MiMi
Mitigation of the environmental impact from mining waste

The MISTRA-programme MiMi
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MiMi - Sulfide oxidation in mine waste deposits
A review with emphasis on dysoxic weathering

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Preface

Beginning January 1998, the Swedish Foundation for Strategic Environmental Research (MISTRA) began funding the research program *Mitigation of the Environmental Impact from Mining Waste* (MiMi) for an initial period through the year 2000. The MiMi program will concentrate on finding new and improved methods to mitigate the environmental problems related to mining operations and the disposal of mining wastes. State-of-the-art knowledge and experience in different scientific and engineering disciplines will form a starting point. The MiMi program will establish a close cooperation between different research groups, industry, environmental authorities and engineering specialists in order to find efficient and practical methods for remediation.

Within the MiMi program, the subproject on *Weathering and Redox Processes* is initiated with this review of the most recent research findings in the area of sulfide weathering processes in sulfidic mine waste deposits. This study includes both a literature search and a survey of Swedish and international experts, so as to include, as much as possible, the enormous amount of data in this field which is not widely distributed in international journals. The goals of this review are (1) to address the current data available on sulfide oxidation under dysoxic and anoxic conditions and (2) to propose areas of focused research within the MISTRA research program *Mitigation of the Environmental Impact from Mining Waste*. The proposal for focused research in the MiMi program will be addressed in a statement under separate title.

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1 Introduction

1.1 Sulfide mineral oxidation

Where ever mining activities expose sulfide mineral deposits and extract base and precious metals from sulfidic ore bodies, the atmospheric oxidation of sulfide minerals is a potential source of acid mine drainage. Pyrite (FeS_2) is the most common sulfide mineral present in sulfide ore deposits, and is thus the most common source of acid mine drainage from mine workings and mine waste deposits. In the presence of atmospheric oxygen, pyrite oxidation can be written as:

$$FeS_{2(s)} + 7/4O_{2(g)} + H_2O_{(aq)} \Rightarrow Fe^{2+}{}_{(aq)} + 2SO_4{}^{2-}{}_{(aq)} + 2H^{+}{}_{(aq)} \quad (1)$$

This equation is generally accepted as the overall reaction for pyrite oxidation by oxygen, even though the reaction in fact consists of a series of complex subreactions with reaction mechanisms that, in some cases, are not completely understood. Pyrite oxidation is a natural process that occurs in many 'pristine' environments free from mining activities. However, pyrite oxidation in mine waste deposits is enhanced because of the greater accessibility of air through mine wastes relative to the unmined mineralizations, and the greater surface area of sulfides subsequent to ore processing.

In addition to atmospheric oxygen, other oxidants may contribute to the oxidative dissolution of sulfide minerals. Ferric iron is the most important oxidant (Nordstrom and Southam, 1997), while under certain conditions, nitrate may also function as an electron acceptor (Postma *et al*., 1991). In the continued presence of oxygen, ferrous iron produced during pyrite oxidation (equation 1) will oxidize to ferric iron:

$$Fe^{2+}{}_{(aq)} + 1/4O_{2(g)} + H^{+}{}_{(aq)} \Rightarrow Fe^{3+}{}_{(aq)} + 1/2H_2O_{(aq)} \quad (2)$$

Ferric iron may subsequently hydrolyze and precipitate as ferric oxyhydroxide:

$$Fe^{3+}{}_{(aq)} + 2H_2O_{(aq)} \Leftrightarrow FeOOH_{(s)} + 3H^{+}{}_{(aq)} \quad (3)$$

Ferric iron is a strong oxidant of pyrite, such that at low pH (< 4.5) and under sterile conditions, it is widely accepted that Fe^{3+} oxidizes pyrite much faster than O_2 (*cf*. equation 1):

$$FeS_{2(s)} + 14Fe^{3+}{}_{(aq)} + 8H_2O_{(aq)} \Rightarrow 15Fe^{2+}{}_{(aq)} + 2SO_4{}^{2-}{}_{(aq)} + 16H^{+}{}_{(aq)} \quad (4)$$

This reaction also proceeds more rapidly than the abiotic oxidation of dissolved Fe^{2+} to Fe^{3+} (equation 2). For this reason, ferrous iron oxidation (equation 2) is considered the rate-limiting step in abiotic pyrite oxidation.

Mine waste deposits are far from sterile and contain an abundance of bacteria, including iron- and sulfide-oxidizing bacteria such as *Thiobacillus ferrooxidans*. The presence of *T. ferrooxidans* and other bacteria can accelerate the rate of Fe^{2+} and sulfide mineral oxidation by O_2 by several orders of magnitude. For both the abiotic and microbial processes, sulfide oxidation will be limited by the availability of oxygen as either a direct oxidant of sulfide (equation 1) or for the oxidation of ferrous iron (equation 2).

1.2 Prevention of acid mine drainage

As the oxidation of pyrite is either directly (by oxygen) or indirectly (by ferric iron) driven by the accessibility of oxygen to sulfide surfaces or oxidation horizons, conventional techniques for the prevention of acid mine drainage seek to minimize the transport of oxygen to the sulfide minerals. As the movement of oxygen into mine tailings generally proceeds by diffusion (advection and convection may play greater roles in rock waste dumps), covering the mine waste with water or water-saturated layers results in a reduction of O_2 diffusion into the wastes, thereby limiting sulfide oxidation (Nicholson *et al*., 1989). Sulfide oxidation is limited since the diffusion coefficient for oxygen in air is greater by a factor of 10^4 than the diffusion coefficient for oxygen in water (Evangelou *et al*., 1998), thus limiting the access of O_2 to sulfide surfaces.

Other techniques for limiting sulfide oxidation have been tested, such as the addition of bactericides to the wastes (Parisi *et al*., 1994; Schippers *et al*., 1998), but these are not common prevention techniques. Novel prevention technologies, such as the passivation of pyrite surfaces with ferric phosphate (Evangelou, 1994; Kalin *et al*., 1997) or silica (Evangelou et al., 1998) surface precipitates, have been tested in bench-scale experiments.

