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ORE LEACHING BY BACTERIA ◆1777

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INTRODUCTION

The elements iron and sulfur circulate in the biosphere through specific paths from environment to organisms and back to the environment. Certain paths only involve microorganisms and it is here that biological reactions of relevance in leaching of metals from mineral ores occur (28). Of major importance are bacterial oxidations of iron, elemental sulfur, and mineral

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sulfides; these organisms have evolved an unusual mode of existence and it is known that their oxidative reactions have assisted man from times of the early Phoenicians and Romans. Natural metal leaching has been practiced in Spain, Peru, Canada, France, the United States, and other countries, without knowing the contributions of microorganisms in the processes (13, 130). Only during the last three decades have we begun to understand microbial metal dissolution phenomena.

The writers of this review were encouraged by the expanding interest in both basic and applied aspects of bacterial leaching. During the last four years, three international conferences, held on different continents, have focused on various facets of microbial ore extractions (101, 106, 115). There have also been recent reviews of the subject area which emphasize the potential future revolutionary changes bacteria could have on the mining of metallic minerals (13, 92, 107, 130, 142, 143, 145).

THEORY AND APPLICATION OF BACTERIAL LEACHING

Metals can be dissolved from insoluble minerals directly by the metabolism of microorganisms or indirectly by the products of their metabolism. The biological reactions involved in extractive metallurgy are primarily oxidations of sulfur or mineral sulfides. Many metals may be leached from the corresponding sulfides and it is this process that has been utilized in the commercial leaching operations using microorganisms. Uranium ores do not exist as sulfides; they usually occur as insoluble oxides; whether or not they are directly attacked by bacteria is still unproven. Uranium is, however, closely associated with pyrite (FeS_2) and is affected through indirect action by bacteria forming ferric sulfate:

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4. \qquad 1.$$

The uranyl sulfate can be recovered from solution by ion exchange or solvent extraction (54).

A generalized reaction is often used to express the biological oxidation of a mineral sulfide involved in leaching (130).

$$MS + 2O_2 \xrightarrow{\text{microorganism}} MSO_4 \qquad 2.$$

where M is a bivalent metal.

The leaching process is the end result of the bacteria acting upon the mineral sulfide, which serves as an energy source in the presence of other nutrients.

Direct Mechanism

Some microorganisms are capable of direct oxidative attack on mineral sulfides. Scanning electron micrographs have revealed that numerous bacteria attach themselves to the surface of sulfide minerals in solutions supplemented with nutrients (10, 11, 141). Results indicate that bacteria dissolve a sulfide surface of the crystal by means of cell contact. Lundgren & Tano (83) have discussed the nature of the cell surface of a mineral sulfide-oxidizing bacterium and have presented a model to explain iron and sulfide oxidation. Microbial cells have also been shown to attach to metal hydroxides (75).

Silverman (120) concluded that at least two roles were performed by bacteria in the solubilization of minerals. One role involved the ferric-ferrous cycle (indirect mechanism), whereas the other involved physical contact of the organism with the insoluble sulfide crystals and was independent of the ferric-ferrous cycle. Insoluble sulfide minerals can be degraded by microorganisms in the absence of ferric iron under conditions that preclude any likely involvement of a ferrous-ferric cycle (98). Present evidence from research using soluble sources of sulfide and reduced iron substrates supports the idea that iron and sulfide can be simultaneously oxidized by bacteria (8, 38, 77, 78) and that both oxidative processes contribute to metal leaching.

Although many aspects of the direct attack by bacteria on mineral sulfides remain unknown, it is apparent that specific iron and sulfide oxidizers must play an important role. Microbial involvement is influenced by the chemical nature of both the aqueous and solid crystal phases (11). Bacteria appear to attach specifically to the sulfide moiety of mineral rock surfaces, which are the regions that contain the energy supply for the bacteria. Attachment and sulfide oxidation result in pitting of the mineral surface. The extent of surface corrosion varies from crystal to crystal and is related to the orientation of the mineral (10, 11, 141). A metabolite that aids the dissolution of the mineral sulfide may also be produced (10). This substance was postulated to act either by: (*a*) oxidation of Fe^{2+}, (*b*) solubilization of molecular sulfur on the surface of crystals, or (*c*) direct attack on crystallized mineral sulfide surfaces.

Attachment of bacteria to surfaces of pyrite (FeS_2) and chalcopyrite ($CuFeS_2$) is followed by biological reactions. For pyrite, the following reactions are believed to take place:

$$FeS_2 + 3\tfrac{1}{2}O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4, \quad 3.$$

$$2FeSO_4 + \tfrac{1}{2}O_2 + H_2SO_4 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O, \quad 4.$$

$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2S, \quad 5.$$
$$2S + 3O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2H_2SO_4. \quad 6.$$

The reactions with chalcopyrite are:

$$2CuFeS_2 + 8\tfrac{1}{2}O_2 + H_2SO_4 \xrightarrow{\text{bacteria}} 2CuSO_4 + Fe_2(SO_4)_3 + H_2O, \quad 7.$$
$$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S. \quad 8.$$

The sulfur formed in Reaction 8 can be oxidized by Reaction 6.

