

Review

Beginnings of rational bioleaching and highlights in the development of biohydrometallurgy: A brief history

H.L. Ehrlich*

Department of Biology, Rensselaer Polytechnic Institute, Troy, NY 12180-3590 USA

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ABSTRACT

The origin of a rational (scientific) approach to extraction of metal values from ores with the aid of microorganisms (bioleaching) is traced. The removal by microbiological means of ore constituents that interfere with metal extraction (biobeneficiation), an outgrowth from bioleaching, is also traced. © 2004 SDU. All rights reserved.

Keywords: Bioleaching; Biobeneficiation; Metal sulfide ores; Acid mine drainage; Acidophilic iron-oxidizing autotrophs

1. INTRODUCTION

Bioleaching is an industrially exploitable process involving the mobilization of metals from ores with the help of some prokaryotic and/or eukaryotic microorganisms. Historical records indicate that the process has been exploited empirically since ancient times, but without any recognition of a biological contribution to the process (Ehrlich, 2001). Miners simply noted that watering piles of sulfidic ores or ore tailings containing copper led in time to mobilization of the copper, which could then be recovered from solution as cupric sulfate or, after appropriate further chemical treatment, as metallic copper. Not until the middle of the twentieth century was convincing evidence obtained which showed that microbes were active participants in leaching copper and some other metals from ores. This led to a concerted effort to identify the who and how among these microorganisms, which is continuing to this day.

2. THE DISCOVERY OF ACIDOPHILIC, AUTOTROPHIC IRON-OXIDIZING BACTERIA IN ACID DRAINAGE FROM BITUMINOUS COAL MINES

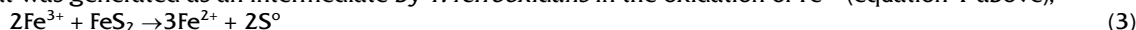
The first direct evidence of an involvement of bacteria in the generation of acid mine drainage (AMD) from the oxidation of pyrite in seams of bituminous coal deposits was obtained in some investigations of the AMD problem associated with bituminous coal mines in the eastern United States (Appalachia). In 1947, Colmer and Hinkle concluded that iron oxidation in AMD was microbially promoted. In 1950 Colmer *et al.* isolated an acidophilic, autotrophic iron-oxidizing bacterium from bituminous coal mine AMD. Its iron-oxidizing physiology was studied in more detail by Temple and Colmer (1951), who named the organism *Thiobacillus* (later renamed *Acidithiobacillus* by Kelly and Wood, 2000) *ferrooxidans*. Leathen *et al.* (1956) isolated an acidophilic, autotrophic iron oxidizer, which they named *Ferrobacillus ferrooxidans* because, unlike *T. ferrooxidans*, it did not oxidize elemental sulfur in their experiments. Later this organism was shown to be a strain of *T. ferrooxidans* (Unz and Lundgren, 1961; Ivanov and Lyalikova, 1962; Hutchinson *et al.*, 1969). Various studies implicated these iron-oxidizing acidophiles in the oxidation of pyritic materials associated with some coal deposits (e.g., Leathen *et al.*, 1953b; Silverman *et al.*, 1961). Leathen *et al.* (1953b) reported some action by the bacteria on "sulfur balls", which are pyritic inclusions in bituminous coal, on marcasite, the orthorhombic polymorph of iron pyrite (FeS_2), but not on iron pyrite, whereas Silverman *et al.* (1961) reported action on iron pyrite and marcasite. In general, it was believed that the role of *T. ferrooxidans* and *F. ferrooxidans* was to generate ferric iron (Fe^{3+}) from dissolved ferrous iron (Fe^{2+}), the ferric iron then serving as chemical oxidant of FeS_2 . The oxidation of pyrite by the ferric iron generated fresh ferrous iron, as shown by the following reactions

* Corresponding author. E-mail: ehrlih@rpi.edu



Singer and Stumm (1970) viewed the bacterial oxidation of Fe^{2+} as the rate controlling reaction in pyrite oxidation, because in the absence of the iron oxidizers at an acid pH below 5.0, ferrous iron oxidation was very slow.