In addition to restricting oxygen diffusion in waste dump pore spaces, a water-saturated barrier also imposes an upper limit on oxygen concentrations for sulfide oxidation. The solubility of oxygen in water will determine its maximum concentration in the deposit porewater. For a given temperature, the variation of oxygen solubility with partial pressure obeys Henry's law:

$$[O_{2\,(aq)}] / pO_2 = k_H \qquad (5)$$

where k_H is Henry's law constant (k_H = 1.26 mol m^{-3} atm^{-1} at 25°C for O_2; Sposito, 1989). Atmospheric air contains O_2 at a partial pressure (pO_2) of 0.209 atm (also: pN_2 = 0.781 atm, pAr = 0.0093 atm, $pCO_2 = 3.1\times10^{-4}$ atm), so the solubility of oxygen in water at 25°C is 260 µM (~8.5 mg O_2 L^{-1}). As shown in Figure 1, this solubility increases with decreasing temperature. In this study, conditions characterized by $[O_{2\,(aq)}] > 0.5$ mg L^{-1} are considered *oxic*, and by $[O_{2\,(aq)}] < 0.1$ mg L^{-1} *anoxic*. The intermediate region (0.1 mg L^{-1} < $[O_{2\,(aq)}]$ < 0.5 mg L^{-1}) is termed *dysoxic* (*cf. dysaerobic*; Rhoads and Morse, 1971; Raiswell and Berner, 1985).

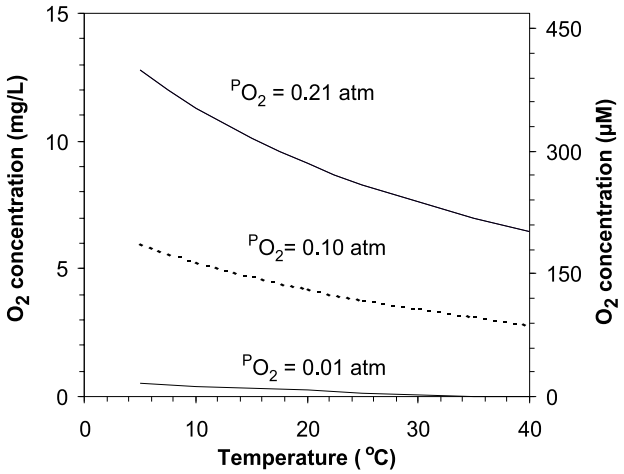

Figure 1: O_2 solubility in water as a function of temperature and O_2 partial pressure.

1.3 Purpose of study

As noted above, current measures for the minimization of sulfide oxidation commonly require the inundation of mine wastes or the installation of water-saturated covers. However, the complete inhibition of pyrite oxidation by flooding or covering may never be possible because of the difficulty in the total exclusion of O_2, thereby maintaining the availability of Fe^{3+} as an oxidant. For example, dissolved oxygen measurements from the flooded Saxberget tailings impoundment in Sweden (Ljungberg *et al*., 1997; Holmström, 1998) indicate that the water column is well-mixed down to depths as great as 9 m. Surface water is saturated with dissolved oxygen, and oxic conditions extend down to a depth of 15 mm into the underlying sediments (*i.e*. tailings). Within this sediment interval, oxygen is consumed by sulfide oxidation. In addition, the maintanence of even anaerobic conditions under a cover may not preclude bacterially-mediated sulfide oxidation. There is increasing evidence that iron-oxidizing bacteria such as *T. ferrooxidans*, under anaerobic or microaerophilic conditions, can fix CO_2 and use a variety of metabolic pathways for the acquisition of energy, including the oxidation of ferrous iron, elemental sulfur, and sulfide minerals (*e.g.* Goodman *et al*., 1981; Pronk and Johnson, 1992).

Because of trends in the mining industry for the sub-aqueous disposal of mine wastes, this study reviews the current data available on sulfide oxidation under dysoxic / anoxic conditions. There have been a number of recent studies that have thoroughly reviewed the field of sulfide oxidation, and the reader is referred to those papers for additional reading (Lowson, 1982; Nordstrom, 1982; de Haan, 1991; Otwinowski, 1994; Evangelou and Zhang, 1995; Nordstrom and Southam, 1997; Nordstrom and Alpers, 1998).

2 Microbial ecology of mine wastes

2.1 Nutrient strategies and microbial diversity

Mine dumps and tailings may seem to be extreme environments, but they can only be considered 'moderately' extreme since microorganisms show an abundance and variety of species, even though complex organisms are extremely few or absent (Goodman *et al*., 1981). Ledin and Pedersen (1996) present a review of the roles of microorganisms in mine waste systems. Mine waste deposits possess a complex microbial ecology consisting of numerous auto- and heterotrophic microorganisms, as well as fungi and prokaryotes (*e.g*. yeast). Heterotrophic organisms (*e.g*. humans, sulfate-reducing bacteria) gain energy from the oxidation of organic compounds; autotrophs (*e.g*. *Thiobacilli*) obtain carbon for cellular growth through carbon dioxide fixation. The environment in mine waste deposits is generally depleted in organic compounds, and autotrophic microbes are thus thought to dominate. However, organic compounds are produced as metabolic excretion products from living biomass, during cell lysis, and through the metabolic activity of fermentative bacteria that degrade carbohydrates and proteins in dead biomass (e.g. *Thiobacilli* from the upper horizons of the tailings). These organic products provide nutrients for the heterotrophic growth of, for example, sulfate-reducing bacteria (Pronk and Johnson, 1992).

In the upper oxygenated horizons of mine waste deposits, acidophilic *Thiobacillus ferrooxidans* thrives on the abundance of ferrous iron in this low pH environment, but other bacteria are certainly also present in significant number. In the anoxic zone, typically below the water table, viable sulfate-reducing bacteria (SRB) and acidophilic heterotrophs have been identified (Groudev *et al*., 1978; Goodman *et al*., 1981; Fortin *et al*., 1995, 1996; Fortin and Beveridge, 1997). Although SRB require organic carbon for an energy source, which is generally considered rather limited in mine tailings, low-molecular weight compounds such as acetate and formate have been measured in tailings at concentrations up to 1mM (Fortin *et al*., 1996). Mixed populations of sulfate-reducing and sulfide-oxidizing bacteria may certainly coexist in mine wastes (*e.g*. Fortin *et al*., 1996), and may even benefit from each other. For example, Groudev *et al*. (1978) suggested that hydrogen sulfide produced via sulfate reduction by SRB was used by sulfur-oxidizing *T. thioparus* as an energy source.

