

# THE ROLE OF MICROORGANISMS IN ACID ROCK DRAINAGE

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## *Abstract*

Microorganisms are directly or indirectly involved in many chemical alterations and in the decomposition of minerals required for their growth. Microbial dissolution of sulfide minerals under acidic conditions can produce over 1,000 times more Acid Rock Drainage (ARD) than chemical action alone. Microbial actions can cause precipitation of minerals, adsorption or release of metals, and formation/destruction of organic-metallic complexes. Soluble minerals can serve as oxidizing substrates, electron donors/acceptors in redox reactions, and can be directly involved in cell metabolism. Microorganisms and microbial oxidation-reduction reactions and their contribution to mineral mobilization and stabilization will be discussed.

## *Introduction*

In the environment, pyrite is oxidized by oxygen and/or ferric iron and microbial activity can accelerate the rate of this oxidation. A rate law derived by Williamson, et al, (1) indicate the abiotic rate of pyrite oxidation increases with increasing oxygen concentration and increases slightly as pH decreases. In the environment, the rate of sulfide mineral oxidation increases as pH decreases into a range conducive to bacterial mediation of ferrous iron oxidation (2,3,4). Microbial pyrite oxidation rates begin to exceed chemical oxidation rates at around pH 3.5 - 4. The microbial mediated rate of pyrite oxidation by ferric iron is roughly two to three orders of magnitude faster than the abiotic oxidation by

oxygen at pH 2 (5). Both abiotic and biotic rates tend to increase with increasing temperature. A specific mining site is currently under study to better characterize the microbial populations involved and their potential contribution to rock pile stability and/or instability. Site rock pile materials contain trace amounts of sulfides other than pyrite, such as chalcopyrite, galena, and sphalerite. Other than the iron fraction of the chalcopyrite, oxidation of these sulfides will not produce acid. Additional minerals present at the site such as a large amount of gypsum and some carbonate materials may also contribute to weathered rock pile stability or instability. This study examines the microbial population present within the rock piles, surrounding rock outcrops, soils, and surface crust materials. This information will be correlated with various geochemical and geophysical parameters being gathered to evaluate the overall influence of various microbial populations on the weathered rock pile materials. Additionally, correlations will examine the potential influence of the different microbial populations found on rock pile stability or instability.

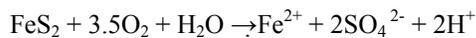
The proposed investigation has a focus on the following interrelated issues:

- Understanding the microbial influence on the rock pile weathering processes, both at the surface and within the mine rock piles
- Measuring the changes in microbial population(s) responsible for the observed weathering and crust formation processes occurring at the surface and within the rock piles over time
- Examining the interrelationship between the microbiology and geochemistry at specific sites and determining their combined affects on the

weathering processes and on the long-term physical stability of the rock piles

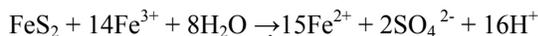
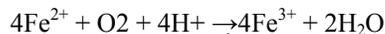
### Microbiology

Acid is typically formed by the oxidation of pyrite (FeS<sub>2</sub>) containing reduced iron and sulfur species. When solid-phase pyrite in rock is exposed to oxygenated water (6), the following chemical reaction occurs:



As a result of this oxidation, the concentration of both reduced iron and acid in drainage waters increases significantly. In addition, the simultaneous presence of reduced iron and oxygen promotes the growth of acidophilic, autotrophic iron- and sulfur-oxidizing bacteria. These include: *Acidithiobacillus species more commonly know as Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, and others such as *Sulfolobus acidocaldarius*, and the colorless sulfur-oxidizing bacteria (7).

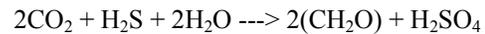
Although these and other bacterial species may play an important role in mineral leaching, *T. ferrooxidans* has often been used as a model bacterium for studying iron oxidation (8). It is widely believed that *T. ferrooxidans* and other iron oxidizers act as a catalyst for acid production by constantly oxidizing reduced iron (Fe<sup>2+</sup>) to ferric iron (Fe<sup>3+</sup>) which then acts as a chemical oxidant of pyrite, thereby liberating more reduced iron, sulfate, and large amounts of acidity as shown in the following two reactions (9).