Early studies on AMD also revealed a presence of *Thiobacillus* (now *Acidithiobacillus*) *thiooxidans* (Carpenter and Herndon, 1933; Colmer and Hinkle, 1947; Leathen *et al.*, 1953a; Temple and Delchamps, 1953). This organism, which like *T. ferrooxidans* is an acidophilic autotroph, was, however, found unable to oxidize ferrous iron. It did exhibit its previously known ability to oxidize reduced forms of sulfur, especially elemental sulfur (S^0) associated with pyritic constituents of bituminous coal, such as "sulfur ball". Temple and Delchamps suggested that the source of S^0 in sulfur ball was the chemical oxidation of pyrite by ferric iron that was generated as an intermediate by *T. ferrooxidans* in the oxidation of Fe^{2+} (equation 1 above),



T. thiooxidans oxidized this S^0 to sulfuric acid,



thereby contributing to the acid load in AMD

3. DISCOVERY OF *T. FERROOXIDANS* AND *T. THIOOXIDANS* IN AMD FROM COPPER MINES

The published reports of microbial promotion of oxidation of pyrite in bituminous coal seams led Loren C. Bryner of the Department of Chemistry and Jay V. Beck of the Department of Bacteriology at Brigham Young University in Provo, Utah, USA to ask if *T. ferrooxidans* might not also be detectable and active in AMD from sulfidic copper mines because the ore bodies of such mines always include pyrites in addition to other metal sulfides. They and their students did, indeed, recover *T. ferrooxidans* and *T. thiooxidans* from mine drainage issuing from a waste rock dump in Bingham Canyon, an open-pit mine outside Salt Lake City, Utah, operated by Kennecott Copper Corporation (Wilson, 1952; Davis, 1953; Bryner *et al.*, 1954, Bryner and Jameson, 1958). Bryner and Jameson (1958) also found similar bacteria in drainage from ore waste dumps at a copper mine in Cananea, Sonora, Mexico.

4. ABILITY OF THE ACIDOPHILIC, AUTOTROPHIC, IRON-OXIDIZING ISOLATES FROM COPPER MINE DRAINAGE TO PROMOTE OXIDATION OF COPPER SULFIDES

Laboratory tests in air-lift columns by Bryner, Beck, and their students revealed the ability of pure or mixed cultures containing iron-oxidizing *Thiobacillus ferrooxidans* from copper mine drainage to oxidize pyrite (FeS_2), chalcopyrite (CuFeS_2), chalcocite (Cu_2S), bornite (Cu_5FeS_4), tetrahedrite ($\text{Cu}_8\text{Sb}_2\text{S}_7$), each in mineral form, and covellite (CuS) in mineral form and as reagent grade CuS (Bryner *et al.*, 1954). Tests also revealed promotion of oxidation of molybdenite by *T. ferrooxidans*, especially if in the presence of pyrite (Bryner and Anderson, 1957; Bryner and Jameson, 1958). The nutrient solution used in initial experiments included (in g/l) ammonium sulfate (0.15), potassium chloride (0.05), magnesium sulfate (0.50), potassium hydrogen phosphate (0.05), and calcium nitrate (0.01) (Bryner *et al.*, 1954). It was amended in subsequent experiments by adding a few additional salts (Bryner and Anderson, 1957; Bryner and Jameson, 1958). Initial pH adjustments of the medium ranged from 2.6 to 3.0. Oxidizable sulfidic mineral constituents of the ores or minerals in contact with any of the nutrient solutions were the energy sources for *T. ferrooxidans*; CO_2 from the atmosphere was the carbon source.