Although *T. ferrooxidans* is often considered the primary bacterial oxidant of pyrite in mine wastes, laboratory experiments have shown that mixed cultures oxidize pyrite, reduced iron, and sulfur faster than single-species cultures (Wakao *et al*., 1982; Lizama and Suzuki, 1989a, Hallmann *et al*., 1992; Sasaki *et al*., 1998). For example, the interaction of *Leptospirillum ferrooxidans* with chemoorganotrophic bacteria like *Acidiphilium* sp. may lead to an enhanced leaching by the iron-oxidizer, perhaps due to 1) the degradation of organic compounds by the *Acidiphilium* sp. which may otherwise be inhibitors for *L. ferrooxidans* growth, and 2) the enhanced production of exopolymers which increase the ability for cell attachment to pyrite surfaces (Hallmann *et al.*, 1992). In addition, there may be a number of iron-oxidizing strains in the mine waste deposits and the populations may vary with time. Southam and Beveridge (1993) employed lipopolysaccharide chemotyping to show that a single *T. ferrooxidans* strain colonized a young tailings deposit (Copper Rand, Canada), while an older tailings deposit (Lemoine, Canada) was colonized by six strains. This increase in diversity probably exists since it is almost impossible to maintain pure cultures in the natural environment.

In addition to their effect on sulfide oxidation and dissolution, bacteria in mine waste deposits are effective nucleation sites for metal adsorption and precipitation (Schultze-Lam *et al*., 1996; Fortin and Beveridge, 1997). Under oxic conditions, iron oxyhydroxide precipitates often form on the *T. ferrooxidans* surfaces (Southam and Beveridge, 1992; Fortin *et al*., 1995), providing a

large surface area for the further nucleation and the adsorption of other heavy metals. In contrast, iron sulfide may precipitate on the surfaces of sulfate-reducing bacteria under anoxic conditions below the water table or below a hard pan (Fortin *et al*., 1996), also providing a large surface area for trace metal adsorption (Fortin *et al*., 1995). Secondary metal sulfides of lower solubility (*e.g*. CuS, ZnS) may also precipitate in the tailings, or may form by cation substitution for iron in pre-existing iron sulfides (Blowes and Jambor, 1990; Machemer and Wildeman, 1992).

2.2 Iron- and sulfur-oxidizing bacteria

T. ferrooxidans is a chemolithotrophic (autotrophic) bacteria. It oxidizes reduced inorganic compounds including ferrous iron and sulfides, using oxygen as an electron acceptor, in order to gain energy for the reduction of carbon dioxide (carbon dioxide fixation). The outer membrane of *T. ferrooxidans* (Gram-negative bacteria) does not contain electron acceptors (Haddock and Jones, 1977) and functions instead as a passive diffusion barrier for the movement of reduced iron, such that Fe^{2+} oxidation by oxygen proceeds within the periplasm. The outward diffusion of ferric iron, which participates in sulfide oxidation, is balanced by the inward diffusion of ferrous iron (Nordstrom and Southam, 1997). The oxidation of iron by *T. ferrooxidans*, however, yields low levels of energy so that even under conditions of 100% metabolic efficiency, 18.5 moles of iron need to be oxidized to assimilate one mole of carbon (Silverman and Lundgren, 1959). Other common autotrophic bacteria in mine waste environments include *T. thiooxidans* and *L. ferrooxidans* (see Table 1).

Table 1: Bacteria and Archaea(*) spp. known to be associated with iron and/or sulfur oxidation. Adapted from Ledin and Pedersen (1996) and Nordstrom and Southam (1997). Abbreviation: CA chemolithotrophic autotroph

Bacteria	Energy (e^-) source	PH Optimum	Nutrient Strategy
Acidophilic mesophiles			
Thiobacillus ferrooxidans	H_2S, sulfide minerals, S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$, Fe^{2+}	2.0 – 3.0	Obligate CA
T. thiooxidans	S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$	2.0 – 3.0	Obligate CA
T. albertis	H_2S, $S_2O_3^{2-}$	3.5 – 4.0	Obligate CA
T. acidophilus	S^0, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$	2.5 – 3.0	Facultive CA
Leptospirillum ferrooxidans	Fe^{2+}, sulfide minerals	~ 2	Obligate autotroph
Acidiphilium spp.	Fe^{3+}, …	2.5 – 5.9	Chemo-heterotroph
Neutrophilic mesophiles			
T. thioparus	H_2S, sulfide minerals, S^0, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$	6.6 – 7.2	Obligate CA
T. denitrificans	H_2S, S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$	6.8 – 7.4	Obligate CA
T. intermedius	S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$	5.5 – 6.0	Facultive mixotroph
T. neopolitanus	H_2S, sulfide minerals, S^0, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$	6.5 – 6.9	Obligate CA
Acidophilic thermophiles			
*Acidianus brierleyi**	Fe^{2+}, S^0, sulfide minerals	1.5 – 2	Facultive CA
*Sulfolobus solfataricus**	S^0	~ 4.5	Facultive CA
*S. ambivalens**	S^0	--	Facultive CA
*S. acidocaldarius**	Fe^{2+}, S^0	2 – 3	Facultive CA

T. ferrooxidans activity is a function of pH, attaining a maximum at approximately pH 3.2 (Jaynes *et al*., 1984), which can be correlated to a maximum in the rate of pyrite oxidation. At pH < 2.5, pyrite oxidation is diminished because of physiological limitations on the bacterial production of Fe^{3+}, while above pH 3.5, the pyrite oxidation rate is limited by the decreasing solubility of Fe hydroxides (*cf*. equation 3). In studies of tailings deposits where neutral pH conditions persist because of the presence of carbonate minerals, populations of the neutrophilic species *T. thioparus* (see Table 1) were 1 – 6 orders of magnitude higher than the population of acid-tolerant *T. thiooxidans* and acidophilic *T. ferrooxidans* (Blowes *et al.*, 1998). A similar observation was reported by Goodman *et al*. (1981), where high concentrations of high pH, sulfur-oxidizing *Thiobacilli* (*i.e.* not *T. thiooxidans*) were measured at the top of the Rum Jungle dumps during the wet season, at levels 10 to 1000 times higher than numbers of *T. ferrooxidans*.