Although the catalytic role of bacteria in these reactions is generally accepted, surface attachment is not obligatory for leaching of pyrite or chalcopyrite. The presence of sufficient numbers of bacteria in the solutions in juxtaposition to the reacting surface is adequate to indirectly support the leaching process.

Indirect Mechanism

Silverman & Ehrlich (121) have discussed the role of ferric sulfate solutions and oxygen in the oxidation of mineral sulfides. Minerals such as galena (PbS), chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), and sphalerite (ZnS) are oxidized more rapidly than pyrite and marcasite (FeS_2), whereas covellite (CuS), chalcocite (Cu_2S) and molybdenite (MoS_2) are oxidized more slowly (121, 137, 138). Ferric ion, either alone or in combination, is the most important chemical species involved in the indirect attacks on sulfide minerals (44). Reactions to explain the involvement of ferric iron are:

$$\text{(aerobic) } MeS + 2Fe^{3+} + H_2 + 2O_2 \rightarrow Me^{2+} + 2Fe^{2+} + SO_4^{=} + 2H^+, \quad 9.$$
$$\text{(anaerobic) } Fe_2(SO_4)_3 + FeS_2 \rightarrow 3FeSO_4 + 2S. \quad 10.$$

In the presence of iron-oxidizing bacteria, the ferrous iron produced by these reactions can be oxidized to ferric ion, thereby establishing a cyclic process. Thus the oxidative attack involves two steps: (*a*) the chemical interaction of ferric iron with the sulfide material (123) and (*b*) the regeneration of ferric iron by the bacteria.

There is a very slow rate of abiotic ferrous iron oxidation at low pH values, but the oxidation is greatly accelerated in the presence of iron-oxidizing bacteria; a factor of 10^5 to 10^6 times has been estimated (146). There is little doubt that under some conditions ferric iron plays a positive role in indirect mechanisms of oxidative attack on sulfide metals, and the possibility exists that, with copper-containing minerals, complex cuprous ions may play a similar role (107). Duncan & Walden (41) have shown that the addition of ferrous sulfate has no effect upon the release of copper from chalcopyrite.

MICROBIAL INVOLVEMENT

Overview

The contribution of microbiological activity to the degradation of sulfide minerals has been unequivocally demonstrated (27). The dominant organisms are those possessing the ability to oxidize iron and reduced sulfur compounds and to tolerate high metal iron concentrations and low acidity (6, 7, 37, 145, 148). To a lesser extent, the involvement of a number of heterotrophic bacteria, fungi, yeasts, algae, and protozoa are found (21, 36, 46, 89). Ralph (107) has presented a reasonable explanation of the varied microbial population associated with sulfide oxidations and has proposed a likely microbial succession in sulfide environments which is influenced by physical and chemical characteristics of the mineral components. Attention has been given to the importance of the pH of the environment; below pH 4.5, organisms that can derive energy from the oxidation of ferrous iron predominate. At pH values around 4.5, a filamentous iron-oxidizing bacterium, *Metallogenium,* can grow (153, 154). The organism's role in leaching reactions is unknown, but it may function as an important pH-succession organism in the environment. Above pH 5, where the chemical oxidation of ferrous iron is rapid and where the effectiveness of the coupling of energy for the ferrous-ferric reaction is diminished, organisms such as *Gallionella* may be extremely important (62, 63). Details of the specific actions of these heterotrophic or mixotrophic microorganisms is sparse, and a full understanding of the organisms' role in sulfide oxidation must await results of further laboratory studies utilizing novel culture procedures (107).

The principal acid-generating microorganism affiliated with mineral leaching is *Thiobacillus ferrooxidans,* which oxidizes both reduced sulfur and iron compounds in acid conditions. Spherical lobate thermophilic chemolithotrophs (7, 14, 15, 73) associated with sulfide minerals can also oxidize both reduced iron and sulfur compounds. The taxonomy of these is not yet clear. Thermophilic, iron-oxidizing thiobacillus-like bacteria have also been isolated (16, 17, 18). Norris & Kelly (104) have discussed the role of different thiobacilli in pyrite oxidation when cultured alone and in mixed culture.

Iron-Oxidizing Thiobacilli

More is known about the general physiology and metabolism of iron-oxidizing thiobacilli than any other organisms associated with metal extraction. Studies have been motivated by applied interest in commercial leaching and in the chemolithotrophic character of these bacteria. Recent reviews of the biochemistry and physiology of *T. ferrooxidans* have focused

on the organism's role in hydrometallurgical processes (13, 107, 128, 130) and its involvement in the biogeochemical processes of iron cycling (82).