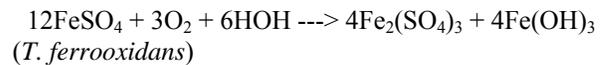
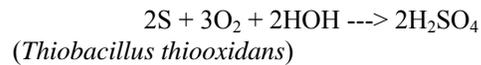
Oxidation of Fe<sup>2+</sup> serves as the rate-limiting step in biologically mediated acid production (4). Because bioavailable organic carbon tends to be relatively scarce in most watersheds with acid drainage, *T. ferrooxidans* and other iron oxidizers often compete quite well with other bacterial species for oxygen and nutrients.

From analysis of various other acid producing rock piles and their associated waters, it is expected that the site microbes involved in the oxidation of

sulfide to elemental sulfur belong principally to the genera *Thiobacillus*, *Leptospirillum*, *Sulfolobus*, and the colorless sulfur-oxidizing bacteria, with *Thiobacillus sp.* most likely being the dominant genus (7,10,11). For the following two "generic" reactions, the first is typical of oxidation of sulfide to sulfur, and the second of oxidation of sulfide to sulfate. The rate-limiting step is mediated by the enzyme sulfite oxidase (enzyme cofactors are Mo and Fe).



### Oxidation of Sulfur and Related Compounds



Sulfur bio-oxidation takes place from a pH of 8.5 to 1.9, however, the species involved will vary, each in turn oxidizing the sulfur to sulfate. Therefore, a "succession" of microbial species takes place as the pH of the soil is lowered by the production of sulfate (7, 11). A similar type of microbial "succession" can be observed with relationship to site and/or rock pile temperatures, and various other environmental parameters.

Acid generated by pyrite oxidation will increase dissolution of other minerals present. The various lithologies piled at the mine site also contain carbonate minerals (e.g. calcite) and silicate minerals (e.g. plagioclase, K-feldspar, hornblende, biotite, augite).

Table 1: Optimum pH for several *Thiobacillus* species.

<i>T. intermedius</i>	pH 1.9 to 7.0
<i>T. thiooxidans</i>	pH 2.0 to 3.5
<i>T. ferrooxidans</i>	pH 2.5 to 4.0
<i>T. thioparus and T. denitrificans</i>	pH 7.0 to 8.5

### Weathering

Calcium and magnesium carbonate minerals dissolve rapidly, and their presence and dissolution can maintain circumneutral pH conditions despite the presence and oxidation of iron sulfide minerals. Relative to calcium and magnesium carbonate minerals,

silicate minerals dissolve very slowly and will maintain neutral pH conditions only if the iron sulfide content is low (12). The rate of silicate mineral dissolution increases with increasing mineral surface area and decreasing pH (13).

Much of the existing research on rates of mineral dissolution has been conducted on pure minerals. In rocks, these minerals are present in a matrix of minerals. This occurrence affects the surface area of individual minerals available for reaction (dependent on grain size and mode of occurrence), their reactivity as influenced by surface coatings, and the composition of solutions contacting the minerals. Even for an individual lithology stored in a pile, these variables will change with time (14).

Weathering can have at least two counter influences on slope stability. First, dissolution of feldspars, biotite and other silicate minerals results in reduction of the silicate mineral grain size and generation of clay minerals, both of which can adversely affect rock-pile stability. Second, iron oxyhydroxides generated by pyrite oxidation, as well as other reaction products, can aid in the cementation of soil grains. Formation of such aggregates will tend to increase rock-pile stability. Both of these processes appear to be operational at the mine site and have been observed in many mining sites. An example increasing rock pile stability, often mentioned by a colleague, is that after twenty years, a mine-rock pile located in the mining zone at the Bingham Copper Pit had been so strongly cemented it had to be drilled and blasted to be removed. Cementing mechanisms also occurs in less arid areas.