Pyrite oxidation is a strongly exothermic reaction, and the pore space temperature in mine waste deposits is generally greater than the atmospheric temperature (Nordstrom and Alpers, 1998, and references therein). Iron- and sulfur-oxidizing bacteria isolated from mine waste deposits are predominantly meso- or thermophiles (see Table 1), and the heat produced by sulfide oxidation contributes to advantageous growth conditions. *T. ferrooxidans* and *L. ferrooxidans* are mesophilic bacteria with optimal temperature in the range 28 – 30°C, growing at nearly the same rate between 20 and 40°C. In mixed cultures below 20°C, however, *T. ferrooxidans* will outgrow *L. ferrooxidans* because of its shorter generation time in this temperature range (Hallmann *et al*., 1992). *Thiobacilli* have been reported to oxidize ferrous iron, elemental sulfur, and sulfide minerals at temperatures down to 4°C (Ahonen and Tuovinen, 1989, 1990, 1991, 1992). Schrenk *et al*. (1998) showed that *L. ferrooxidans* was the dominating bacteria associated with ore body leaching at the Iron Mountain site (California, USA), where conditions were generally > 40°C and pH was 0.7 to 1.0. *T. ferrooxidans* was not directly associated with the oxidative dissolution of the main ore body, but was found rather in cooler, higher pH regions.

2.3 The role of bacteria

As shown on Table 1, most acidiphilic bacteria located in the upper horizons of mine waste deposits are capable of utilizing a variety of reduced sulfur species as electron sources for carbon fixation, while some may also use reduced iron (*e.g. T. ferrooxidans*, *L. ferrooxidans*). For the bacterial oxidation of sulfur and iron to occur in these environments, the reactions must be thermodynamically feasible. Although microorganisms cannot change thermodynamic relationships, their action as catalysts increases the rates of chemical reactions.

The oxidation of sulfide minerals in mine waste deposits can proceed via a number of pathways, as illustrated in Figure 2. Solid-phase sulfide may be abiotically oxidized by O_2 (pathway 1, equation 1); this has been a common pathway investigated in laboratory experiments. However, in natural systems, microorganisms are omnipresent mediators in sulfide oxidation reactions (*i.e*. pathways 2 – 5). The role played by iron- and sulfur-oxidizing bacteria is multi-faceted. In systems containing iron, *T. ferrooxidans* may exclusively oxidize Fe^{2+} to Fe^{3+} to gain energy, although reduced S is also a potential source of energy, with O_2 used as an electron acceptor (pathway 2). Ferric iron may oxidize pyrite (equation 4) with or without microbial mediation (Figure 2, pathways 4 and 3, respectively). Finally, other oxidants may be present in the mining environment, such as nitrate (pathway 5), and nitrate-reducing bacteria may contribute to pyrite oxidation.

There has been a long-standing debate on whether the bacterial oxidation of sulfides proceeds via an indirect or direct mechanism. With the indirect mechanism, ferric iron directly oxidizes the pyrite in a geochemical, abiotic reaction (equation 4, pathway 3). In contrast, the direct contact mechanism requires bacterial adhesion to the pyrite surface. The overall oxidation reaction takes on the form of equation 1, as oxygen is utilized as an oxidant by sulfur-oxidizing

bacteria. The various reaction pathways, as well as evidence for the indirect and direct mechanisms, will be discussed in later sections of this report.

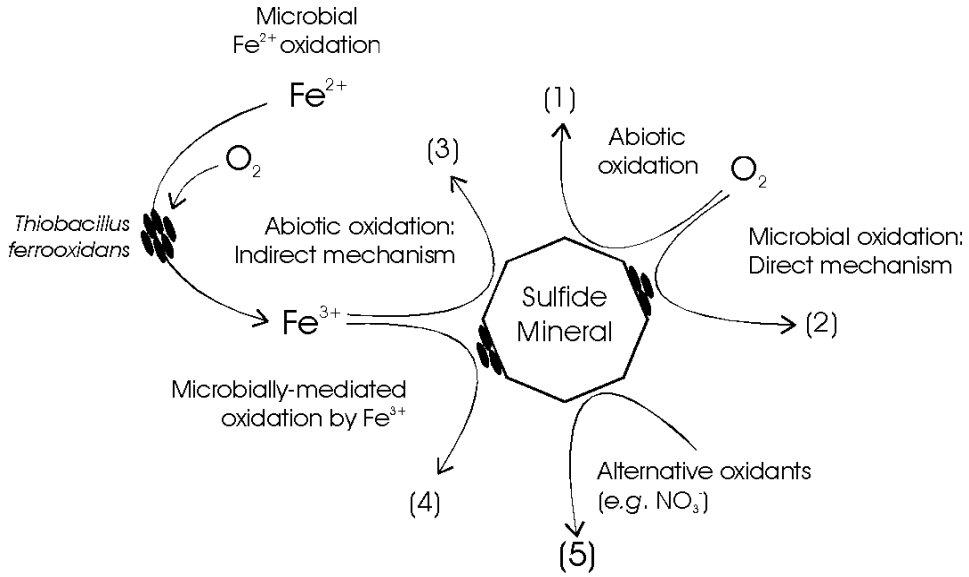

Figure 2: Alternative pathways in the oxidative weathering of sulfide minerals.