The influence of microorganisms on metal leaching is physiological (e.g. as catalysts) and is a consequence of the organism's mode of metabolism. This metabolism involves the oxidation of a suitable substrate and is carried out at the expense of certain nutrients taken into the bacteria in support of growth. The energy source for *T. ferrooxidans* is reduced iron, which is stable in acid solutions. Physiologically, the following events occur: ferrous iron is oxidized with the release of electrons (20, 26, 77)

$$2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^{-}, \qquad 11.$$

the reducing potential of which is then available for the reduction of molecular oxygen

$$\tfrac{1}{2}O_2 + 2e^{-} + 2H^{+} \rightarrow H_2O. \qquad 12.$$

The pair of electrons transferred yields enough energy to insure the formation of ATP when coupled to oxidative phosphorylation:

$$ADP + P_i \rightarrow ATP. \qquad 13.$$

In the process of electron transfer there are a number of Fe^{2+}-dependent cytochrome reductions. Although *T. ferrooxidans* grows optimally at pH 2, its internal pH approaches neutrality and maintains a transmembrane pH gradient of 4.5 (30–32). Ingledew and associates (67, 68) have calculated the membrane potential to be –10 mV and the electrochemical proton activity difference to be 256 mV; this value is adequate to support the formation of ATP. Energy conservation has been shown in *T. ferrooxidans* to result from the classical chemiosmotic ATPase reaction which couples the entry of protons down the transmembrane pH gradient to the synthesis of ATP (67, 68). The following overall reactions involving iron are expressions of the cell's metabolism:

$$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O, \qquad 14.$$

$$2Fe_2(SO_4)_3 + 12H_2O \rightarrow 4Fe(OH)_3 + 6H_2SO_4. \qquad 15.$$

Ferric sulfate, the product of the oxidation, reacts with water to form ferric hydroxide and sulfuric acid. Reaction 15 is spontaneous and leads to a net increase in acid in the environment. Furthermore, the reaction is important in maintaining an electron-proton balance during the oxidation of iron. Various ferric hydroxide complexes result, as reactions occur be-

tween ferric hydroxide and sulfates. These reactions lead to the formation of jarosite, which appears as yellowish and brownish red precipitates following the oxidation of iron and is commonly referred to as "yellow-boy." The hydroxide-sulfate complexes have a buffering capacity that controls the pH of the environment, thereby affecting the reactions of iron. Some energy substrates of *T. ferrooxidans* are insoluble even at low pH values, and this makes their biochemical oxidation difficult. In these environments, the direct association of the organism with the substrate is important. Further, the localization of the appropriate oxidases in the cytoplasmic membrane provides the proper topology to allow electron transfer to occur at the cell's periphery. A rationale for the oxidation of insoluble substrates by procaryotic organisms has been offered (48).

Two other important reactions for autotrophic growth, the formation of reducing power and the fixation of CO_2, are dependent upon the energy-generating processes of metabolism. The reduction of pyridine nucleotides (NADH, NADPH) provides the organism with reducing power and is generated from the oxidation of ferrous iron. Energy is also required to move the electrons against a redox potential gradient of +.38V (cytochrome *c*) to –.34V for the NAD/NADH couple (pH 2.9).

$$NAD^+ + 2e^- + 2H^+ + 2ATP \rightarrow NADH + H^+ + 2ADP + 2Pi, \quad 16.$$

$$NADH + H^+ + NADP^+ \rightarrow NAD^+ + NADPH + H^+. \quad 17.$$

This aspect of the metabolism of *T. ferrooxidans* has received very little study.

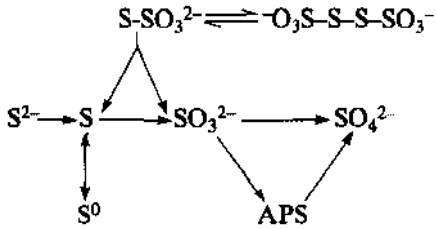
The CO_2 fixation reactions in *T. ferrooxidans* are coupled to the production of energy from Fe^{2+} oxidation. The two major reactions are controlled physiologically, and all of the organism's need for carbon can be supplied through CO_2 fixation (52, 88). The pathway for CO_2 assimilation is a reductive pentose phosphate cycle that includes two characteristic enzymatic reactions: (*a*) phosphorylation of ribulose 5-phosphate, catalyzed by phosphoribulose kinase, to yield ribulose 1,5-diphosphate, and; (*b*) ribulose-1,5 bisphosphate carboxylase, which catalyzes a reaction between one molecule of CO_2 and one molecule of ribulose 1,5-diphosphate with a concomitant dismutation to yield two molecules of 3-phosphoglyceric acid (35, 88).

Under certain growth situations, the carbon needs of the organism can be met by compounds other than CO_2. Although a number of laboratory studies have shown the adaptability of iron-grown cultures to the use of organic substrates for energy and carbon, little is known about the organism's capacity for heterotrophy and/or mixotrophy in nature. It is assumed that prior to growth on organic substrates, synthesis of new en-

zymes is necessary. Enzyme synthesis would depend upon substrate concentration, pH, temperature, and other environmental parameters (147). It has been shown that a number of enzyme systems in iron-grown cells are repressed or induced, depending upon growth conditions (125, 147). When *T. ferrooxidans* is adapted to heterotrophy, the organism can permanently lose its ability to oxidize iron, which is unusual among microbial adaptation mechanisms. The possibility exists that such bacterial physiological variations are really due to mixtures of closely associated organisms possessing these diverse characteristics that predominate when the organisms are grown under different environmental conditions. One of these was isolated, characterized, and named *Thiobacillus acidophilus* (57, 105). Different strains of *T. ferrooxidans* vary in their ability to oxidize metal sulfides (119). These variations are reflected in differences in the base ratios of the DNA of populations grown on different substrates (117).

