Microbiology and mining—practices and problems

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Introduction

Economic considerations, environmental regulation, and diminishing ore grades are some factors directing the mining industry to explore new mining and environmental restoration practices. Some of these new technologies are utilizing microbiology for recovery of mineral values, restoration of wastewaters, and reclamation of mining sites. However, some novel mining processes are creating unique environmental conditions, and there are now frequent reports of microbially related problems in mineral processing.

This paper reviews microbial mineral leaching and mine wastewater restoration processes, proposes techniques for biological renovation of in-situ uranium sites, and discusses microbial problems occurring during in-situ uranium leaching.

Bacterial leaching

Copper

Dump leaching of copper has been practiced for centuries, but the first large-scale development was probably in Rio Tinto, Spain, about 1750. Today, dump leaching is extensively practiced in the western United States and accounts for about 11.5 percent of the total copper production of this country (Wadsworth, 1975). The role of bacteria in dump leaching was recognized in 1957 (Bryner and Anderson, 1957). The principal microorganisms involved in the extraction process are Thiobacillus ferroxidans and Thiobacillus thiooxidans. Both are aerobic rod-shaped bacteria, 0.5 x 1.0 μm in size, which require an acid environment of about pH 1.5 to 3.0 and utilize carbon dioxide as their sole source of carbon. The microbes function in the temperature range of 18° to 40°C. T. ferroxidans derive energy for growth from the oxidation of ferrous iron:

\[2\text{FeSO}_4 + 1/2 \text{O}_2 + \text{H}_2\text{SO}_3 \rightarrow \text{Fe}_2(\text{SO}_4)_3 + \text{H}_2\text{O}\]  

Although iron oxidation occurs in air, T. ferroxidans can increase the rate by 500,000 times (Lacey and Lawson, 1970). T. thiooxidans oxidize sulfur, accordingly:

\[\text{S}^0 + 3/2 \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4\]  

Both organisms are found in large numbers—up to 10⁷ per gram of ore—in copper dump leaching operations. The ferric iron produced by T. ferroxidans is directly responsible for the dissolution of metals (M) from metallic sulfide (MS) minerals,

\[\text{MS} + 2\text{Fe}^{3+} \rightarrow \text{M}^{n+} + 2\text{Fe}^{2+} + \text{S}^0\]  

and the elemental sulfur produced by this reaction is oxidized by T. thiooxidans.

Several specific metal dissolution reactions are:

\[\text{CuFeS}_2 + 2\text{Fe}_2(\text{SO}_4)_3 \rightarrow 4\text{FeS}_3 + 5\text{Fe}_2\text{SO}_4 + 2\text{S}^0\]  

(Calcochide)

\[\text{Cu}_2\text{S} + 2\text{Fe}_2(\text{SO}_4)_3 \rightarrow 4\text{FeS}_3 + 5\text{CuSO}_4 + 4\text{Fe}_2\text{SO}_4 + 2\text{S}^0\]  

(Caldocite)

The sulfur generated by these chemical reactions can passivate the mineral surface and inhibit further leaching. One function of T. thiooxidans is believed to be the oxidation of the sulfur to soluble sulfurous acid, thus allowing leaching to continue. The involvement of bacteria in such metal dissolution reactions has been called “indirect” since the bacteria generate the oxidizing agent, ferric iron, and provide a suitably acid environment to maintain the oxidized metal species in solution.

The thiobacilli, as these organisms are collectively called, also directly affect metal dissolution. The mechanism of attack has not been elucidated, but it is widely believed that the metal molety itself is oxidized by the microbes. Some specific reactions in which the bacterial leaching is “direct” include:

\[2\text{CuS} + \text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{Cu}_2\text{SO}_4 + 2\text{H}_2\text{O}\]

Uranium

Bacterial leaching of uranium is an indirect process. T. ferroxidans oxidizes ferrous iron and pyrite according to reactions (1) and (7) to produce the oxidant, ferric iron, which reacts with reduced uranium:

\[\text{UO}_2 + \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{UO}_2\text{SO}_4 + 2\text{FeSO}_4\]