3 Ferrous iron oxidation

Evidence for ferrous iron oxidation is usually pervasive at mine sites: the formation of yellow-red iron precipitates is readily apparent in the upper horizons of mine deposits and in near-lying surface water. These precipitates are often composed of ferric oxides and oxyhydroxides (*e.g.* goethite, α-FeOOH; ferrihydrite, $Fe_5HO_8 \cdot 4H_2O$) and ferric sulfates [*e.g.* jarosite, $KFe_3(SO_4)_2(OH)_6$; schwertmannite, $Fe_{16}O_{16}(OH)_y(SO_4)_z \cdot nH_2O$] (Cornell and Schwertmann, 1996). The pH of this mine drainage water is generally quite acidic (pH < 3), as Fe^{3+} hydrolysis and precipitation releases protons to the environment (equation 3). Field observations of Fe^{2+} oxidation indicate that this is a relatively rapid process, with rates ranging up to 3.3×10^{-6} mol L^{-1} s^{-1} (Kirby and Elder Brady, 1998). Singer and Stumm (1968, 1970) reported an *abiotic* rate of 2.7×10^{-12} mol L^{-1} s^{-1} at pH values under 4, indicating that the abiotic Fe^{2+} oxidation rate is very slow under acidic conditions and is independent of pH. Singer and Stumm (1970) found that *T. ferrooxidans* increased the ferrous iron oxidation rate by 10^6 over the abiotic rate, from ~3×10^{-12} mol L^{-1} s^{-1} to about 2×10^{-7} mol L^{-1} s^{-1}. This indicates that the field-observed rates are for bacterially mediated oxidation. As is apparent here, the abiotic oxidation rate has limited relevance, because iron- and sulfur-oxidizing bacteria are ubiquitous in ground and surface waters.

Based on the compilation of a number of studies, an average microbial oxidation rate for ferrous iron was chosen by Nordstrom and Alpers (1998) of 5×10^{-7} mol L^{-1} s^{-1}. For *T. ferrooxidans*, if the nutrient concentrations become high enough, then the bacterial growth rate becomes constant and independent of nutrient concentration. Thus, the ferrous iron oxidation rate can become independent of the ferrous iron concentration and the rate becomes zero-order. During the lag phase and exponential growth phase of the bacteria, the growth is dependent on ferrous iron concentration and a first-order or pseudo-first-order reaction rate can be observed. For the rate of 5×10^{-7} mol L^{-1} s^{-1}, this assumes nutrient-saturated conditions and zero-order rates (Nordstrom and Southam, 1997).

As many prevention and remediation strategies for acid mine drainage involve acid neutralization (*e.g.* lime addition to tailings, limestone drains), it is also important to consider Fe^{2+} oxidation rates at circumneutral pH. Acidophilic bacteria such as *T. ferrooxidans* require acidic conditions for cell growth, such that at neutral pH the abiotic rate exceeds the microbial rate. Although the abiotic rate of Fe^{2+} oxidation rises rapidly at neutral to alkaline pH (Singer and Stumm, 1970), the Fe^{3+} concentrations and hence the rate of pyrite oxidation decreases greatly due to the precipitation of ferric hydroxide (equation 3). Regardless, recent findings have shown that Fe^{3+} is the preferred oxidant of pyrite even at circumneutral pH (Moses and Herman, 1991), and the major role of O_2 is to oxidize Fe^{2+} and thereby sustain the pyrite oxidation cycle (Evangelou and Zhang, 1995). In such environments, neutrophilic bacteria (*e.g.* *T. thioparus*) may dominate among the iron- and sulfur-oxidizing bacteria.

For the abiotic oxidation of ferrous iron by molecular oxygen, Lowson (1982) summarized rate constants for this reaction over wide pH, temperature, solution composition, and concentration ranges. At low pH (<2) and at 25°C, Lowson (1982) indicated that the reaction rate (equation 6) was generally second order with regard to ferrous iron concentration and first order with regard to oxygen partial pressure, with k varying from 6.5×10^{-7} to 4.0×10^{-6} M^{-1} atm^{-1} s^{-1}, depending on the particular electrolytic medium and the ionic strength used. With increasing pH, Lowson (1982) indicated that the reaction rate is more pH dependent and less dependent on Fe concentration, as indicated by equations 7 (pH range 2 to 5) and 8 (pH >5).

$$-d[Fe^{2+}]/dt = k\,[Fe^{2+}]^2\,{}^{P}O_2 \qquad \text{pH < 2 (Lowson), 3.5 (Singer and Stumm)} \quad (6)$$

$$-d[Fe^{2+}]/dt = k\,[Fe^{2+}]\,{}^{P}O_2\,[OH^-] \qquad \text{pH 2 - 5} \qquad (7)$$

$$-d[Fe^{2+}]/dt = k\,[Fe^{2+}]\,{}^{P}O_2\,[OH^-]^2 \qquad \text{pH > 5 (Lowson), 4.5 (Stumm and Lee)} \qquad (8)$$

At pH < 3.5, Singer and Stumm (1970) indicated that the reaction proceeds at a rate independent of pH, as shown in equation 6, for solutions dominated by sulfate and choride anions. For pH > 4.5, Stumm and Lee (1961) presented a rate law as shown in equation 8, where k is 8×10^{13} M^{-2} atm^{-1} min^{-1} at 25°C. As shown above, the rate of ferrous iron oxidation greatly increases above pH 5 where the rate becomes second order with respect to hydroxyl concentrations.

Pesic *et al*. (1989) constructed rate expressions for both the abiotic and biotic oxidation of Fe^{2+}. At sub-neutral pH, the abiotic oxidation of Fe^{2+} by O_2 can be expressed by equation 9, while the bacterially-mediated oxidation (*T. ferrooxidans*) is expressed by equation 10:

$$-d[Fe^{2+}]/dt = k\,[Fe^{2+}]^2\,{}^{P}O_2\,[H^+]^{-1/4} \qquad (9)$$

$$-d[Fe^{2+}]/dt = 1.62 \times 10^{11}\,[C_{bact}]\,[Fe^{2+}]\,{}^{P}O_2\,[H^+]\,e^{-(58.77/RT)} \qquad (10)$$

where concentrations are denoted by brackets, k is a rate constant (5.1×10^{-7} $M^{-1.25}$ atm^{-1} s^{-1} at 30°C and pH ca. 1; Matthews and Robins, 1972), ${}^{P}O_2$ is the oxygen partial pressure, C_{bact} is the bacteria concentration, T is absolute temperature, and R is the universal gas constant. According to Pesic *et al*. (1989), the abiotic oxidation of ferrous iron is rather insensitive to pH (equation 9), which is in agreement with the rate law established by Singer and Stumm (1970, equation 6; Lowson, 1982).

