%) of ore fully suspended in the medium. An illustrative example is provided by the work of Helle and Onken (*47*). Pachucas operate in a similar fashion to the air-lift fermentor, but there is no inner column. Air enters at the conical base of the reactor, aerates the medium, and suspends and circulates the mineral particles. Pachucas are useful reactors for building up culture stocks.

Probably the preferred batch test reactor is the stirred pot. Stirring can be controlled precisely using defined impellers. The pots are usually fitted with baffles to improve mixing and aeration. Parameters can be measured continuously and adjusted. Results obtained from the use of 1-liter pots can be related to pilot plant operations.

Continuous leaching can also be carried out on a laboratory scale, with one or more tanks (in series or in parallel) and with recycling of solids, if necessary. The difficulties that arise in long-term operation of these systems are usually due to wear on the equipment due to the action of the mineral particles, problems in maintaining complete suspension and homogeneity of the mineral, and accurate transfer of relatively small volumes of mineral suspensions from the feed tank to the reactor tanks and then to waste. Examples of the use of continuous reactors include the leaching of pyrite (*28*), zinc sulfide (*42*), and gold-bearing pyrite or arsenopyrite (*72, 89, 102, 112*).

The modeling of heap or dump leaching can be carried out in columns packed with material ground to the appropriate size. The leaching solution is allowed to percolate down the column, and then is pumped back to the top for recycling, with samples removed for various analytical operations. Alternatively, the medium can be pumped up from the bottom of the column, thus minimizing the compacting of the material. These experiments are usually of long duration, which may introduce complications. Choi and Hopkin (*29*) studied the leaching of copper ores over a 21-month period.

A variety of novel reactors have been described in the literature. These include examples wherein the role of the bacteria is the continuous regeneration of Fe(III) in acidic solution, which is then reacted separately with the sulfidic mineral. In these experiments, the bacteria may be attached to rotating films or disks, and solutions of Fe(II) sulfate are passed over them (*101*).

Classical methods for culturing *T. ferrooxidans* gives low cell yields, but cell numbers may be increased dramatically by growing the organism on Fe(II), with continuous electrochemical reduction of the Fe(III) so formed. This prevents any inhibitory effects associated with the buildup of high concentrations of Fe(III).

2. *Monitoring Growth of the Organism*

Autotrophic organisms may be grown either in a medium containing Fe(II) and a reduced sulfur compound or in a medium containing pyrite or other sulfide ores. The latter option is desirable if mineral-oxidation studies are anticipated. Growth rates on minerals are likely to be lower, as the release of Fe(II) from the mineral may be rate determining. Growth on minerals may also be restricted by the deposition of materials on the mineral surface, such as sulfur (Section III,A,1,b) or jarosites (Section III,C,3).

It is important that the growth of cultures be measured and related to the rate of mineral oxidation, and, if possible, that the numbers of cells on the mineral surface and in suspension in the medium be determined. The growth of cultures on a mineral-containing medium cannot be followed by turbidity or Coulter Counter measurements due to interference from the mineral particles. Microscopic counting has been used to evaluate bacterial numbers in the supernatant solution, but cells attached to the mineral surfaces cannot be counted nor can active and inactive cells be differentiated. The most-probable-number method has been widely used, although this is error prone, and the uptake of $^{14}CO_2$ and ^{32}P-labeled compounds has been used as a measure of bacterial activity.

Growth can be followed indirectly by monitoring the increase in concentration of the metal solubilized from the mineral, for example, iron from pyrite and copper from chalcopyrite. The formation of Fe(III) in cultures growing on Fe(II) sulfate can be measured by colorimetric and UV methods, and also from the Fe(III)/Fe(II) redox potential, when the concentration of Fe(III) can be determined from the Nernst equation. Assessment of growth can in theory also be made from the change in pH of the medium, but this method is subject to many errors.