The observations of microbially promoted oxidation of copper sulfide and some other metal sulfides by Bryner, Beck and their students were very soon followed by similar reports from others in the USA, Canada, and Europe (Marchlewitz and Schwartz, 1961; Kramarenko, 1962; Razzell, 1962; Razzell and Trussell, 1962; Ehrlich, 1962, 1963a, 1964; Sutton and Corrick, 1963; Kuznetsov *et al.*, 1963; Szolnoki and Bognár, 1964; Lyalikova and Kulikova, 1965). Indeed, the occurrence of this biooxidation process at appropriate sites has since been found to be global (Rawlings, 1997). No qualitative difference in metal-mobilizing activity from metal sulfides was reported between strains of *T. ferrooxidans* (*F. ferrooxidans*) isolated from AMD from bituminous coal mines and from AMD from metal sulfide ore bodies. The mechanisms by which *T. ferrooxidans* mobilized the metal and/or metalloid constituents in the sulfide minerals were considered to be (a) direct oxidative attack, and/or (b) indirect attack by ferric oxidant regenerated microbiologically from ferrous iron formed when ferric iron reacts chemically with a metal sulfide (Silverman and Ehrlich, 1964; Ehrlich and Fox, 1967). Mechanism (b) has been favored as the exclusive mechanism by some investigators up to now (e.g., Sand *et al.*, 1995, 2001). However, some recent evidence strengthens the view that mechanism (a) can also be operative (Ehrlich, 2002; Yarzabal *et al.* 2002a, b). Environmental conditions undoubtedly determine which of the two mechanisms is operative predominantly in a specific process, and this may vary over time.

5. PHYSIOLOGICAL STUDY OF IRON OXIDATION BY *T. FERROOXIDANS*

Although Temple and Colmer published the original description of the physiology of ferrous iron oxidation at acid pH by *T. ferrooxidans* in 1951, the laboratory observations on which their physiological description was based were not made under optimal growth conditions. Leathen *et al.* (1951, 1956) published similar observations for *F. ferrooxidans*, which, as previously mentioned, was later shown to be a strain of *T. ferrooxidans*. A more detailed physiological study of *F. ferrooxidans* strain TM was published by Silverman and Lundgren (1959a, b). They devised an improved culture medium for culturing *F. ferrooxidans*, which they named 9K medium (Silverman and Lundgren, 1959a). The 9K designation in the name of the medium refers to its iron concentration, which is 9g/l. Its composition is as follows (g/l): (NH₄)SO₄ 3.0; KCl 0.1; K₂HPO₄ 0.5; MgSO₄·7H₂O 0.5; Ca(NO₃)₂ 0.01; FeSO₄·7H₂O 44.22; the pH of the medium is adjusted to 3.5 with 10N H₂SO₄. This medium continues to be widely used by many investigators. The sole energy source in it is ferrous iron, which may be replaced by other oxidizable inorganic energy sources such as metal sulfides. The medium is an extensive modification of the medium described by Leathen *et al.* (1956). Other media for cultivating *T. ferrooxidans* and other acidophilic iron oxidizers were formulated in later years (Tuovinen and Kelly, 1973; Johnson and McGinness, 1991).

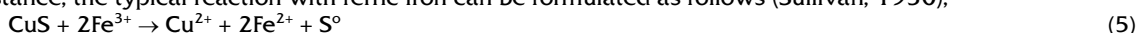
In their physiological characterization of *F. ferrooxidans* TM, Silverman and Lundgren performed manometric studies in which they measured oxygen consumption on ferrous iron by resting cell suspensions of the organism (Silverman and Lundgren, 1959b). These studies revealed that their strain was able to oxidize 500μmoles of Fe²⁺ more rapidly than 250μmoles. The optimum pH for Fe²⁺ oxidation by their strain in these experiments ranged from pH 3.0 to 3.6. The optimum temperature for Fe²⁺ oxidation by their resting cells was 37°C whereas the optimum temperature for growth was ~28°C, with no growth occurring at 37°C. From their studies, they calculated the average efficiency of CO₂ fixation by resting cells of their organism to be 20.5±4.3%.

6. SIGNIFICANCE OF THE PRESENCE OF *THIOBACILLUS THIOOXIDANS* IN AMD FROM BITUMINOUS COAL MINES AND METAL SULFIDE ORE BODIES