Like the biochemistry of iron oxidation, the biochemistry and physiology of inorganic sulfur oxidation by thiobacilli is well characterized. Silver (117) and Ralph (107) have summarized the relevance of sulfur oxidations to metal leaching. The various reduced sulfur compounds studied include soluble compounds such as S^{2-}, H_2S, $S_2O_3^{2-}$, SO_3^{2-}, $S_4O_6^{2-}$, $n(S\text{-}S)^{2-}$. Insoluble sulfur compounds include elemental sulfur and many sulfide minerals (113).

A general scheme relating most of the known oxidative reactions of sulfur compounds by thiobacilli is:



When sulfide or sulfur, common substrates in sulfide mineral leachings, is the substrate for oxidation, it enters the central pathway. Sulfide oxidation proceeds in two stages; in the first stage, sulfide loses two electrons mediated by a sulfide oxidase, and polymerization of the resulting sulfur atoms occurs. The oxidation of the short-chain polysulfide to polymeric sulfur compounds then follows (99). The polysulfides are thought to be membrane associated. Membrane fractions prepared from acidophilic sulfur-grown *T. ferrooxidans* oxidize soluble sulfide (126). Sulfur is oxidized to sulfite, which in turn can be oxidized by one of two pathways.

Although most of the attention of microbial leaching has centered upon the oxidation of iron and sulfur, it is important to remember that successful leaching depends as well upon other aspects of growth of the appropriate organisms. Besides an energy source and carbon (CO_2), *T. ferrooxidans* requires nitrogen, phosphate, sulfate, and trace metals; the general nutrition has been reviewed (13, 47, 130, 149).

LEACHING METHODS

Research Applications

The object of small-scale laboratory investigations is to determine the extent and rate of the alteration of the minerals in these ores. To this end, the laboratory investigations should be geared initially towards the optimum leaching conditions and subsequently towards simulating methods for proposed commercial implementation. Early laboratory studies used stationary vessels of crushed ore and liquid media containing bacteria (109), or ore-filled columns with or without solution percolation (24, 51, 90); neither method provided sufficient oxygen transfer. Respirometer vessels were also used which, although providing sufficient aeration, were limited by the extremely small sample volume (5). Vessels agitated either by stirring (40) or by shaking (43) provided superior aeration. Larger scale experiments could also be conducted in tanks and fermentors (29, 95) in which the leaching parameters could be controlled (59).

Extraction in columns, either with or without circulation of the leaching medium, simulates heap and dump leaching. Although percolation results in better transfer of the dissolved nutrients to the bacteria and facilitates the removal of solubilized products and toxic waste materials, percolation still might not fulfill the high oxygen requirements of the bacteria. Dual stage column percolation experiments have been performed in which the column effluents were treated to remove metabolic products and to regenerate a suitable leaching solution, as well as the bacteria suspended therein (34, 91), thereby giving better yields.

Commercial Applications

The predominant commercial methods used in bacterial extraction of ores are dump and heap leaching (56, 116). In dump leaching, very large quantities of low-grade, run-of-the-mine ores or waste rock are deposited on impermeable ground, usually in valleys. Leach solutions are introduced either naturally or more often by spraying, flooding, or injecting through vertical pipes. The fluid percolates to the bottom where the pregnant solution is collected for concentration of the metal values. Heap leaching is

similar but smaller in scale, using finer and more concentrated ore deposited in mounds on prepared drainage pads. Heap leaching is also used for the extraction of metal values from the swell of underground mines. Metal values are also extracted from the stopes of underground mines.

Leaching solutions are brought in contact with the broken ore by percolation (54), alternate flooding and dewatering (152), spraying and hosing (86, 87, 116), or by the injection of leach solutions (152). The pregnant liquors are then recovered either from the sumps or at recovery wells. This method is applicable not only in new mines but also in abandoned operations in which up to 30% of the metal values may remain underground in the walls and pillars.

Tank and vat leaching can be done on ores of sufficiently high grade and values to justify the higher capital costs incurred in this procedure. Although currently not practiced in the extraction of metals, procedures have been proposed (59, 64, 95, 96, 132).

Parameters Affecting Bacterial Leaching

Like all other life processes, the leaching of ores by microbes is influenced by environmental factors. These will be dealt with individually.

TEMPERATURE All processes based on the transformation of minerals by microorganisms are confined to a limited range of temperatures, despite the fact that growth of various bacteria has been observed between −18 and 105°C (151). Increasing temperature results not only in the usual augmentation in the chemical reaction rate but also, within limits, in faster microbial metabolism. Although the lower temperature limits of the metabolic activity of the iron-oxidizing bacteria have not as yet been established, active bacterial iron oxidation in soils and in mines has been noted at about 10°C, and the lower limit of this activity is generally accepted to be the freezing point of water. Optimal leaching of metal sulfide ores and oxidation of ferrous iron by these bacteria has been determined to occur between 25 and 45°C (29, 93); 55°C is the limit of biological oxidation, and only chemical oxidation occurs above this temperature. The temperature coefficients (Q_{10}) and the energies of activation (E_a) have been determined, between 23 and 32°C, to be around 2 and to be between 11.7 and 16.3 kcal/mol respectively for ZnS (140), $FeSO_4$ (60, 81), Cu_2S, and CuS (112); the energies of inactivation (E_i) for these substrates, between 40 and 45°C, are between 53.3 and 61.5 kcal/mol respectively (60, 112, 140). Thermophilic iron-oxidizing bacteria have also been isolated from sulfide mineral-bearing environments that might represent a natural succession to *T. ferrooxidans,* as localized temperatures increase because of the exothermic oxidation of pyrite (6). Both rod-shaped and lobate spherical iron and sulfur-oxidizing bacteria have

been isolated from these and other sources that possess temperature minima of 30 to 55°C and maxima of 50°C to more than 90°C (17).