Direct estimates of biomass have been attempted by analyzing for molecules or elements that must be of microbiological origin, such as total organic carbon, organic nitrogen, protein, DNA, or ATP. Standard methods are available for all these determinations but their application to autotrophs is complicated by the low cell counts and by interference from biooxidized material. Recently, immunological methods have received attention (*4*).

a. Protein Determination. Usually such assays are only reported for supernatant solutions. Samples are treated with alkali to precipitate Fe(II) and Fe(III) and to solubilize the protein, which is then determined by the Lowry method [via the formation of a heteropoly-(molybdenum) blue]. Bacteria on the mineral surface may be removed by treatment with detergent or by boiling in alkali prior to the determination, giving a measure of total cells (*28*). Unfortunately, the Lowry method suffers major interference from the products of biooxidation of sulfide minerals, a problem that appears to be largely unrecognized. The formation of yellow solutions on treatment of samples with alkali is due to the presence of polysulfides, an interfering species, and the presence of arsenic(III) from the biooxidation of arsenopyrite results in the reduction of the heteropoly(molybdenum) blue (*37*). Shrestha (*109*) has used an alternative assay, involving Coomassie blue, to measure total and supernatant protein.

b. Fluorescence Microscopy. Several groups of workers (*3*, *5*) have used fluorescent antibodies to determine pyrite-oxidizing microorganisms. The method of Apel *et al.* (*3*) for the determination of *T. ferrooxidans* was extended by Muyzer *et al.* (*91*) to include a DNA-fluorescence staining technique that allowed the enumeration of *T. ferrooxidans* in a population of acidophilic bacteria. A disadvantage of these methods appears to be their lack of sensitivity (e.g., 10^8 cells g^{-1}). The technique has also been modified (*5*) to distinguish respiring bacteria. Here, fluorescent antibody (FA) staining was combined with reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium (INT) chloride in the FAINT procedure for differentiating growing from nongrowing bacteria.

Epifluorescence microscopy, using certain nucleic acid dyes that fluoresce under blue light, has been used to assay *T. ferrooxidans* attached to particles and free cells. The changes in the relative amounts of free and bound cells during the leaching of pyrite can then be monitored. It was noted that the cells were green during active leaching of pyrite, but turned orange with time (*126*), an observation that may have practical value.

3. *Some Factors Affecting Bacterial Growth and Mineral Oxidation*

a. Nutrients. The normal growth medium provided for autotrophic bacteria is the 9K medium of Silverman and Lundgren (*111*), although the "weak medium" of Norris (*98*) is also commonly used. In some cases, the need for CO_2 is met by that present in the air supply, but the growth of moderate thermophiles is limited unless additional CO_2 is supplied (0.5% v/v is usually sufficient). Although some strains of *T. ferrooxidans* can fix nitrogen, ammonium is the usual nitrogen source and is limiting at concentrations less than 0.1 m*M* (*121*). Both phosphate and sulfate are essential nutrients. At higher pH values some phosphate may be lost from solution by precipitation with Fe(III), so phosphate may become growth limiting. Sulfate is required for biosynthetic and other enzymatic reactions but is commonly present at high concentrations during growth.

Requirements for bulk and trace metals are not fully characterized, although it is assumed that these would be provided by the mineral and aqueous environments of the bacteria. However, this could depend upon the speciation of the metal ions, and, in turn, the pH and the presence of potential ligands. There is little information in the literature on the enhancement of growth of chemolithotrophs by the addition of trace elements. A continuous culture of *Sulfolobus* strain BC oxidizing tetrathionate was found to be molybdenum limited (*27*), whereas replacement of laboratory water by water from a bore hole resulted in a dramatic increase in the growth rate on pyrite of a moderately thermophilic mixed culture. This effect has been attributed to trace metals (*37*). The apparent requirement of moderate thermophiles for yeast extract, once assumed to be an indicator of heterotrophic growth, may reflect the presence of trace metals in the yeast extract. Yeast extract can also serve as a source of chelating agents to complex toxic metals.

b. Toxicity. The growth of chemolithotrophic organisms may lead to the solubilization of high levels of toxic metal ions from ores, particularly if the leach solution is recycled. Acidophilic bacteria, notably *T. ferroxidans,* are fairly resistant to these toxic effects because potential uptake sites for cations on the cell surface are protonated in acidic media. Silver and molybdenum (as molybdate) appear to be particularly toxic, although molybdenum can be growth limiting at lower concentrations (*27*). Many studies on metal toxicity to acidophilic thiobacilli are available, but it is not possible to give threshold values as no account has been taken of the speciation of the metals under these conditions. It may well be that the metals form insoluble complexes with phosphate, which will reduce their toxic effects. Table III gives the concentrations of metal ions at which oxidation of Fe(II) by *T. ferroxidans* can occur (*76*). The tolerance to UO_2^{2+} is sufficient to allow uranium leaching.