Ferric iron is regenerated by the bacteria, and the sulfuric acid necessary to maintain the oxidized uranium in solution is added and/or generated by the bacterial oxidation of pyrite (reaction 7). The microbial process has been used as a scavenger of uranium values from mined-out areas and low-grade stopes in the Elliot Lake region of Canada. Bioextraction is now applied as the principal means of recovering uranium from heaps and fractured stopes at one Elliot Lake mine (McCready, 1976). Acid leach solutions are percolated through the underground ore, and the oxidizing agent, ferric iron, is regenerated biologically. The uranium ores of northern Ontario are amenable to bacterial leaching because of the substantial pyrite concentrations. However, the uranium ores of the Rocky Mountain region and south Texas are secondary uranium formations, deposited in the front configuration, and thus lack the large concentrations of pyrite essential for effective bacterial leaching. These depos-
its are amenable to in-situ, or bore-hole, mining (Shock and Conley, 1974) by either acid or carbonate leaches. Limited research has been conducted on the bacterial leaching of these roll-front ores, but preliminary investigations (C. Brierley, 1978b) on ore from the Anaconda Company Jackpile-Paguate mines of New Mexico indicate that suitable energy sources are unavailable for the bacteria, and that the organisms lose viability when in protracted contact with the ore. Present studies of this ore amended with suitable bacterial energy sources are evaluating the effect of hydrostatic pressure on the bacteria during the leaching process (A. Torma, J. Brierley, and C. Brierley, unpublished data). Such laboratory studies are an attempt to simulate the water pressure exerted underground during leaching. Extensive laboratory and field testing is needed before valid conclusions can be made on the use of bacteria for in-situ leaching of western U.S. uranium reserves.

Other leaching microorganisms

Bacterial leaching is not confined to the microorganisms *T. thiooxidans* and *T. ferrooxidans*. Several other microbes affect the dissolution of metallic sulfides by oxidation of iron and sulfur and direct attack of sulfide minerals. Among the most unique are the extremely thermophilic (heat-loving) and acidophilic (acid-loving) bacteria. The first of these microbes to be characterized was *Sulfobolus acidocaldarius* (Brock, et al., 1972). Other related forms have been subsequently characterized (C. Brierley and J. Brierley, 1973; DeRosa, et al., 1975). These spherical bacteria are about 1 μm in diameter, require an acid environment, and oxidize reduced sulfur and iron compounds. They obtain carbon from either simple organics or carbon dioxide, and function aerobically or anaerobically. These microbes are unique in that they are obligately thermophilic, requiring temperatures between 45° and 80°C; some species function at a temperature as high as 90°C. The natural habitats of these organisms are acidic hot springs, and although they have not been specifically identified with any commercial leaching operations, extensive laboratory tests confirm their capability to enhance the extraction of metals from the recalcitrant minerals chalcopyrite and molybdenite (C. Brierley, 1977; J. Brierley and C. Brierley, 1978; C. Brierley, 1978a; C. Brierley, 1974).

A few years after the discovery of the extremely thermophilic, acidophilic bacteria, LeRoux et al. (1977) reported the discovery of *Thiobacillus*-like microorganisms which require temperatures between 30-55°C. Although these organisms oxidize iron like *T. ferrooxidans* and are morphologically and physiologically similar, they require small quantities of simple organic compounds. First isolated from Icelandic, acidic thermal springs, thermophilic thiobacilli have been found to occur naturally in a large-scale leach test facility (J. Brierley and Lockwood, 1977) and copper leach dumps at Chino mines, Santa Rita, New Mexico (J. Brierley, 1978), and Bingham Canyon, Utah (C. Brierley, et al., 1979).

It is premature to ascertain the significance of thermophilic bacteria in mineral leaching. Temperature profiles of copper leach dumps show areas in excess of 80°C due to the exothermic oxidation of pyrite (Beck, 1967). Such regions could be the site of active thermophilic bacterial populations. However, studies to date have neither quantitated these populations nor evaluated their contribution to metal solubilization.

Restoration of mine sites and wastewaters

Microorganisms are now used in the restoration of mine and mill effluents, and current research indicates that some bacteria may play an active role in the restoration of in-situ uranium leaching sites.

Restoration of in-situ uranium mines

In-situ leaching of uranium has flourished in the uranium regions of south Texas and Wyoming. The most frequent leaching agents used in these regions are carbonate and bicarbonate ions with hydrogen peroxide as the oxidizing agent to form the highly soluble uranyl dicarbonate and tricarbonate ions (Garrels and Christ, 1965).

\[2\text{UO}_2 + 2\text{H}_2\text{O} + 4(\text{NH}_4)\text{CO}_3 \rightarrow 2(\text{NH}_4)\text{UO}_2(\text{CO}_3)_2 + 4\text{NH}_4\text{OH} \]  \hspace{1cm} (10)

\[\text{UO}_2 + \text{H}_2\text{O} + 3(\text{NH}_4)\text{CO}_3 \rightarrow (\text{NH}_4)_2\text{UO}_2(\text{CO}_3)_2 + 2\text{NH}_4\text{OH} \]  \hspace{1cm} (11)

Since the hydroxyl formed by these reactions will attack the uranyl carbonate complex, it is neutralized by the addition of bicarbonate.