The relationship between these various abiotic and microbial rate laws is illustrated in Figure 3. According to various reported rate data (Stumm and Lee, 1961; Singer and Stumm, 1970; Lowson, 1982; Pesic *et al*., 1989), microbial ferrous iron oxidation will dominate in acidic environments where bacteria are abundant (*e.g*. mine wastes). A great proportion of the reported field rates in acid mine drainage environments fall within this area (Kirby and Elder Brady, 1998, and references therein). At higher pH, the abiotic rate law of Stumm and Lee (1961) is generally found to apply (equation 8). The elevated bacterially-mediated rates at low pH correspond to the pH region of optimal growth for *T. ferrooxidans* (*i.e*. pH ~3.2; see above) and other acidophilic bacteria common to mine wastes (*cf*. Table 1).

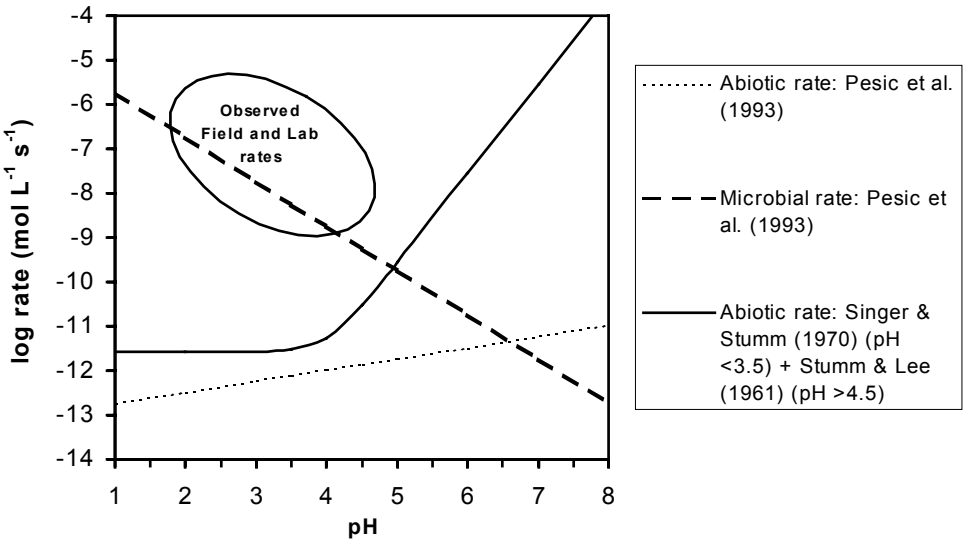

Figure 3: Abiotic vs. microbial rates of Fe^{2+} oxidation. 'Observed field and laboratory rates' applies to field rates observed in acid mine drainage (AMD) environments, and laboratory rates determined at conditions commonly observed in AMD environments (Kirby and Elder Brady, 1998). Rate curves drawn for $[Fe^{2+}]$ = 1 mM, $^{P}O_2$ = 0.21 atm., and $[C_{bact}]$ = 1.5 mg/mL.

4 Pyrite Oxidation

4.1 Abiotic pyrite oxidation mechanisms

Pyrite oxidation takes place when the mineral is exposed to an oxidant and humidity; this is a surface-controlled reaction. Pyrite oxidation involves chemical, microbiological, and electrochemical reactions that depend on site-specific environmental conditions. Thus, there is no single rate law available to describe the overall kinetics of pyrite oxidation, which is controlled by concentrations of O_2, Fe^{3+}, and CO_2, pH, bacterial activity, galvanic cells, temperature, and sulfide surface area and crystallinity. The oxidation rates for many different sulfides and with various oxidants have been determined under experimental conditions (see Nordstrom and Alpers, 1998, Table 6.3), although studies of pyrite oxidation by oxygen (Goldhaber, 1983; McKibben and Barnes, 1986; Moses et al., 1987; Nicholson et al., 1988; Moses and Herman, 1991) and ferric iron (Wiersma and Rimstidt, 1984; McKibben and Barnes, 1986; Moses et al., 1987; Moses and Herman, 1991) are the most numerous. It cannot be assumed that the reaction rates and mechanisms for the oxidation of other sulfide minerals are comparable with the rate of pyrite oxidation. Indeed, studies have shown substantial variations in the rate laws, reaction orders, and activation energies for the oxidation of galena, sphalerite, chalcopyrite, pyrrhotite, and arsenopyrite by ferric iron (Ahonen and Tuovinen, 1992; Rimstidt *et al*., 1994). Because of the prevalence of pyrite studies in the literature, this review will focus on pyrite oxidation.

The mechanism for pyrite oxidation is uncertain, partly because of the complexity of the Fe-O-S redox system. A number of studies suggest that it is the Fe-S bond that weakens during oxidation rather than the S-S bond in pyrite (Moses and Herman, 1991; Sato, 1992). The initial step in pyrite oxidation is thus the release of Fe^{2+} to solution, resulting in an S-rich surface. Under acidic conditions, the initial dissolution of pyrite has been confirmed by surface studies (Sasaki, 1994; Nesbitt and Muir, 1994; Sasaki *et al*., 1995) which indicate that a surface layer of disulfide, monosulfide, and polysulfides is gradually oxidized to thiosulfate and sulfate (Nesbitt and Muir, 1995; Guevremont *et al*., 1998). Pyrite oxidation is generally considered to be initiated by reaction with molecular oxygen. The reduction of water can be written as the following cathodic half-cell reaction:

$$O_2 + 4H^+ + 4e^- = 2H_2O \qquad E^o = 1.229\ V \qquad (11)$$

Sato (1992) proposed that pyrite is initially oxidized with the loss of two electrons:

$$FeS_2 = Fe^{2+} + S_2 + 2e^- \qquad E^o = -\ 0.805\ V \qquad (12)$$

Diatomic sulfur may subsequently oxidize to thiosulfate and sulfate. Sato (1960) indicated, however, that oxygen reduction is the sum of the formation (equation 13) and dissociation (equation 14) of hydrogen peroxide, and that H_2O_2 may actually be the initial pyrite oxidant.