In general, anionic species are more inhibitory to *T. ferrooxidans* than are cations. Toxicity associated with arsenic is of major concern, particularly in the biooxidation of gold-bearing arsenopyrite, wherein levels of soluble arsenic greater than 200 m*M* may be reached when working at 15–20% pulp density (Section V). Arsenic(III) as arsenious acid is substantially more toxic than arsenic(V) (arsenate). The effects of arsenate toxicity can be alleviated by growing the culture at high phosphate concentrations, as arsenate enters the cell by the phosphate-uptake pathway. However, bacteria adapt well to increasing concentration of arsenate (and to the toxic metals) and surprising tolerances may be achieved through selection of naturally resistant cells. Other anions are also inhibitory to *T. ferrooxidans* due to interference with phosphate or sulfate uptake and metabolism.

The application of standard genetic manipulation techniques to *T. ferrooxidans* to impart resistance to these toxic effects is hindered from a practical viewpoint by low cell densities and the difficulties associated with growing colonies on solid media (Section III,C). In particular, however, the production of genetically modified cells has not been accomplished because it has not been possible to return into the cell the DNA plasmids containing the genes that code for metal resistance

TABLE III

CONCENTRATION OF METAL AT WHICH *Thiobacillus ferrooxidans* CAN OXIDIZE Fe(II)

$[M^{n+}]$:	Hg^{2+}	Ag^+	Tl^+	UO_2^{2+}	Cr^{3+}	Zn^{2+}	Cu^{2+}	Co^{2+}	Mn^{2+}	Al^{3+}
Conc. (m*M*):	0.005	0.01	1	10	100	150	160	170	180	370

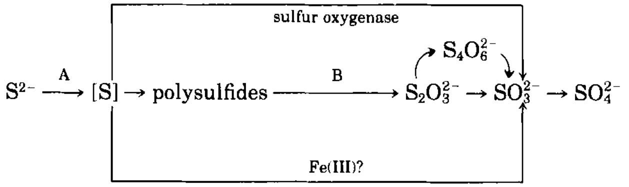
(*48, 49*). Plasmids are present in most strains of *T. ferrooxidans*, although these have not been implicated in resistance to metals, apart from resistance to UO_2^{2+} (*84, 85*). These plasmids could serve as vectors to carry genes back into *T. ferrooxidans* cells.

c. Control of pH. The pH of the leaching medium must be kept in the range that is optimum for mineral oxidation by the culture under use. The pH may be perturbed on addition of the ore, as this may consume acid. Increase of pH may cause serious problems due to deposition of insoluble precipitates onto the ore. This may necessitate regrinding of the ore to expose fresh surfaces. Ores with high levels of arsenic may cause precipitation of Fe(III) arsenate at $pH > 2$. A more general problem is the precipitation at $pH > 2.5$ of hydroxo-sulfato complexes of Fe(III) either in amorphous form or as crystalline jarosites, $M[Fe_3(SO_4)_2(OH)_6]$. For these reasons, to provide a cleaner reaction system, it is best to have the organisms growing at as low a pH as possible. Adaptation of the organism will take place over a time period if a pressure to grow at low pH is placed upon it.