Geochemical environments (15) can be described as follows:

#### **Oxic Environments**

- Dissolved oxygen > 30  $\mu\text{M}$
- $\text{Mn}^{2+}$  below detection

#### **Suboxic Environments**

- Dissolved oxygen > 1  $\mu\text{M}$ , < 30  $\mu\text{M}$
- $\text{Fe}^{2+}$  below detection
- $\text{Mn}^{2+}$  detectable

#### **Anoxic Environments**

- Dissolved oxygen < 1  $\mu\text{M}$ )
- Anoxic – Sulfidic
- $\text{H}_2\text{S}$  > 1  $\mu\text{M}$
- Anoxic – Non-sulfidic
- $\text{H}_2\text{S}$  < 1  $\mu\text{M}$

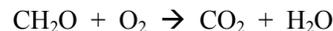
#### **Post-Oxic**

### **Methanic**

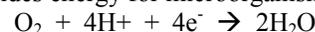
These environments are based on measured dissolved oxygen and  $\text{H}_2\text{S}$  and strongly tied to redox reactions. Concentrations of ions, Eh, and pH have major effects on microorganisms and can control the formation of authigenic minerals.

Iron oxyhydroxides generated by pyrite oxidation, as well as other reaction products, can aid in the cementation of soil and sand materials. Formation of these types of aggregates would tend to increase rock pile stability. Ferricretes – represent iron transported by reducing acid waters and precipitated as oxyhydroxides at higher Eh (above 0.4) and/or pH (above 6) more oxidizing, less acidic conditions. These conditions represent major shifts in redox chemistry and possibly in the site microbiology. Both weathering and microbial actions and influences can have counter influences on slope stability. It will be interesting to examine the microbial populations present in these areas and the possible contributions they may have to rock pile stability and/or instability.

The basic reaction for oxidizing organic matter



Oxygen is an electron acceptor, and reducing the oxygen provides energy for microorganisms.



The higher the redox potential, the higher the energy yield, the more negative is the Gibbs free energy and the greater the benefit to microorganisms utilizing the energy.

#### ***Sequence of Reduction Reactions***

If a system is effectively a closed system and there is abundant carbon to support microbial activity, then there is a well defined sequence of reduction or redox reactions affecting the inorganic species present in the system. For each of the major redox elements there is a specific range of pE for the initiation of the reduction of the element; pE is a measurement of the tendency of an environment to accept or donate electrons. The half reactions presented in Table 2 are coupled with the oxidation of carbon. In this way, the redox elements act as electron acceptors in carbon oxidation.

In oxygen rich environments, aerobic microorganisms use oxidized forms of Fe, S, Mn, and

some metal oxianions. Nitrate respiration below pE 8 produces products that include  $\text{NO}_2^-$ ,  $\text{N}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{NH}_4^+$ , and other metal oxianions with denitrification producing nitrogen gases. Solid phase manganese reductions can occur in the presence of  $\text{NO}_3^-$ . However, solid phase iron reduction, does not occur in the presence of  $\text{NO}_3^-$  or  $\text{O}_2$  and the Mn:Fe ratio is an indication of whether a denitrifying environment is present. Anaerobic microbes produce products that include  $\text{H}_2\text{S}$ ,  $\text{HS}^-$ ,  $\text{S}_2\text{O}_3^{2-}$ . Changes in pE induce both chemical reduction sequences as well as sequences in microbial ecology.

- **Aerobic microorganisms** that utilize oxygen do not function below pE of 5
- **Denitrifying bacteria** function in the pE range of +10 to 0
- **Sulfate-reducing bacteria** live at pE's below 2

This leads to the concept of redox ladders. Redox ladders assume that redox conditions are relatively constant and the factors controlling the abundance of electron donors and acceptors determine the Eh. The different steps shown are usually catalyzed by microorganisms with organic carbon being the most common electron donor. Electron acceptors include dissolved oxygen and nitrate and other electron acceptors can originate in weathered rock materials such as sulfate, ferric iron, manganese, carbon dioxide and metal oxianions.

Oxidized metals such as selenium, arsenic, sulfur, phosphate, potassium, magnesium, calcium, manganese, iron, uranium, cadmium, copper, and zinc can be used as electron acceptors by microorganisms and are reduced in the process. Mining rock piles and process waters vary greatly in the types of microbial populations, ions present, pH, redox, temperature, salinity, and carbon and nitrogen available as nutrients. Figure 1, and Tables 2 & 3 describe some environmentally important concepts and redox reactions for both chemical and microbial processes.