pH *T. ferrooxidans* is active in the pH range of 1.5–5 (120) with a maximum of about 6 (90) and a minimum of about 1 without adaptation of the culture (145). Optimum pH is between 1.0 and 2.5 for the oxidation of ferrous iron (60, 85), zinc sulfide (140), cobalt sulfide (129), chalcopyrite (90, 110, 111), chalcocite (112), covellite (66, 112), galena (76, 127), and pyrite (2). *T. ferrooxidans* has been adapted to grow at lower pH values (71, 146) at which the formation of basic ferric sulfate jarosite is decreased.

Spherical thermophilic bacteria of the *Sulfolobus* type have been shown to be active between pH values of 1 and 6 (19) with pH optima between 2 and 3 (20, 33) when they use elemental and reduced sulfur and iron compounds as substrates. A number of thermophilic thiobacilli have also been isolated with various pH optima between 2 and 9 (80) when the substrates are ferrous iron, sulfide minerals, elemental sulfur, and reduced sulfur compounds.

OXIDATION-REDUCTION POTENTIAL The oxidation-reduction potential (*Eh*), is a measure of the tendency of a substance to accept or donate electrons and is used to measure the oxidizing ability of a given environment. The indirect oxidation that results in solubilization of minerals by ferric iron is highly dependent upon this factor (44). During the oxidation of ferrous iron by *T. ferrooxidans,* the oxidation-reduction potential might be expected to increase toward that of the ferric-ferrous system, which is +747 mV at 25°C. In reality, this value is rarely approached, except at very low iron concentrations or at high acidity, because oxidized iron is precipitated from the medium as jarosite. *Eh* values between +190 and +550 mV have been measured during this oxidation (58). Similarly, the *Eh* has been observed between +220 and +515 mV (102, 111, 112) and between +340 and +540 mV (25, 54, 55, 95) during the oxidation of metal sulfides and the extraction of uranium, respectively, by the iron-oxidizing thiobacilli.

COMPOSITION OF THE LEACHING MEDIUM Growth media for the iron-oxidizing thiobacilli have been described (13, 23, 79, 122, 130, 149); these media contain excess concentrations of all the required nutritional salts, including ammonia, sulfate, potassium, phosphate, magnesium, and calcium. Other trace element requirements are usually fulfilled by impurities in the constituents of the media. The energy source, ferrous sulfate, may be replaced by minerals containing iron or sulfur, and the carbon source is supplied in the air as carbon dioxide. Although ferric iron has been suggested to serve as an oxidant (21), oxygen is generally regarded as the only

agent to fulfill this role. These growth media have been widely used in laboratory studies, but they are obviously too expensive for use in large scale mining operations. From an economic standpoint, it is desirable to identify those nutrients needed to be added to the leaching solution to form a practical leaching medium. The organism's requirement for sulfur and acidic environment is fulfilled by sulfate, a product or byproduct of the oxidation of sulfide minerals and ferrous iron respectively. Potassium, calcium, and magnesium are also present in operational leaching media in excess of the minimal requirements needed for growth. Phosphate and ammonia are the only medium constituents not ubiquitous to the leaching solutions. However, phosphate is supplied whenever the mineral apatite is present in the ores, and ammonia may be residual from blasting or milling operations. Nutritional studies have shown that the concentrations of these latter two components affect the rate and yield of zinc extractions (140, 148).

The iron-oxidizing thiobacilli are strictly aerobic, and limitations in oxygen availability affect the rates of ferrous iron oxidation (60) and metal extraction (59). Optimization of aeration has been accomplished by increasing the mass transfer of oxygen (59, 60). The volumetric oxygen transfer coefficient (K_{1a}) decreases from 200 h^{-1} in sterile conditions to 46 h^{-1} during the growth of *T. ferrooxidans* at the expense of ferrous iron as substrate (59, 127).

The concentration of carbon dioxide affects leaching; when the levels were increased, ferrous iron oxidation (9, 100, 114) and metal extraction from sulfide minerals (22, 140) were stimulated. Conversely, when carbon dioxide was removed, the rate of pyrite oxidation decreased (24).

Ferric iron is not only the product of the oxidation of ferrous iron and iron-containing sulfide minerals, but it is also a very effective oxidant of both sulfide and oxide minerals (44). Extraction of copper from covellite and chalcocite is stimulated by concentrations of 10^{-4} to 10^{-2}M iron (66, 112). Thus, both the direct and indirect mechanisms can occur simultaneously. Excess ferric sulfate precipitates cover the ore surfaces, thus impeding bacterial and chemical oxidation (60, 69, 93, 116). Regrinding of the leach residue allows the resumption of leaching on the freshly exposed surfaces (96, 111, 129).