\[\text{NH}_4\text{HCO}_3 + \text{NH}_4\text{OH} \rightarrow (\text{NH}_4)_2\text{CO}_3 + \text{H}_2\text{O} \]  \hspace{1cm} (12)

Although solutions other than ammonium carbonate and ammonium bicarbonate can be used, the ammonium cation is preferred since it is less expensive and doesn’t produce the swelling of clays sometimes noted with the sodium ion. However, the ammonium ion does have a tendency to adsorb to base exchange sites associated with clays in the mineral formation. Ground water passing through the formation tends to mobilize the ammonium ion, and there is the potential problem of the conversion of ammonium to nitrite and nitrate. The latter compounds have little affinity for minerals and are freely soluble. Nitrite and nitrate are also hazardous to human health.

Laws for the restoration of mine sites are now being formulated in affected states. Present government regulations for Texas require that the water at the mine location be restored to base line ground-water quality and the mine site be returned to its original state after solution mining has ceased (Walsh et al., 1979; Whittington and Taylor, 1979).

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*Volume 1, Number 3, August 1979*

*Editor: Neila Pearson*

*Published quarterly by New Mexico Bureau of Mines & Mineral Resources a division of New Mexico Institute of Mining & Technology*  
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Editorial matter: Contributions of possible material for consideration in future issues of NMG are welcome. Materials cannot be returned unless accompanied by return postage. Address inquiries to Neila Pearson, Editor New Mexico Geology, New Mexico Bureau of Mines & Mineral Resources, Socorro, NM 87801.  
Circulation: 1,200  
Printer: University of New Mexico Printing Plant
Bacteria have recently received considerable attention as agents for restoration of in-situ mine operations leached with ammonium carbonate solutions. The organisms specifically considered for this function are Nitrosomonas, which oxidize ammonium to nitrite,

\[
\text{NH}_4^+ + 3/2 \text{O}_2 \rightarrow \text{2H}^+ + \text{H}_2\text{O} + \text{NO}_2^- \quad (13)
\]

and Nitrobacter, which oxidize nitrite to nitrate.

\[
\text{NO}_2^- + 1/2 \text{O}_2 \rightarrow \text{NO}_3^- \quad (14)
\]

**FIGURE 1—THE BIOLOGICAL NITROGEN CYCLE.**

Other bacteria are capable of denitrification reactions, i.e., the reduction of nitrate to reduced nitrogen species. Some microbes reduce nitrate to gaseous nitrogen while others reduce nitrate to nitrous oxide or ammonium ion. The end product is species dependent. The energy needs for denitrification can be an organic energy source for most microbes and thiosulfate or elemental sulfur, a source for the organism Thiobacillus denitrificans. As shown in the biological nitrogen cycle as it occurs in soil (fig. 1), it is feasible to oxidize ammonium to nitrate and then reduce nitrate to a gas.

Some preliminary research has been completed on the use of Nitrosomonas and Nitrobacter for uranium mine site restoration (David Johnson, personal communication). These studies indicate that biological nitrification can occur in column experiments using uranium core and synthetic ground water. It is entirely feasible that bacteria could be used to oxidize both mobilized ammonium and adsorbed ammonium to soluble nitrate. The nitrate could then be easily flushed out of the mine site. There are many factors to be critically examined. Among these are the introduction of the nitrifying bacteria into the formation, the retention of viability of the bacteria in the in-situ site, the continued activity of the bacteria, their ability to oxidize adsorbed ammonium ion, and possible problems that bacteria in the formation might create such as reduction in permeability.

**Restoration of mining and milling waters**

Many organisms have the capacity of accumulating metals either by binding at the organisms' surfaces or by intracellular uptake of the ions. Likewise, biological agents can readily transform metals by solubilization, precipitation, valency changes, and conversion to organo-metallic compounds (Kelly, et al., 1979). Because such transformations occur on a geochemically significant scale, the use of bacteria has long been considered a scheme for metal recovery from dilute solutions such as seawater. Rather than mineral depletion being a motivating factor, environmental constraints and economic conditions were primarily responsible for active development of biological processes for recovery of metals from dilute solutions. One of the most successful applications of the process is the use of algae to remove both soluble and particulate lead from the mill tailings of several mining ventures in the "new lead belt" of Missouri (Gale and Wixson, 1977; 1979). In one operation a tailings pond allows heavy particulates to settle, and the effluent from the pond passes through a series of shallow meanders. Algae are encouraged to grow in the meandering stream, and analyses of the algae and aquatic vegetation indicate that these agents effectively accumulate or entrap the heavy metals released in the effluent from the tailings pond. A settling pond and baffled outlet prevent the algae from discharging into the receiving stream. Vegetation, identified to function effectively in this wastewater treatment, include Cladophora, Rhioclonium, Hydrodictyos, Spirogyra, Potamogeton and occasional blooms of Oscillatoria.