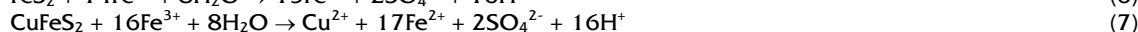
The acidophilic chemoautotroph *Thiobacillus thiooxidans*, which was originally isolated from soil that was enriched with flowers of sulfur and described by Waksman and Joffe (1922), was recognized as a companion of *T. ferrooxidans* in AMD by Colmer and Hinkle (1947), Leathen (1953), Bryner and Jameson (1958), Marchlewitz and Schwartz (1961). Because *T. thiooxidans* is unable to oxidize ferrous iron, its growth in AMD has been explained as the result of its ability to oxidize reduced forms of sulfur associated with bituminous coal and metal sulfide ore deposits. Temple and Delchamps (1953) and Leathen *et al.* (1953a) demonstrated some sulfuric acid production by *T. thiooxidans* at the expense of marcasite, a polymorphic form of FeS₂, but not at the expense of "sulfur balls" from bituminous coal. Although Temple and Delchamps (1953) attributed the sulfuric acid generation by *T. thiooxidans* mainly to oxidation by the organism of elemental sulfur (S⁰) formed as an intermediate during chemical oxidation of pyrite by Fe³⁺ (equation 3 above), some sulfuric acid may also be formed by this organism from thiosulfate and polythionates that may originate as intermediates in pyrite oxidation (e.g., Schippers *et al.*, 1996). There is, however, a question of stability of thiosulfate at acid pH. It breaks down spontaneously to SO₃²⁻ and S⁰ under such a condition.

7. INITIAL SCIENTIFICALLY BASED (RATIONAL) DEVELOPMENTS OF BIOLEACHING

Soon after demonstration of the presence of *T. ferrooxidans* in AMD from the sulfidic copper ore body in Bingham Canyon, Utah and the Chino mine in New Mexico, at the time operated by Kennecott Copper Corporation, and the ability of this organism to mobilize copper from such minerals as chalcopyrite, covellite, chalcocite, and bornite reported by Bryner, Beck and their students (Bryner *et al.*, 1954; Bryner and Anderson, 1957; Bryner and Jameson, 1958), Kennecott designed and patented a heap-leaching process for low-grade sulfidic ores and ore tailings (Zimmerley *et al.*, 1958). They based the process on the metal-mobilizing activity of *T. ferrooxidans* as it was then understood from the studies at Brigham Young University. The basic process design involved spraying barren solution (acidified water containing ferric iron) onto heaps of crushed ore or ore tailings. As this solution penetrated the low-grade ore or tailings heaps, it contacted the metal sulfide in the ore, allowing the ferric iron in the solution to oxidize the metal sulfide abiotically. As a result the metal was mobilized to enter the solution. In the case of covellite (CuS), for instance, the typical reaction with ferric iron can be formulated as follows (Sullivan, 1930),



Acid (sulfuric) was also generated in the oxidation of some metal sulfides, especially pyrite (FeS₂) and chalcopyrite (CuFeS₂),



The sulfuric acid caused weathering of the host rock or gangue, consisting of aluminosilicate minerals, resulting in further exposure of embedded sulfidic ore minerals. Growth of naturally present *T. ferrooxidans* and *T. thiooxidans* in the ore heaps, and thus metal sulfide oxidation, was stimulated in this treatment. The mobilization of the metal values in the ore converted the barren solution sprayed on the heaps into pregnant solution, which emerged at the base of the leaching heaps and was collected in specially constructed sumps. Copper-containing pregnant solution was processed in reactors called launders in which it was brought in contact with scrap iron resulting in an electrochemical reaction that led to precipitation of copper metal according to the reaction,



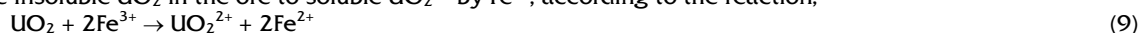
This process was called cementation.

The precipitated copper was separated from unreacted scrap iron, washed and sent to a smelter for conversion into high-grade copper metal. The residual solution (barren solution) in the launder after removal of the copper was transferred to an oxidation pond to convert the ferrous iron that it had acquired in the launder (Reaction 8) to ferric iron by the oxidizing action of *T. ferrooxidans*. This organism was present in the barren solution coming from the launder, having originated in the leach heaps. The oxidized barren solution from the oxidation ponds was then sprayed onto the ore heaps for further leaching action, completing the leach cycle.

One major modification in the recovery of copper from pregnant solution, introduced in later years, was to substitute solvent extraction and electrolysis for cementation. The copper produced by electrolysis (cathode copper) was of the highest grade and needed no further purification, unlike copper obtained by cementation. A second important modification of the heap leaching process was to ensure an adequate air supply to the interior of the ore heaps by air injection (Schnell, 1997). A third modification was an adjustment of the configuration of heaps in terms of height and general shape to prevent slumping.