Ferrous iron and metal sulfide oxidations by iron-oxidizing thiobacilli are remarkably insensitive to metal ion concentrations. These bacteria can tolerate aluminum (0.37M), zinc (0.15M), cobalt (0.17M), manganese (0.18M), copper (0.16M), chromium (0.1M), and uranium (0.01M) (13, 145, unpublished results). Tolerances are lower to silver (10^{-9}M to 10^{-5}M), mercury (0.05M), and molybdenum (0.03M), and the oxides of selenium,

tellurium, and arsenic are inhibitory (13). High concentrations of both ferrous and ferric ions cause a kinetically complex inhibition of ferrous iron oxidation (72).

Although low concentrations of surfactants accelerate the oxidation of copper sulfide minerals (22, 39), higher concentrations are inhibitory (49, 134, 150) because they reduce surface tension (134) and decrease the mass transfer of oxygen (1). Flotation and solvent extraction reagents are also inhibitory (136, 144), as are various sugars (118) and keto acids (12).

PARTICLE SIZE AND SUBSTRATE CONCENTRATION Greater yields and extraction rates of metals from ores occur when the specific surface area of the ore is increased by reducing particle size (130). Size reduction increases the availability of substrate, and in practice, the degree of lixiviation is directly proportional to the surface area of the particles. Decreasing particle size of low grade ores below a critical dimension can, however, increase the surface area of the gangue relative to the substrate and is thus equivalent to the dilution of the substrate (49). The concentration of solid substrate is expressed as the pulp density or as the solid : liquid ratio. Increasing the mass results in more available substrate and thus tends to increase the absolute quantity of metal values solubilized. Extraction rates may decrease, however, when the solid : liquid ratio is high because of the interference of the solids with the mass transfer of nutrients, especially oxygen and carbon dioxide, to the organisms (130).

MICROBIAL METAL EXTRACTIONS

As stated previously, a number of minerals may be oxidized directly by bacteria or indirectly by ferric ions. Both mechanisms usually operate simultaneously in nature and in commercial metal extractions.

Copper

The most commonly investigated minerals of copper are chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), and covellite (CuS). All can be leached by both direct bacterial attack and/or by ferric iron as the oxidant. For the iron-containing mineral chalcopyrite, both mechanisms occur simultaneously and are difficult to separate. The following sequence of reactions describes chalcopyrite oxidation in the presence of bacteria.

$$48\ CuFeS_2 + 204\ O_2 + 24\ H_2SO_4 \rightarrow 48\ CuSO_4 + 24\ Fe_2(SO_4)_3 + 24\ H_2O, \quad 18.$$

$$12\ CuFeS_2 + 24\ Fe_2(SO_4)_3 \rightarrow 12\ CuSO_4 + 60\ FeSO_4 + 24\ S^0, \quad 19.$$

$$60\ FeSO_4 + 30\ H_2SO_4 + 15\ O_2 \rightarrow 30\ Fe_2(SO_4)_3 + 30\ H_2O, \quad 20.$$

$$24\ S^0 + 24\ H_2O + 36\ O_2 \rightarrow 24\ H_2SO_4, \quad 21.$$

$$30\ Fe_2(SO_4)_3 + 120\ H_2O \rightarrow 20\{H[Fe(SO_4)_2 \cdot 2\ Fe(OH)_3]\} + 50\ H_2SO_4, \quad 22.$$

$$60\ CuFeS_2 + 255\ O_2 + 90\ H_2O \rightarrow 60\ CuSO_4 + 20\{H[Fe(SO_4)_2 \cdot 2\ Fe(OH)_3]\} + 20\ H_2SO_4. \quad 23.$$

It is doubtful that the initial attack on this mineral is through dissolution, owing to its very low solubility (61). *T. ferrooxidans* has been reported to oxidized monovalent copper, as well as iron and sulfur (53, 103). Iron and copper exist in chalcopyrite in both oxidized and reduced valencies (155); these and the sulfide moiety are readily oxidized either by bacteria directly (Reaction 18), by ferric iron initially present (Reaction 19), or by ferric sulfate formed by bacterial oxidation (Reaction 20). The resulting ferrous iron and elemental sulfur can subsequently be oxidized by bacteria (77, 78) as shown in Reactions 20 and 21. During the initial phase of oxidation (Reactions 18–20), sulfate is consumed and the pH tends to rise, causing the precipitation of ferric sulfate and basic sulfate salts of copper such as antlerite (119). Ferric sulfate is hydrolyzed in a slow reaction (69) to secondary iron minerals, such as jarosite, with the regeneration of sulfate (Reaction 15).

In early studies of chalcopyrite leaching in single-stage laboratory columns (24), the yield of solubilized copper was generally disappointing. Typical recoveries from this process were 25% in 60 days (108), 40% in 70 days, and 60% in 470 days (90). Razzell & Trussell (109, 110) recovered 45% of the copper from chalcopyrite using a stationary culture test. These yields were improved using agitation in reaction tanks; recoveries from different experiments ranged from 35% in 33 days (40), 72% in 12 days and 100% in 26 days (39), 59% in 5 days (42), 60% in 4 days and 79% in 6 days (137) and 55% in 12 days (111). Bruynesteyn and coworkers (22) recovered 50–60% of the copper from chalcopyrite in 4–6 days using an aerated tank. Yields could be improved to 80–96% by regrinding and leaching the residues (22, 111).