D. THE MECHANISMS OF OXIDATION OF SUBSTRATES

1. The Oxidation of Sulfur Compounds

Most studies have been concerned with the mechanism of oxidation of reduced sulfur compounds in solution. The oxidation of sulfide to sulfate, an eight-electron process, must involve the formation of intermediate species (probably in two-electron steps). Sulfur(0), thiosulfate, tetrathionate, and sulfite are all likely intermediates between sulfide and sulfate, while polysulfides may also be formed. It is probable that several distinct pathways exist and these may vary with organism [Eq. (10), A = sulfide oxidase, B = siroheme]. These reactions have been reviewed by Kelly (*61–63*). Oxidation of sulfide gives sulfur atoms, which may polymerize or react further to give polysulfide species. Sulfur is accumulated in the cell wall and in the periplasm. The sulfur globules produced extracellularly by *T. ferrooxidans* consist of a hydrophobic S_8 nucleus covered by a coat of long-chain polythionates (*114*), explaining why sulfur can be transported into the cell. Several pathways for the oxidation of sulfur have been proposed. A sulfur oxygenase from *S. brierleyi* catalyzes oxidation of sulfur to sulfite (*36*), while it is claimed that for *T. ferrooxidans* and other organisms there is a sulfur–Fe(III) oxidoreductase that uses Fe(III) as an intermediate electron carrier in the oxidation of sulfur to sulfite (*115*). For *T. ferrooxidans* there is contrary evidence for the involvement of a cytochrome bc_1 electron transfer system (*30*). A further proposal is for the extracellular oxidation of sulfur by the siroheme-containing sulfite reductase. The oxidation of thiosulfate to sulfite is catalyzed by *T. versutus* without the formation of free intermediates (*63*) by a periplasmic multienzyme complex. In other thiobacilli, the oxidation of thiosulfate involves the formation of tetrathionate. The final step, the oxidation of sulfite to sulfate, can occur in thiobacilli via the adenosine phosphosulfate pathway or via a sulfite–cytochrome *c* oxidoreductase (*61–63*).



(10)

2. *The Oxidation of Fe(II)*

This topic has been reviewed by Ingledew (*55*). The major components of the respiratory chain for *T. ferrooxidans* are a cytochrome oxidase of the a_1 type, cytochromes *c*, and the blue copper protein rusticyanin. Initial electron transfer from Fe(II) to a cellular component takes place at the outer surface of the plasma membrane in the periplasmic space. The rate of electron transfer from Fe(II) to rusticyanin is too slow for rusticyanin to serve as the initial electron acceptor. Several proposals have been made for the primary site of iron oxidation. Ingledew (*56*) has suggested that the Fe(II) is oxidized by Fe(III) bound to the cell wall: the electron then moves rapidly through the polynuclear Fe(III) complex to rusticyanin or an alternative electron acceptor. Other proposals for the initial electron acceptor include a three-iron–sulfur cluster present in a membrane-bound Fe(II) oxidoreductase (*39, 88*), a 63,000 molecular weight Fe(II)-oxidizing enzyme isolated from *T. ferrooxidans* (*40*), and an acid-stable cytochrome *c* present in crude extracts of *T. ferrooxidans* (*14*).

E. PROSPECTS FOR BIOLEACHING OF BASE METALS

As discussed in Sections IV and V, industrial exploitation of chemolithotrophic bacteria has been confined to low-grade copper ores, to uranium ores, and to the enhanced recovery of gold. Nickel and cobalt seem to be promising candidates for future exploitation, as large reserves of low-grade ores are available. At least two classes of nickel sulfide ores are refractory to conventional techniques. These materials, and several cobalt sulfides, have proved amenable to oxidation with *T. ferrooxidans* (*107*). The minerals galena (PbS) and sphalerite (ZnS) often occur in association with each other. Bioleaching may be competitive with conventional processes for the extraction of Zn and Pb for low-grade ores. Other possibilities for leaching include the sulfides of antimony and gallium.

Although molybdate is toxic to *T. ferrooxidans,* the organism catalyzes the oxidation of molybdenite (MoS_2) to molybdate. *Sulfolobus* catalyzes this reaction strongly and is much more resistant to the toxic action of molybdenum (*19*).