Microbial, geochemical, and geophysical correlations will be examined to determine if these correlations can be related to effects of the microbiology on rock pile weathering. Characterization of site materials and microbiology should also provide insight into methods for increasing rock pile stability and preventing or enhancing water penetration into the rock pile.

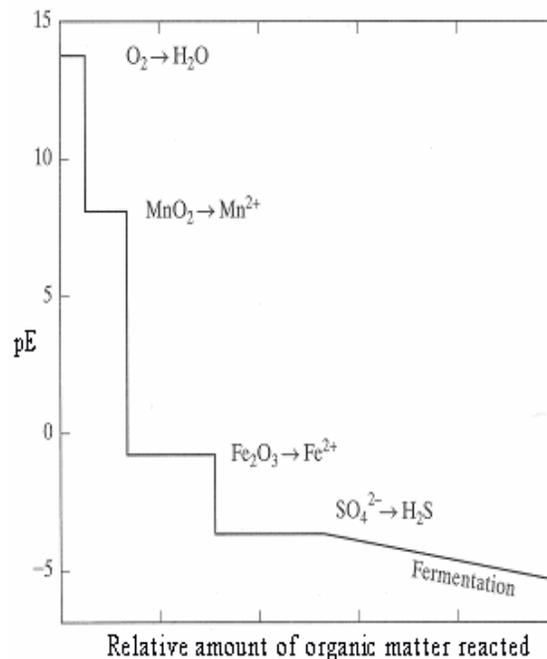


Figure 1. Redox ladder showing sequential utilization of electron acceptors maintaining a redox potential at specific intervals until that acceptor is gone.

Table 2. Some important redox reactions in soil and weathering mine rock pile environments.

Rxn	$E_h^0$ (volts)
$\text{Mn}^{3+} + e^- = \text{Mn}^{2+}$	1.51
$\text{MnOOH}_{(s)} + 3\text{H}^+ + e^- = \text{Mn}^{2+} + 2\text{H}_2\text{O}$	1.45
$1/5\text{NO}_3^- + 6/5\text{H}^+ + e^- = 1/10\text{N}_{2(g)} + 3/5\text{H}_2\text{O}$	1.245
$1/2\text{MnO}_{2(s)} + 2\text{H}^+ + e^- = 1/2\text{Mn}^{2+} + \text{H}_2\text{O}$	1.23
$1/4\text{O}_{2(g)} + \text{H}^+ + e^- = 1/2\text{H}_2\text{O}$	1.229
$\text{Fe}(\text{OH})_{3(s)} + 3\text{H}^+ + e^- = \text{Fe}^{2+} + 3\text{H}_2\text{O}$	1.057
$1/2\text{NO}_3^- + \text{H}^+ + e^- = 1/2\text{NO}_2^- + 1/2\text{H}_2\text{O}$	0.834
$\text{Fe}^{3+} + e^- = \text{Fe}^{2+}$	0.711
$1/2\text{O}_{2(g)} + \text{H}^+ + e^- = 1/2\text{H}_2\text{O}_2$	0.682
$1/8\text{SO}_4^{2-} + 5/4\text{H}^+ + e^- = 1/8\text{H}_2\text{S} + 1/2\text{H}_2\text{O}$	0.303
$1/6\text{N}_{2(g)} + 4/3\text{H}^+ + e^- = 1/3\text{NH}_4^+$	0.274
$1/8\text{CO}_{2(g)} + \text{H}^+ + e^- = 1/8\text{CH}_{4(g)} + 1/4\text{H}_2\text{O}$	0.169
$\text{H}^+ + e^- = 1/2\text{H}_{2(g)}$	0.000

Organics	Oxygen Status	Dominant Microbial Reaction	Components Removed (Aqueous Phase)	Components Added (Aqueous Phase)	Components Added (Solid Phases)
Almost None	Oxic	Aerobic Respiration	O <sub>2</sub>	HCO <sub>3</sub> <sup>-</sup>	Metal Oxides
	Sub-Oxic		Dissolved Metal Ions	NO <sub>3</sub> <sup>-</sup> H <sub>3</sub> PO <sub>4</sub> H <sup>+</sup>	Hematite Goethite Ferrihydrite Birnessite
Present	Anoxic Sulfidic	Sulfate Reduction	SO <sub>4</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Metal Sulfides,
			Dissolved Metal Ions	HS <sup>-</sup> H <sub>3</sub> PO <sub>4</sub> H <sup>+</sup>	Pyrite Mackinawite Chalcocite Rhodochrosite
Low (Minor)	Post Oxic	Nitrate, MnO <sub>2</sub> , Fe (III) Reduction	NO <sub>3</sub> <sup>-</sup>	NH <sub>3</sub>	Silicates
			Dissolved Metal Ions	N <sub>2</sub> Fe <sub>2</sub> <sup>+</sup> (Fe <sub>3</sub> <sup>+</sup> ) Mn <sub>2</sub> <sup>+</sup>	Siderite Vivianite Rhodochrosite