Until about a decade ago, bioleaching was economically practicable only for metal extraction from low-grade ores. Then bioleaching of ore concentrates became industrially feasible because of advances in stirred reactor designs (Brierley, 1997; Dew and Miller, 1997), which made significantly faster leaching rates possible.

Not too long after an initial understanding of the nature of bioleaching of low-grade copper sulfide ores with *T. ferrooxidans* had been gained, in-situ leaching of uraniferous ores began to be explored. In early examples of bioleaching of uraninite ore residues, a nutrient solution to stimulate the growth of indigenous *T. ferrooxidans* was sprayed on the walls of uranium mine stopes after ore recovery by conventional mining had been completed (e.g., Harrison *et al.*, 1966; Gregor, 1966; Anonymous, 1967). In later examples, a nutrient solution was injected into rubblized ore bodies (McCready and Gould, 1990). The function of *T. ferrooxidans* in the uranium leaching process was to regenerate Fe^{3+} from the Fe^{2+} formed in the oxidation of the insoluble UO_2 in the ore to soluble UO_2^{2+} by Fe^{3+} , according to the reaction,



The pregnant solution containing dissolved uranyl (UO_2^{2+}) was then treated biologically or chemically to re-reduce the soluble UO_2^{2+} to the insoluble UO_2 (McCready and Gould, 1990).

8. MIXED MICROBIAL FLORA IN ACID MINE DRAINAGE FROM METAL SULFIDE ORE BODIES

Whereas Bryner, Beck, and their students mentioned only the presence of *T. ferrooxidans* and *T. thiooxidans* in the AMD from the copper mine in Bingham Canyon, others reported finding fungi including yeasts in AMD from copper and coal mines (Colmer and Hinkle, 1947; Ashmead, 1955; Razzell and Trussell, 1963). Marchlewitz and Schwartz (1961) identified members of the fungal genera *Pullularia*, *Penicillium*, and *Spicaria* and the pink yeasts *Rhodotorula glutinis* and *R. rubra* in coal mine AMD. Ehrlich (1963b) reported the presence two protozoan organisms, one an amoeba and the other a flagellate resembling *Eutreptia* in addition to yeast similar to *Trichosporon* and *Rhodotorula* in a sample of pregnant solution having a pH of 2.5 and containing 0.8g Cu and 1.06g total Fe per liter from an underground sump of an in-situ leaching operation. Acid-tolerant protozoa had been previously noted in very acid AMD from coal mines by Lackey (1938). Ehrlich (1963b) reported that the amoebae in the copper mine AMD, which he examined microscopically, contained bacteria and yeasts in their vacuoles, suggesting that the amoebae were feeding on them. He was able to culture the flagellate in the presence of iron-oxidizing bacteria in 9K iron medium (Silverman and Lundgren, 1959a), the appearance of the bacteria always preceding the flagellate by more than one week on subculture. It was unclear whether the flagellate fed on the bacteria holozoically or saprozoically. Collectively these findings suggested the existence of a complex biocenosis in AMD. Some insight into how bacterial members in this biocenosis may interact with each other can be gained from a recent study by Marchand and Silverstein (2003).

When the native flora associated with an ore is the source of the active leaching agents in a heap-, dump, or in-situ leaching process, a microbial succession may occur until the process is stabilized (e.g., Brierley, 1999).