IV. Large-Scale Commercial Applications

A. DUMP LEACHING OF COPPER ORES

This process has been used commercially for some time in several countries. About 15% of the annual production of copper in the United States is produced by bacterial leaching techniques. Low-grade ore, with less than 0.4% copper content, is dumped on a site with an impermeable floor to prevent contamination of ground water by acidic leach solutions. The Kennecott Copper Corporation dump at Bingham Canyon, Utah contains about 4×10^{12} kg of material. Aeration facilities and a spray system are provided. The process is begun by spraying acidified water over the top of the dump. The liquid slowly percolates through the dump, carrying dissolved O_2 and CO_2 and providing conditions for the proliferation of various acidophilic bacteria naturally present in the dump. Oxidation of sulfidic material results in a gradual decrease in pH, with sequential domination by organisms with decreasing pH maxima for optimal growth. Iron-oxidizing bacteria of the genus *Metallogenium,* active in the pH range 3.3–4.5, could be involved in the early stages unless acidic leaching solutions are used. The microbiology of large dumps is complex, with different populations present in various parts of the dump, which may well differ in temperature.

The bacteria oxidize and break up the copper minerals, the Cu(II) thus formed dissolving in the acidic liquors. The leach solution is collected at the bottom of the dump, at which stage it will contain about 2 g liter^{-1} of copper. The copper is extracted by treating the liquors with scrap iron (or by electrolysis) and the barren leach solution, which is an acidic solution of Fe(III), is pumped to the top of the dump for recirculation (*17*).

The leaching of large dumps is a slow process, requiring up to 20 years for complete extraction of the copper. Some of the problems associated with dump operation are obvious: the supply of O_2; overheating, with possible death of the bacteria; the liquors may fail to percolate evenly through the dump; and toxic metals may be solubilized. New dumps could be engineered more satisfactorily, but there is little incentive to build these while supplies of higher quality ores are available and commodity prices remain low. There are, however, many advantages in dump leaching: roasting of sulfide ores is avoided, the technology is simple and can be operated in remote locations, and copper can be extracted from low-grade ores without greatly increased costs. Reduced availability of better quality ores will be a major factor in encouraging dump leaching.

B. Heap Leaching

Heap leaching differs from dump leaching in scale. A heap contains about 10^8 kg of material and is usually a long, narrow structure, about 2 m high, which is well aerated from air lines laid in place before construction. The leaching process is carried out under better controlled conditions, with effective aeration and less chance of overheating. Heap leaching is usually carried out on higher quality ores or on concentrates. The time for complete extraction of the metal is reduced to months.

C. In-Place Leaching

This process is carried out underground, in disused or working mines in which a substantial proportion of the metal may remain in the walls and pillars of the mine. The mine workings are flooded selectively, left for 4–6 months, and the metal-rich acidic leachate, produced by the action of the native population of bacteria, is pumped out for recovery of the metal. Copper has been recovered successfully from a number of disused mines by this method. About 8000 tons of copper are produced annually by bacterial leaching techniques from the Miami Mine in Arizona, where conventional mining has ceased. In some cases the process has been applied to virgin ore bodies by drilling and using explosives to break up the ore body.

In-place leaching can only be applied in geologically favorable cases, that is where the rock is porous to allow the percolation of the leaching solution, and in the absence of minerals, such as carbonates, that would neutralize the acid. In-place leaching is particularly favorable in terms of energy costs and environmental issues. It is important that the flow of the leach liquor is monitored carefully.

In-Place Leaching of Uranium

The principles underlying this method have been given in Section II. The bacteria oxidize the pyrite present in the ore to give an acidic solution of Fe(III). This oxidizes UO_2 to UO_2^{2+}, which dissolves in the acidic leach solution. *Thiobacillus ferrooxidans* is able to catalyze this reaction directly, but not rapidly enough to compete with oxidation by Fe(III).

Leaching of uranium was carried out successfully in several Canadian mines in the 1960s, by spraying the stope walls with acidic mine drainage waters and recovering the uranium from the liquors. In 1964 and 1965, a total of 127,000 lb of U_3O_8 was recovered by this technique from the Milliken Mine, which had suspended conventional mining operations earlier. Currently, two Canadian uranium mines are carrying out in-place bacterial leaching of crushed ore. In one case, the success of the bacterial leaching process allowed the in-place treatment of 4,000,000 tons of ore that could not be treated by conventional technologies. Costs were lower than with the conventional process. No tailings dumps are necessary on the surface, and, because the leached ore remains underground, there are no environmental consequences. A full account has been given by McCready and Gould (*86*).