$$O_2 + 2H^+ + 2e^- = H_2O_2 \qquad E^o = 0.682\ V \qquad (13)$$

$$H_2O_2 + 2H^+ + 2e^- = 2H_2O \qquad E^o = 1.772\ V \qquad (14)$$

Although oxygen is needed for the release of iron from pyrite lattices, the literature clearly indicates that the primary pyrite oxidant in both abiotic and biotic systems is ferric iron rather than molecular oxygen (Singer and Stumm, 1970; Goldhaber, 1983; Wiersma and Rimstidt, 1984; McKibben and Barnes, 1986; Moses *et al*., 1987). While it has been generally accepted that oxidation by ferric iron is only relevant at acidic pH, considering the low solubility of ferric iron at neutral pH (*cf*. equation 3), recent investigations (Moses *et al.*, 1987; Moses and Herman, 1991; Brown and Jurinak, 1989) demonstrated that Fe^{3+} may be a very effective oxidant at both acidic pH *and* circumneutral pH. The results of Moses *et al*. (1987) showed that pyrite oxidation in the presence of Fe^{3+}, as opposed to dissolved oxygen, is favored over the pH range 2 to 9, and that even a low concentration of Fe^{3+} (in the absence of O_2) was effective in oxidizing pyrite.

Moses *et al*. (1987) proposed that Fe^{3+} may interact with pyrite through the transfer of a hydroxy radical from the $Fe(H_2O)_5(OH)^{2+}$ complex to S_2^{2-}; OH^- in $Fe(III)(OH)_n^{3-n}$ on the pyrite surface is most likely involved in the electron transfer process between the disulfide and ferric iron. The increase in the abiotic pyrite oxidation rate as a function of pH may in fact be related to the increase in the availability of surface OH^- (Evangelou and Zhang, 1995). The model of Moses *et al*. (1987) was extended in the work of Moses and Herman (1991), who observed that ferrous iron is removed from solution by adsorption on pyrite surfaces. In their model (see Figure 4), the mechanism for abiotic pyrite oxidation involves the transfer of electrons from dissolved oxygen to pyrite via the adsorbed $Fe(H_2O)_6^{2+}$, which is cyclically oxidized to Fe(III) by O_2 and back again upon accepting another electron from the pyrite. Sulfur in pyrite is successively oxidized until it forms a metastable sulfoxy anion (*e.g*. thiosulfate) and detaches from the pyrite surface.

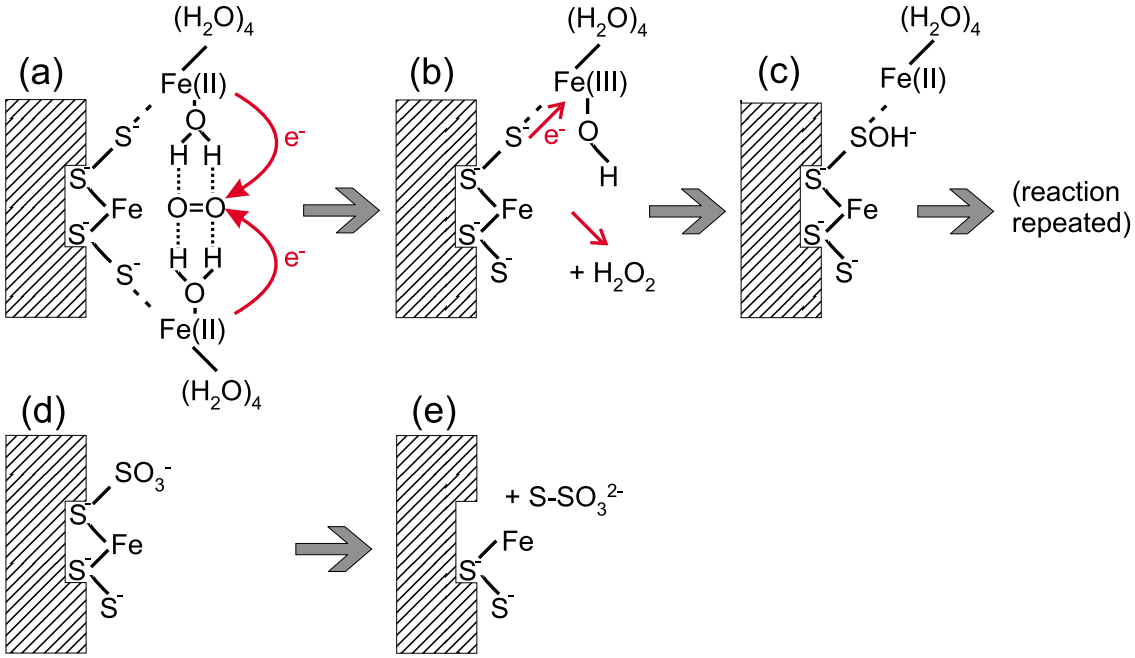

Figure 4: Schematic sequence of reaction steps for the oxidation of pyrite sulfur, adapted from Moses *et al*. (1987) and Moses and Herman (1991). (a) adsorbed Fe(II) forms a hydrogen-bonded termolecular complex with dissolved oxygen (O_2), and electrons are transferred from two Fe(II) atoms to O_2. The O_2 is reduced, producing H_2O_2, which may further react with sulfides until reduced to water. One of the six waters of hydration on Fe(II) has been lost to accommodate the bond for the Lewis acid-base surface complex. (b) Electrons are transferred rapidly from pyrite to the adsorbed Fe(III) that results from (a). (c) an OH group has been transferred from the adsorbed aquo-Fe(III) in response to the electron transfer shown in (b), creating a sulfhydroxyl group ($-SOH^-$). After rehydration, the Fe(II) in step (c) can be oxidized again as in step (a), resulting in the generation of progressively more oxidized S species on the pyrite surface. As the sequence progresses, sulfhydroxyl groups are dehydrated, resulting in sulfoxy groups:

$$-- S(OH)_2^- \Rightarrow -- SO^- + H_2O$$

The above reactions are repeated until a SO_3 group remains (d). This detaches from the surface (e) with an adjacent S atom, releasing thiosulfate ($S_2O_3^{2-}$) to solution.