9. FIRST MODEL STUDY OF THE ORIGIN OF ACID MINE DRAINAGE FROM A PYRITIC COAL SPOIL

In 1978, A. P. Harrison Jr. constructed a coal spoil heap in his laboratory to study the stages in the origin of AMD. He heaped a homogeneous mixture consisting of 1 part crushed, sifted coal, 2 parts shale, and 8 parts subsoil from the overburden of a coal deposit on a plastic tray. The heap was 50cm in diameter and 25cm high. He inoculated the heap by pouring 20L of an emulsion of acid soil, drainage water, and mud from the spoil of an old coal strip mine on the bottom of the plastic tray. The heap absorbed the emulsion, which migrated upward by capillary action. Evaporation losses were corrected periodically by addition of distilled water to the liquid on the tray. Samples collected at the base of the heap revealed the presence of heterotrophic bacteria, whose population density reached a peak of about 10^7 cells/g within 2 weeks after the start of the experiment. Although the pH dropped from 7 to 5 after 8 weeks, the heterotrophs were still dominant. Between 12 and 20 weeks, the size of the population decreased by an order of magnitude while the pH dropped to a little below 5. This pH drop was attributed to a burst of growth of sulfur-oxidizing bacteria, which, however, died off progressively while the heterotrophs increased once more to just below 10^7 /g. Near the top of the heap, heterotrophs were dominant for the first 15 weeks but then decreased from 10^6 to 10^2 cells/g, while the pH decreased to 2.6. The drop in pH correlated with a rise in the population of sulfur- and iron-oxidizing bacteria (*T. thiooxidans* and *T. ferrooxidans*). The sulfur-oxidizers predominated at first over iron-oxidizers, but only briefly. Molds, ciliated and flagellated protozoa, algae, a moss and an arthropod were noted mostly when the pH was closer to neutrality. A mineral efflorescence appeared at the surface of the heap, consisting mainly of sulfates of Mg, Ca, Na, Al, and Fe. The magnesium sulfate was a hexahydrate. The leached cations derived mostly from the coal, but some magnesium was also derived from the overburden material.

This study clearly showed that AMD formation involves the participation of a succession of organisms involving heterotrophs and acidophilic S- and Fe-oxidizing autotrophs. At the time that Harrison carried out his investigation, the identification of different acidophilic S- and Fe-oxidizing autotrophs in AMD had just barely begun (see below). It would thus seem to be important to repeat Harrison's experiment using currently available molecular techniques, which had not yet been thought of and developed at the time that Harrison carried out his study, to obtain a much more inclusive qualitative and quantitative picture of microbial participants in the AMD forming process.

10. DISCOVERY OF PREVIOUSLY UNRECOGNIZED ACIDOPHILIC IRON- AND SULFUR-OXIDIZING MICROORGANISMS IN AMD BELONGING TO THE DOMAIN BACTERIA

The report on the isolation of *Leptospirillum ferrooxidans* from a copper deposit in Armenia by Markosyan (1972) (Balashova *et al.*, 1974) made clear that *T. ferrooxidans* was not the only mesophilic, acidophilic, autotrophic iron-oxidizer active in metal sulfide ore oxidation. Interestingly, *L. ferrooxidans*, unlike *T. ferrooxidans*, is unable to oxidize reduced forms of sulfur. This organism was subsequently reported to occur in AMD in different parts of the world, and it became apparent that more than one species of *Leptospirillum* existed (Harrison and Norris, 1985; Johnson *et al.*, 1989; González-Toril *et al.*, 1999). A report in 1993 by Sand *et al.* made clear that in some heap leaching operations, *L. ferrooxidans* can be as important or more important than *T. ferrooxidans*.

In 1978, Golovacheva and Karavaiko reported the isolation of a moderate thermophile, *Sulfobacillus thermosulfidooxidans*, which was a sporeformer capable of oxidizing reduced forms of sulfur as well as ferrous iron. Its optimum growth temperature of $\sim 50^\circ\text{C}$ contrasts with the optimum for *T. ferrooxidans* and *L. ferrooxidans*, which is in the range of $25\text{-}30^\circ\text{C}$ and characterizes them as mesophiles. A more thorough discussion of mesophilic autotrophic bacteria of significance in bioleaching can be found in Rawlings (1997), and one of thermophilic bacteria active in bioleaching can be found in Norris (1997).

Much more recently, ferrous iron- and reduced-sulfur oxidizing heterotrophs have been reported in connection with bioleaching. An overview of this group was published by Johnson and Roberto (1997).

What is becoming clear is that bioleaching of metal sulfide ores involves a much more complex community of microorganisms than initial findings suggested, and that under different environmental conditions, different organisms may dominate the process.