A combination biological/chemical treatment system is presently operational in the Grants, New Mexico uranium district for the removal of selenium, molybdenum, radium, and uranium from mine wastewater. The system consists of a series of settling ponds to remove heavy particulate matter; some waters are subjected to an ion exchange plant to remove uranium. Barium chloride is subsequently added to precipitate sulfate and radium. The wastewaters are retained in three consecutive algae ponds before discharge. This wastewater treatment system is now under study (C. Brierley and J. Brierley, unpublished data). It is evident from preliminary data that, although algae and aquatic vegetation are present, large populations of anaerobic, sulfate-reducing bacteria in the sediments may participate in trace element removal. These organisms reduce sulfate to hydrogen sulfide

\[
\text{SO}_4^{2-} + 10\text{H}^+ \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O} \quad (15)
\]

using hydrogen or simple organic molecules as energy sources. Analyses of sediments indicate significant accumulations of uranium and molybdenum. The mechanisms for ion removal have not been defined, but it is evident from early studies that both geochemical and biological reactions are likely operative.

**Microbial problems in in-situ uranium leaching**

Thus far only the beneficial uses of microorganisms in the mineral industry have been discussed, but it is apparent that new technology in mining practices is resulting in increased incidences of microbial fouling. The problems are blatantly obvious, the causes are less evident, and the answers are not immediately apparent.

Mine sites that are leached with carbonate solutions and hydrogen peroxide sometimes experience plugging. Backflushing of the wells reveals the presence of massive, macroscopic growths of microorganisms. Preliminary identifications indicate these organisms to be common soil bacteria. Some plugging of mine sites occurs that cannot readily be attributed to either physical or biological factors. In such instances it may be necessary to test for microbial activity, since these agents may be producing gases which could form an airlock in the formation. Examples of such activity are the production of hydrogen sulfide by the sulfate-reducing bacteria (reaction 15) or the production of gaseous nitrogen by Thiobacillus denitrificans:

\[
5\text{SO}_4^{2-} + 8\text{NO}_3^- + 5\text{H}_2\text{O} \rightarrow 4\text{N}_2 + 9\text{SO}_4^{2-} + \text{H}_2\text{SO}_4 \quad (16)
\]

To date little is known why microorganisms sometimes suddenly grow abundantly and uncontrollably in the in-situ uranium leach sites. It may be that chemical and physical conditions are altered to the point where opportunistic microbes find a suitable niche for prolific growth. Hydrogen peroxide, as the oxidant for uranium oxidation, is an oxygen carrier and may supply the aerobic environment needed for microbial development. The causative factors of this situation are as ill-defined as preventative measures. Currently the only action being...
taken against microbial growth is the periodic injection of strong oxidizing agents such as chlorine gas. It may be necessary to find an antimicrobial agent that successfully prevents growth, is inexpensive, environmentally acceptable, and doesn’t interfere with the leaching and uranium recovery processes.

**Conclusions**

Microorganisms can be either a boon or bane to the mining industry. Bacterial leaching has a vital niche in the production of copper and uranium from low-grade ores. Likewise, low-cost energy-conservative techniques for recovery of vagrant inorganic contaminants from industrial wastewater are making increasing use of microbes.

Further investigation will reveal new applications of microorganisms to benefit the uranium industry. The process likely to have the most potential is the bacterial oxidation of ammonium ion remaining in the formation after in-situ leaching with ammonium carbonate solutions.

However, new technologies in uranium mining are evoking problems not previously associated with this industry. Opportunistic microbes are rapidly and uncontrollably proliferating in artificial environments created at the leaching sites and in the uranium recovery and restoration circuits associated with the operations.

Substantial effort is needed to evaluate microbial practices and problems of the mining industry. Evaluations must be comprehensive, including not only the immediate problem or practice, but the basic aspect of the situation. A cooperative effort among many disciplines is needed, for the solutions to the problems are not likely to be uniquely biological.

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**Directory of commercial analytical laboratories in New Mexico**

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**Routine chemical assays** include both wet and instrumental methods (depending on preferences of the individual laboratory) and are analyses of major (1-100%) and minor (.01-1%) elements.

**Geochemical assays** include analyses of trace elements in geological materials (.0001-.01%); these are usually done by instrumental methods.

**Fire assay** is a method of analysis for gold, silver, and platinum group metals using furnace heat and dry reagents.

**Water quality analyses** include the chemical and biological analyses of water constituents but do not include trace contaminants.

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