V. The Biooxidation of Refractory Gold Ores

Gold occurs in nature as the metal, and sometimes can be separated by panning techniques. The microscopic particles of gold present in oxide ores can be efficiently extracted by the well-known cyanide process prior to recovery by carbon-in-pulp treatment. However, as much as 30% of the world's gold reserves is found in refractory minerals such as pyrite, arsenopyrite, and, to a lesser extent, chalcopyrite ($FeCuS_2$) and pyrrhotite (FeS_2). In these cases, the microscopic gold particles are encapsulated by the mineral, or may occur within the pyrite lattice, so that only between 10 and 40% of the total gold present is extracted upon cyanidation. Refractory gold ores will assume greater importance in the future, as new gold deposits, at deeper levels, are increasingly likely to be sulfides rather than oxides.

A. CONVENTIONAL TREATMENT OF REFRACTORY GOLD ORES

This involves the production of a sulfide concentrate by froth flotation to remove oxides and gangue minerals. The concentrate is roasted at 600–800°C to oxidize sulfide and arsenic, if present, to sulfur dioxide and arsenious oxide. The product is cyanided to recover the gold.

These methods are increasingly regarded as unacceptable. In many countries emission of SO_2 into the atmosphere is prohibited on environmental grounds. Arsenious oxide must be trapped, in view of its toxicity. Furthermore, roasting is not always successful, as the gold particles can become reencapsulated in the silicate gangue. New technology has to be developed for the pretreatment of refractory concentrates. The possibilities currently under consideration include biooxidation, using chemolithotrophs to solubilize and remove the sulfidic material (*68*), and chemical oxidation under high pressure or temperature.

B. BIOOXIDATION AND GOLD RECOVERY: CHOICE OF ORGANISM

The biooxidation of refractory gold concentrates has been studied by several groups using stirred tank reactors and small-scale continuous systems, usually with *T. ferrooxidans*. Significant improvement in gold recovery has been achieved (*6–8, 26, 41, 69, 72, 122*).

In refractory concentrates, the gold is not usually distributed homogeneously through the mineral. It may be associated with structurally disturbed features of the mineral or may be present in more than one mineral form, each of which is oxidized at different rates by the bacteria. Therefore, there is not necessarily a linear relationship between the extent of oxidation of the mineral and the fraction of gold recovered. Frequently it is only necessary to oxidize about 70% of the mineral to recover greater than 95% of the gold. This results in considerable economic advantages, as a major part of the operating costs of biooxidation is for neutralization of the acid produced by the oxidation of sulfide. This advantage is not found for alternative methods of oxidation.

A major problem in extending laboratory-scale experiments to large pilot plants is associated with the prevalent use of *T. ferrooxidans*. This mesophile has an optimum temperature for growth of around 30–35°C. The oxidation of pyrite is exothermic, so that cooling of the reactor is often necessary to maintain the optimum temperature. This problem is particularly serious in operations in deserts, where water may be unavailable or prohibitively expensive. One solution involves the use of the extreme thermophile *Sulfolobus*. The cost of heating the reactor to about 70°C may be justified by the greater rate of reaction and the lower capital costs involved in the construction of a smaller reactor. Lawrence and Marchant (*70*) showed that the use of *Sulfolobus* could only be justified if much superior oxidation kinetics compared to *T. ferrooxidans* were achieved. In practice, at industrially acceptable pulp densities (>15%), better performance was achieved with *T. ferrooxidans*. In any case, *Sulfolobus* is not well suited for operations in a stirred tank at high pulp densities in view of its fragile cell wall.

The best solution to these problems seems to involve the use of moderately thermophilic cultures, operating at about 45–50°C. Barrett *et al.* (*6, 7*) have successfully used a moderately thermophilic mixed culture to oxidize gold-bearing pyrite and arsenopyrite. This culture shows a broad dependence of growth rate upon temperature, so that while the usual operating temperature is around 45°C, growth and oxidation of the refractory concentrate will still occur between 35 and 55°C. The culture has satisfactorily adapted to the higher end of the range (>50°C) over a period of some months of use in a pilot plant in Western Australia (*112*).