11. DEVELOPMENT OF A BIOBENEFICIATION PROCESSES FOR FOSSIL FUELS AND PYRITIC GOLD ORES

Biobeneficiation is a term used to denote the use of microbes in the removal, most often by oxidation, of undesirable constituents in fossil fuels such as coal and petroleum, and in ores such as pyritic gold ores. Bituminous coal deposits frequently contain pyritic inclusions (pyrite, marcasite, sulfur balls), smaller amounts of elemental sulfur and sulfate, and some organic sulfur, as well as some undesirable trace elements. The value of the coal is adversely affected by such constituents, especially by the presence of the reduced sulfur compounds, because on combustion they are turned into SO₂ and sulfuric acid that enter the atmosphere via flue gases as highly undesirable atmospheric pollutants (Dugan, 1986). Research on the removal of pyritic inclusions from coal began as soon as the ability of *T. ferrooxidans* to promote pyrite oxidation was discovered and was pursued for many years thereafter (Silverman *et al.*, 1961, 1963; Bos *et al.*, 1986; Beyer *et al.*, 1986; Bos and Kuenen, 1990; Olson *et al.*, 1999). Removal of sulfuric constituents from pulverized coal by flotation after conditioning with *T. ferrooxidans* has also been explored (e.g., Dogan *et al.*, 1986). The problem of microbiological removal of organic sulfur from coal still has not been satisfactorily solved. A model compound for exploring this removal process is dibenzothiophene. An effective process must remove the organic sulfur from coal without degradation of the organic carbon skeleton in which it is contained so as not to affect the calorific value of the coal (Finnerty and Robinson, 1986). More significant progress has been made in development of organic sulfur removal from petroleum by microbes (Foght *et al.*, 1990; Grossman, 1996).

Livesey-Goldblatt *et al.* (1983) proposed a bioleaching method for biobeneficiating pyritic gold ores based on bench-scale experiments using a mixed bacterial inoculum consisting primarily of *T. ferrooxidans*. The function of *T. ferrooxidans* was to remove the pyrite and arsenopyrite partially or completely from the ore by oxidation. *T. ferrooxidans* had been previously shown to be able to oxidize arsenopyrite (Ehrlich, 1964; Groudev, 1981). The pyrite and arsenopyrite in pyritic gold ores interfere with gold recovery by leaching with cyanide or thiourea solutions because these minerals often encapsulate the gold, making it not directly accessible to either lixiviant. The cyanide will also react chemically with the pyrite and arsenopyrite, forming ferro- and ferricyanide, and thiocyanate, which do not mobilize the gold and from which cyanide cannot be recovered for recycling in the leach process. Based on their experiments, Livesey-Goldblatt *et al.* (1983) presented a cost analysis of pyritic gold ore beneficiation by bacterial leaching, which showed it to be more cost-effective than flotation and roasting treatment of the ore. Based on these and subsequent findings, GENCOR, a South-African gold producer, developed an industrial-scale biobeneficiation process for their pyritic gold ores.

12. DISCOVERY OF ACIDOPHILIC OXIDIZERS OF FERROUS IRON AND REDUCED FORMS OF BELONGING TO THE DOMAIN ARCHAEA

The Archaeal acidophilic, chemoautolithotrophic oxidizers of ferrous iron and reduced forms of sulfur include both hyperthermophiles and mesophiles. The optimum growth temperature of the former is above 60°C, that of the latter between 25 and 30°C. The first of these, a hyperthermophile, was found by Brierley in 1966 in water from a hot spring in Yellowstone National Park, U.S.A. It was characterized by Brierley and Brierley in 1973, but its affinity to the Archaea was not recognized at the time because the existence of this prokaryotic domain had not yet been discovered. It was only in 1977 that Woese and Fox demonstrated that the prokaryotes consisted of two domains, the Bacteria and the Archaea. In terms of its morphology and its ability to oxidize elemental sulfur, the Brierleys' organism resembled an oxidizer of elemental sulfur called *Sulfolobus acidocaldarius*, also discovered in a hot spring in Yellowstone National Park (Brock *et al.*, 1972), and it was, therefore, named *Sulfolobus brierleyi*. Interestingly, the iron-oxidizing ability of *S. acidocaldarius* was not recognized until 1976 (Brock *et al.*, 1976). Subsequent molecular phylogenetic analysis revealed significant differences between the two organisms, which led to the reassigning of *Sulfolobus brierleyi* to a new genus, *Acidianus* (Seeger *et al.*, 1986; Norris, 1997).