Other copper sulfide minerals can be leached by *T. ferrooxidans*. Chalcocite is oxidized to copper sulfate with digenite (Cu_9S_5) and covellite (CuS) as intermediates:

$$10\ Cu_2S + 2\ H_2SO_4 + O_2 \rightarrow 2\ Cu_9S_5 + 2\ CuSO_4 + 2\ H_2O, \quad 24.$$

$$2\ Cu_9S_5 + 8\ H_2SO_4 + 4\ O_2 \rightarrow 10\ CuS + 8\ CuSO_4 + 8\ H_2O, \quad 25.$$

$$10\ CuS + 20\ O_2 \rightarrow 10\ CuSO_4, \quad 26.$$

Both digenite and covellite are oxidized by *T. ferrooxidans* to $CuSO_4$ (103, 112). As sulfate is consumed, the pH tends to rise with the precipitation of antlerite and, if not adjusted, will reach values detrimental to bacterial oxidation (119).

Bacterial oxidation on these minerals was first investigated by Bryner and coworkers (24) and later by others (66, 103). They found that copper was solubilized from chalcocite and covellite, both in the absence and presence of iron; the optimum concentration of iron was 4–20 mM (66, 112). Ferric iron can oxidize these minerals to $CuSO_4$ according to the following reactions:

$$Cu_2S + 2\ Fe_2(SO_4)_3 \rightarrow 2\ CuSO_4 + 4\ FeSO_4 + S^0, \quad 28.$$

$$CuS + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2\ FeSO_4 + S^0, \quad 29.$$

$$Cu_9S_5 + 9\ Fe_2(SO_4)_3 \rightarrow 9\ CuSO_4 + 18\ FeSO_4 + 5\ S^0. \quad 30.$$

Generally, less than complete solubilization of the copper from these minerals occurs, which is due to the precipitation of secondary minerals, principally jarosite; the latter deposits on the surface of the mineral particles, blocking the oxidative action of both the bacteria and ferric iron.

Copper sulfides occur naturally, almost always in the presence of iron sulfide minerals, such as pyrite and pyrrhotite, which can also be oxidized by *T. ferrooxidans* and ferric iron (see Reactions 3, 4, 5). Thus, the leaching of sulfide minerals that do not contain iron can be affected either negatively or positively by iron.

Copper selenide (CuSe) is oxidized by *T. ferrooxidans* (135); copper sulfate and elemental selenium are formed according to the reaction

$$2\ CuSe + 2\ H_2SO_4 + O_2 \rightarrow 2\ CuSO_4 + 2\ Se^0 + 2\ H_2O. \quad 31.$$

Uranium

The standard free energy change (20.6 kcal/mol) and the oxidation-reduction potential for the tetravalent-hexavalent uranium system (446 mV) are similar to those of the ferrous-ferric system (17.8 kcal/mol and 747 mV respectively). Uranium oxidation proceeds more rapidly in the presence of iron-oxidizing thiobacilli than in the presence of ferric iron alone (59, 65), suggesting that a supplementary oxidation of uranium occurs which is catalyzed by bacteria. Recent calorimetric (124) and respirometric (70) investigations support the thesis that tretravalent uranium is oxidized by *T. ferrooxidans* according to the reaction:

$$2\ UO_2 + O_2 + 2\ H_2SO_4 \rightarrow 2\ UO_2SO_4 + 2\ H_2O. \quad 32.$$

Insoluble tetravalent uranium is also oxidized to the soluble hexavalent state by ferric sulfate, as stated for Reaction 1.

During the 1960s, uranium extraction mediated by the iron-oxidizing thiobacilli had been investigated in mining operations in Canada (50, 86, 87) and the United States (53) and in laboratory experiments (3, 4, 54, 64, 65, 94, 95, 97). The ferric iron required for the oxidation of the tetravalent uranium is supplied through the biological oxidation of either soluble ferrous iron or iron-containing sulfide minerals present in the uranium ore (4, 58, 64). The applicability of leaching uranium using iron-oxidizing bacteria and the influence of various parameters have been investigated. Using percolation columns, recoveries of uranium up to 90% were achieved from finely ground low-grade ores, i.e. 85% of the uranium was recovered from an ore with particle size of 6.3 mm in 20 weeks (3, 4), 80–90% from an ore with a particle size of 4 mm in 20 weeks (65). Fisher (50) obtained uranium extraction yields of 35–55% and 49–79% in 30 and 60 days, respectively, on ores with particle sizes of 0.8–12.7 mm. Using a two-stage system in which ore crushed to less than 3 mm was leached by percolation at 50°C and the iron reoxidized by *T. ferrooxidans* in a separate reaction vessel at 30°C, Manchee (91) recovered over 90% of the available uranium. The retention time of the replaceable ore-filled columns was 10 days. Derry and colleagues (34) have discussed a similar in-plant process. A six-stage semi-continuous counter-current decantation system was successful in recovering over 90% of the uranium with a retention time of 31 h (95). Uranium solubilization of 67% was achieved in a stirred aerated fermentor in 9 days; recoveries were increased to 87% upon regrinding the residue and releaching it for another 9 days (59). Uranium extraction in shake flasks was also effective; the leaching process was completed in 5–10 days (58, 127).