Figure 3. Some components removed and added to rock pile and soil environments that are influenced by site geochemistry and microbiology (16).

### Microbial Characterization

#### Sampling

Defining the site microbiology entails collecting, isolating, and characterizing microbes from samples collected at specific sites, with specific lithologies and geochemistry. In order to evaluate the microbial influence on the rock pile stability, microbiological samples from site rock pile trenches, associated soils and rock outcrops, crust materials and rock pile vents areas were collected. Laboratory examination of these materials is underway and it is planned to compare microbial populations of the collected samples with microbial starting and ending populations in future accelerated weathering studies. Laboratory microbial characterizations will provide site microbial profiles of selected weathered and un-weathered materials, allowing correlations to be made from the actual site to the accelerated weathering tests.

Microbial enumeration and characterizations underway will define the site microbiology in a general manner and allow correlation of the overall site microbiology with the other site specific geochemical and geophysical characterizations. That is, the microbes characteristically found with these particular rock types or mineralogies and

geochemistry. This may also allow the formation of a base microbial profile to describe microbial activity and or populations in various other site environments. This information will provide relationships needed to examine and assess an overall picture of relationships between specific site geochemistry, microbiology, accelerated humidity cell weathering tests, and models being developed to determine the interplay of these factors in rock pile weathering and rock pile slope stability. Additionally, these assessments may provide insight into rock pile stability that will allow modification of existing and future rock piles to achieve maximum stability with time.

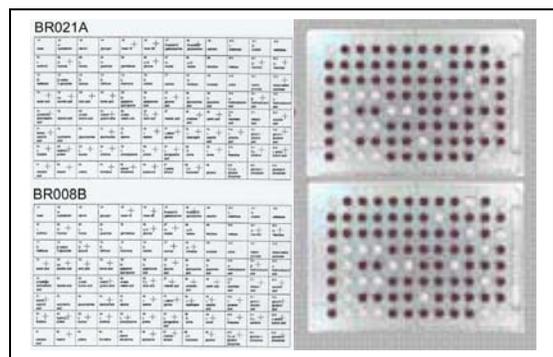
#### Microbial Culturing and Identification

Microbial culturing and identification will be conducted in a manner allowing the determination of the simultaneous presence of multiple bacterial types including: acidophilic, autotrophic iron- and sulfur-oxidizing bacteria such as *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Sulfolobus acidocaldarius*, total heterotrophs, and total iron and sulfate reducing bacteria. This is needed because *T. ferrooxidans* has often been used as the model bacterium for studying iron and pyrite oxidation and it is common to find that acid production by *Thiobacillus ferrooxidans*, *Leptospirillum sp.*,

*Sulfolobus acidocaldarius* and similar species can predominate at the lower pH's; below 3.5. It is also believed that these and other bacterial species play an important role in both mineral leaching and dissolutions that provide opportunities for re-precipitation and crust formation on the surface and within the rock pile. Micro-environmental conditioning, that initially starts lowering the pH below ~6.0, sets-up the environmental conditions required for acid generation that can be dominated by these species. Heterotrophic and other bacterial species can also delay the onset of microbial mediated acid production and/or moderate its effects. These will be determined through various culturing and characterization procedures, e.g., Figures 2 and 3.



**Figure 2.** Reddish-orange colonies of site *Thiobacillus ferrooxidans* growing on an acidified medium containing iron (II). Color due to oxidation of iron (II) iron to (III) ions.



**Figure 3.** Example of a BIOLOG™ plate showing the profile of two different microbes; population metabolic profiles can also be generated with similar analysis.

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