Since biochemical reaction rates tend to increase with a rise in temperature, but unlike chemical rates only in a physiological temperature range, thermophilic Archaea have been and continue to be tested as potentially more efficient bioleaching agents for metal sulfides than their mesophilic counterparts. For instance, Konishi *et al.* (1992) found that in a batch reactor, the thermophile *A. brierleyi* leached chalcopyrite concentrate much faster at 65°C than did the mesophile *T. ferrooxidans* at 30°C. Stott *et al.* (2003) compared the rates of bioleaching of copper from a chalcopyrite concentrate by the mesophile *Acidithiobacillus* (formerly *Thiobacillus*) *ferrooxidans*, the moderate thermophiles *Acidithiobacillus caldus*, *Sulfolobus thermosulfidooxidans*, *Sulfolobus acidophilus*, *Acidimicrobium ferrooxidans*, and the hyperthermophile *Acidianus brierleyi*. Over 50h, Cu leaching was fastest by far with *Acidianus brierleyi*, but the authors concluded that this faster rate was mainly due to the accelerating effect of temperature (70°C) on the chemical reaction of the chalcopyrite with ferric iron and not the biological action, i.e., the biooxidation of ferrous to ferric iron.

The first mesophilic, acidophilic, chemoautolithotrophic iron-oxidizing Archaea were not discovered until very recently. Golyshina *et al.* (2000) discovered *Ferroplasma acidophilum* in a bioleaching pilot operation. Edwards *et al.* (2000) discovered *Ferroplasma acidarmanus* in AMD at a pyrite mine at Iron Mountain, California. Whereas *F. acidophilum* was found to have a pH optimum of 1.7 and to be capable of growing between pH 1.3 and 2.2, *F. acidarmanus* was found to have a pH optimum of 1.2 and to be capable of growing between pH 0 and 2.5. Okibe *et al.* (2003) detected a *Ferroplasma*-like organism along with an *Acidithiobacillus caldus*-like organism, a thermophilic *Leptospirillum* sp., and *Sulfobacillus* sp. in a pilot-scale stirred-tank bioleaching operation involving a polymetallic sulfide ore. *Ferroplasma* became increasingly dominant in the mixed population as the leaching process progressed.

13. CONCLUSIONS

As is apparent from the foregoing review, the history of rational bioleaching started with the discovery of the acidophilic iron-oxidizer *T. ferrooxidans* in AMD. Its ability to promote the oxidation of various metal sulfides and its detection in acid mine waters associated with a variety of metal sulfide ore bodies led to detailed studies of its physiology and genetics so that by now it has become a kind of model organism for students of biomining, which *Escherichia coli* has become to many general microbiologists. However, as should also be apparent from this review, bioleaching processes, especially heap leaching, that involve the natural flora associated with an ore almost never involve just *T. ferrooxidans* but a mixed flora in which *T. ferrooxidans* may not be the most important agent responsible for leaching, if at all. In order to ensure that a leaching agent of choice dominate in an industrial process, running it in a reactor is very desirable. This allows for inoculation of an ore to be processed with a desired culture and for establishing selective growth conditions in respect to pH and temperature, for instance. Such selective conditions help to prevent the organisms in the inoculum from being overgrown by other organisms that are naturally associated with the ore and cannot be eliminated because the ore cannot be presterilized. To control the make up of the active flora in heap bioleaching, massive inoculation with a pregrown culture has been found necessary (Brierley *et al.*, 1995).

Insofar as the formation of AMD is concerned, it should be recognized that for any given ore body that is being mined, it is an evolving process. As the microbiological studies of Harrison (1978) and of Bond *et al.* (2000) suggest, it involves a succession of organisms, of which *T. ferrooxidans* and *T. thiooxidans* may not be the climax organisms. The succession of organisms and the climax organisms that ultimately dominate are determined by such factors as the geology and water chemistry of the deposit and the surrounding terrain and climate. No conclusions should be reached concerning AMD evolution at a specific site from one or a few successive samplings at a single station. Only samples collected at selected sites over extended time involving successive years, starting with the onset of mining can give complete insight into the microbiology of AMD formation at a site.

Bioleaching technology is still not mature.

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