There has also been some interest in heap leaching of low-quality refractory ores. This is a two-stage process, as bacterial leaching of the ore must be followed by leaching with alkaline cyanide to solubilize the gold. The heap must also be washed and neutralized before the cyanide leach can be applied (*103*).

C. BACTERIAL OXIDATION OF REFRACTORY GOLD-BEARING CONCENTRATES

Refractory concentrates usually contain pyrite or mixed pyrite/arsenopyrite. Arsenopyrite is attacked preferentially over pyrite by chemolithotrophs, with substantially greater rates of mineral oxidation. Treatment of concentrates containing high levels of arsenopyrite will lead to the buildup of potentially toxic concentrations of arsenic compounds (usually arsenate) in the medium. However, cultures can be conditioned to withstand arsenate by successive subculturing at increasing concentrations of arsenate. Bacterial resistance to both As(III) and As(V) is governed by separate genes, although in both cases the resistance mechanism involves ATP-driven efflux of the anion (*110*). Several reports describe successful growth of cultures at concentrations of arsenate around 200 m*M* (>15 g liter^{-1} of arsenic) (*7, 89*), and there is no reason why greater tolerances will not be achieved in the long-term operation of reactors. This will allow the use of 20% pulp densities for biooxidation of many arsenopyrite concentrates. One complication at high pulp density is that oxidation of sulfide beyond sulfur is inhibited, giving decreased production of acid. As a result, careful monitoring of the pH of the reactor is necessary. Other factors that affect the efficiency of biooxidation are described in Section III,C.

1. Biooxidation of Refractory Concentrates in the Laboratory

Small-scale tests are necessary to determine the effect on gold recovery of the nature of the concentrate, aeration, stirring, particle size, pulp density, and the extent of oxidation of the mineral. Monitoring the pH and the concentrations of the Fe(II), Fe(III) (possibly from the redox potential), and arsenic species is very important in building up a picture of the biooxidation process. Deviations from this behavior may indicate the onset of complications, allowing remedial action to be taken. The ultimate objective of laboratory testing is a high percentage of gold recovery (greater than 90%) at 20% pulp density with residence times less than 6 to 7 days. The group at King's College has achieved high-percentage gold recovery for a wide range of refractory concentrates, with satisfactory residence times and pulp densities. Table IV shows gold recovery data before and after bacterial treatment of typical concentrates. Longer term continuous biooxidation of these concentrates, with improved conditioning of the culture, will give better results.

2. Refractory Concentrates with High Arsenic Levels

The first full-scale commercial plant will probably deal with an arsenopyrite concentrate, as treatment of such material by roasting is now

TABLE IV

EFFECT OF BIOOXIDATION ON GOLD RECOVERY

		Gold recovery (%) on cyanidation	
Concentrate	As (%)	Before	After bioox
Harbour Lights	14.5	48	93
Indonesian	9.3	12	100
Ghanaian	6.2	30	93
North Kalgurli	0.4	48	92
Clogau St. Davids	0	38	97
Morning Star	0	28	92
Bamboo Creek	0	15	100

difficult to justify. As described above, arsenopyrite is particularly amenable to attack by chemolithotrophs and it is possible to obtain cultures that are resistant to arsenate. A complication arises when the concentration of As(III) builds up in solution, as As(III) is more toxic than As(V). Regular analysis for As(III) by polarography during oxidation of an arsenopyrite concentrate at high pulp densities by a moderately thermophilic arsenate-conditioned mixed culture (*8*) showed that an occasional sudden deterioration in growth was associated with the presence of As(III) at around 30–40 m*M*. On one occasion, a supernatant solution from a dead culture was found to contain 90 m*M* As(III). The toxicity of As(III) is shown in longer lag phases, during which As(III) is oxidized to As(V), and in decreased growth rates. The toxicity of As(III) is probably about three to five times greater than the toxicity of arsenate (*8*). Morin and Ollivier (*89*) also report high levels of As(III) during continuous biooxidation of an arsenopyrite concentrate.