Microorganisms other than *T. ferrooxidans* such as the iron-oxidizing sulfolobus-like thermophiles (13), the thermophilic thiobacilli (80), and other thiobacilli (74) have been proposed to be suitable for uranium extraction.

Other Metals

T. ferrooxidans is able to oxidize stibnite (Sb_2S_3) (119, 133, 135); *Stibiobacter senarmontii* was isolated and shown to use as energy sources stibnite and senarmontite (Sb_4O_6) (81). Stibnite is oxidized directly to antimony (III) sulfate by *T. ferrooxidans* (135); the antimony (III) sulfate is then partially hydrolyzed to insoluble antimony (III) oxide sulfate or oxidized to antimony (V) sulfate, which is subsequently hydrolyzed to insoluble antimony (V) dioxide sulfate.

$$Sb_2S_3 + 6\ O_2 \rightarrow Sb_2(SO_4)_3, \quad 33.$$
$$Sb_2(SO_4)_3 + 2\ H_2O \rightarrow (SbO)_2SO_4 + 2\ H_2SO_4, \quad 34.$$
$$Sb_2(SO_4)_3 + O_2 + 2\ H_2SO_4 \rightarrow Sb_2(SO_4)_5 + 2\ H_2O, \quad 35.$$
$$Sb_2(SO_4)_5 + 4\ H_2O \rightarrow (SbO_2)_2SO_4 + 4\ H_2SO_4. \quad 36.$$

S. senarmontii uses the oxidation of trivalent antimony to the pentavalent form for energy generation (84). Although *T. ferrooxidans* assimilates carbon dioxide in the presence of stibnite (119), it has not been proven whether it is the oxidation of the antimony or the sulfide moiety or both which this bacterium uses as an energy source.

Gallium sulfide (Ga_2S_3) is oxidized to gallium sulfate ($Ga_2(SO_4)_3$) in a reaction analogous to that of stibnite (131). In addition, the sulfides of gallium (131) and bismuth (Bi_2S_3) (130) are oxidized by ferric iron according to the reaction:

$$M_2S_3 + 3\ Fe_2(SO_4)_3 \rightarrow M_2(SO_4)_3 + 6\ FeSO_4 + 3\ S^0. \quad 37.$$

Both simple and complex sulfides of arsenic are reported to be used as substrates by *T. ferrooxidans*. These include orpiment (As_2S_3) (45), enargite [$Cu_3(As, Sb)S_4$] (47), and arsenopyrite (FeAsS) (47). Arsenopyrite is directly oxidized by bacteria:

$$2\ FeAsS + 7\ O_2 + 2\ H_2O \rightarrow 2\ FeAsO_4 + 2\ H_2SO_4 \quad 38.$$

and by ferric sulfate

$$2FeAsS + Fe_2(SO_4)_3 + 4\ H_2O + 6\ O_2 \rightarrow 2\ H_3AsO_4 + 4\ FeSO_4 + 3\ H_2SO_4. \quad 39.$$

The arsenate reacts with the ferric sulfate

$$2\ H_3AsO_4 + Fe_2(SO_4)_3 \rightarrow 2\ FeAsO_4 + 3\ H_2SO_4 \quad 40.$$

to form the insoluble ferric arsenate.

Among other insoluble metal sulfides oxidized by *T. ferrooxidans* are those of cadmium, mineralogically known as greenockite (137), cobalt (sycoporite) (128, 129, 137), lead (galena) (76, 119, 127, 139), nickel (millerite) (42, 43, 107, 127, 128), and zinc (sphalerite) (42, 90, 21, 128, 130, 137). The rates of bacterial oxidation of some of these and of the copper sulfides covellite and chalcocite are dependent upon their solubility constants (138).

A more complex sulfide mineral of nickel, pentlandite, is oxidized directly by *T. ferrooxidans* (130) according to the reaction:

$$8(Ni,Co,Fe)_9S_8 + 141\ O_2 + 26\ H_2SO_4 \rightarrow 36(Ni,Co)SO_4 + 18\ Fe_2(SO_4)_3 + 26\ H_2O. \quad 41.$$

The sulfates of cadmium, cobalt, nickel, and zinc are soluble, whereas that of lead, mineralogically known as anglesite, is insoluble and precipitates (76).

Although molybdenum is generally toxic to *T. ferrooxidans,* this bacterium does oxidize molybdenite (23) by the reaction

$$2\ MoS_2 + 9\ O_2 + 6\ H_2O \rightarrow 2\ H_2MoO_4 + 4\ H_2SO_4. \quad 42.$$

Ehrlich has recently reviewed a number of inorganic oxidations catalyzed by bacteria (48).

CONCLUDING REMARKS

From the literature it is apparent that much has been learned about microbial leaching and the potential of the organisms for leaching. Regardless of the present limited applications of microbes for leaching, the future appears promising. We can look forward to new modifications of the leaching process including: (*a*) redesigning of heap dumps to include finger dumps to exercise natural aeration and artificial aeration with compressed air; (*b*) genetic manipulation of chemolithotrophic bacteria to fit the optimum conditions needed for successful leaching or the application of naturally selected higher-yielding organisms: (*c*) vat leaching to allow more controls for optimizing leaching and improving pollution control and (*d*) the use of ore concentrates for leaching.

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