The mechanism of oxidation of arsenopyrite has not yet been clarified in terms of roles for Fe(III) and bacteria. It is known that Fe(III) can oxidize arsenopyrite with release of As(III) and As(V) (*37, 92*). It is possible that As(III) is normally released into the medium and is then oxidized rapidly to arsenate. Reduction potentials suggest that oxidation of As(III) could be carried out chemically by Fe(III): values at pH 1.5 for $\mathrm{Fe}^{\mathrm{III/II}}$ and $\mathrm{As}^{\mathrm{V/III}}$ are 0.67 and 0.47 V, respectively. Tests under the pH conditions of these experiments showed no indication that this reaction occurred. Alternatively, the bacteria could catalyze the oxidation of As(III) to As(V). This reaction is catalyzed by an acidophilic bacterium isolated by Wakao *et al.* (*124*), although it could not be used as an energy source. The mixed culture was unable to oxidize As(III) when grown on Fe(II) but apparently could achieve this in the presence of pyrite. Subsequent experiments showed that Fe(III) in the presence of pyrite could oxidize As(III), possibly because the reaction took place on the pyrite surface. The role of the bacteria in the oxidation of As(III) is twofold: attack on the pyrite results in a more reactive catalytic surface, and reoxidation of Fe(II) ensures the supply of Fe(III). Ideally, there should be a balance between the rates of formation and oxidation of As(III). High concentrations of As(III) may accumulate when this is disturbed. Contributing factors may include deposition of precipitates on the pyrite (preventing heterogeneous catalysis of the oxidation), particle size, and the relative amounts of arsenopyrite and pyrite present. Deposition of precipitates could result from an increase in pH value, which might also affect the ability of the culture to reoxidize Fe(II). It is important that the concentrations of As(III) should be monitored.

3. Continuous Pilot Plants

Several pilot plants have been successfully operated over periods of 6 months to 2 years (*26, 41, 82, 89, 112*). The equipment is usually simple and conventional (*68*). Often there are two or more tanks in parallel, followed by several tanks in series. These are usually agitated by conventional stirrers. Throughput lies between 0.5 and 2 tons per day. Bactech has operated a pilot plant at the Harbour Lights mine in Western Australia, using an arsenopyrite concentrate with arsenic content averaging 14.5% and gold content between 40–80 g ton^{-1}. The residence time is 6 days, after which about 70% of the arsenic has been oxidized, with greater than 90% recovery of the gold (*112*).

The Fairview Mine of Gencor in South Africa has operated a biooxidation plant since 1986. This accounts for about 40% of the pyrite/arsenopyrite concentrate produced in the mine, taking about 12 tons per day. Gold recovery is greater than 95% after 4 days residence time.

D. THE ECONOMICS OF BACTERIAL OXIDATION

The economics of biooxidation have been compared with those for pressure oxidation and roasting by several groups (*26, 41, 72, 87*). It is generally agreed that bacterial oxidation is the most economical pretreatment method for most ores. The capital costs for setting up the biooxidation plant are lower than those for roasting and pressure oxidation, by factors varying from 50 to 15%. Running costs are similar. However, all ores are different and the case should be examined for each one. This does not take into account the environmental factors, which may ultimately be dominant.

It is noteworthy that costs at the Bactech pilot plant include 35% for neutralization and 35% for power. Neutralization costs would vary considerably from concentrate to concentrate. Neutralization of the liquid wastes is essential for the precipitation of arsenate as the iron(III) compound, which is suitable for storage in view of its stability.

VI. Conclusions and Prospects

At the very least, biooxidation of refractory gold ores will be a valuable additional method for use with certain ore types. It is probable, however, that it will be of major significance. There is scope for the exploitation of this process in many parts of the world. It is understandable that gold producers are cautious at present. Established technology always seems safer. So, it is not to be expected that roasters will be demolished, but when it becomes necessary to build new pretreatment plants biooxidation must be taken seriously. Once a major plant is operating successfully, it is likely that owners of refractory ores will rapidly take up the process. This, in turn, will be a major impetus for research into microbiological and engineering aspects of biooxidation. The acceptance of biooxidation will be accelerated if more stringent environmental protective legislation comes into force.

The future of the leaching of base metals is less easy to predict. There seems to be no immediate prospect of major investment, but time is on the side of biotechnology. As better quality ores are used up, there must be ever greater probability that the leaching of low-grade and inaccessible ores becomes viable.

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