The above-discussed model of pyrite oxidation (Figure 4) by O_2 and $Fe^{3+} – Fe^{2+}$ redox cycling was further refined by Eggleston *et al*. (1996) and Guevremont *et al.* (1998) in a detailed study of pyrite surface structure. In the model by Eggleston *et al*. (1996), electron transfer occurs between Fe^{2+} in sub-surface pyrite and Fe^{3+} in surficial ferric oxide layers; the oxide-like surface species act as intermediates between the underlying pyrite and O_2. The results of Guevremont *et al.* (1998) supported this model, and further suggested that nonstoichiometric regions of pyrite (*i.e*. sites of sulfur vacancy), which are readily oxidized by H_2O even in the absence of O_2, form the $Fe^{3+} – O$ surface phases that facilitate oxidation of the surrounding stoichiometric pyrite surface in the H_2O/O_2 environment.

The greater reaction rate of Fe^{3+} with pyrite, relative to O_2, has been explained by Moses *et al.* (1987) and Luther (1987). Relative to dissolved ferric iron, O_2 is a less likely oxidant, since the probability for a direct reaction between paramagnetic O_2 and diamagnetic FeS_2 is low (i.e. the reaction is spin restricted; Moses *et al*., 1987). Luther (1987) explained, using molecular orbital theory, that the faster rate of pyrite oxidation by Fe^{3+} was the result of Fe^{3+} binding directly to the pyrite surface via the formation of a persulfido bridge, which facilitates the transfer of electrons from highest occupied molecular orbital of S_2^{2-} to the lowest unoccupied molecular orbital of Fe^{3+}. This type of electron transfer via a transition state intermediate (*i.e*. the persulfido bridge) is not possible with O_2. Moses and Herman (1991) supported the mechanistic theory of Luther (1987), as their data indicated that O_2 could react with pyrite, albeit more slowly than Fe^{3+}.

The presence of organic and carbonate compounds has been observed to increase the rate of pyrite oxidation. In the presence of organic ligands, Luther *et al*. (1992) observed an increase in pyrite oxidation with an increase in the concentration of soluble Fe^{3+}-organic complexes and with pH. This observation was explained as that an increase in the Fe^{2+} electron density after binding with oxygen also increases the potential of rapid Fe^{2+} oxidation when Fe^{2+} is in the form of a complex with a ligand containing oxygen as the ligating atom (see later discussion). Similar findings were reported by Evangelou *et al*. (1998a,b), where laboratory studies showed that abiotic pyrite oxidation increases with increasing CO_3^{2-} concentration. The formation of a ferrous-carbonate complex on the surface of pyrite was proposed as responsible for the increase in the pyrite oxidation rate. Infrared spectroscopic studies by Evangelou and Huang (1994) and Evangelou *et al*. (1998b) support the potential formation of pyrite surface-CO_2 complexes, with CO_2 present as either surficial bicarbonate (-HCO_3^-) or carboxylic groups (-COO^-). Pyrite surface CO_x complexes may enhance abiotic pyrite oxidation by promoting the formation of persulfide bridges between pyrite surfaces and ferric iron in solution. The conditions necessary for the formation of -HCO_3^- or -COO^- surface complexes appear to be circumneutral pH and the presence of CO_2 / HCO_3^- in solution.

4.2 Abiotic oxidation kinetics

The intrinsic rate of pyrite oxidation is a controlled by a surface reaction, while the apparent (observed) macroscopic rate is dependent on a certain oxidant's availability. For pyrite oxidation by O_2 and $Fe^{3+}_{(aq)}$, the abiotic rate of pyrite oxidation by Fe^{3+} at low pH significantly exceeds the rate by O_2. However, the rates of pyrite oxidation by the two oxidants converge as pH increases because of the combined effect of the increasing rate at which O_2 can recycle Fe and the decreasing solubility of Fe(III). The dissolution of amorphous $Fe(OH)_3$ is not rapid enough to sustain a maximum rate of pyrite oxidation as the Fe(III) is depleted. As the pH increases, pyrite oxidation becomes more dependent on the Fe-recycling reaction and less dependent on the reservoir of aqueous Fe^{3+} (Moses *et al*., 1987). Thus, according to Moses *et al*. (1987), the oxidation of Fe(II) by O_2 controls the apparent rate of pyrite oxidation from pH 2 - 9.

A general difficulty with many of the oxidation rate studies is that many were performed without an adequate measurement of sulfide surface area, which is required for a quantitative comparison of experimental reaction rates. However, a comparison of oxidation rates measured under similar conditions (pH ~ 2, [Fe(III)] = 10^{-3} *M*, ~ 25°C, oxygen in equilibrium with the atmosphere) shows that the abiotic rate of pyrite oxidation by ferric iron can be up to 2 – 3 orders of magnitude faster than by oxygen (*e.g.* 1.9×10^{-8} mol m^{-2} s^{-1}, Rimstidt *et al*., 1994, and 3.1×10^{-10} mol m^{-2} s^{-1}, McKibben and Barnes, 1986, respectively).

Rate laws – surface area dependence

Moses and Herman (1991) used a rate law which was independent of $[O_2]$ and $[Fe^{3+}]$ to model the oxidation of pyrite by oxygen and ferric iron:

$$r = k\,(\mathrm{A/V})^n \tag{15}$$

where k is the rate coefficient, n is a fitting parameter indicating order of reaction ($n \approx 1$ for their experiments), and A/V is the ratio of the total surface area to reaction solution volume. The results of their study indicated, however, that the reaction dependence on $[Fe^{3+}_{(aq)}]$ is not zero-order. For their studies at circumneutral pH, the rate coefficients for pyrite oxidation by O_2 (with and without added Fe^{2+}) were ca. 5×10^{-10} mol FeS_2 pyrite m^{-2} s^{-1}, while k for the anoxic oxidation of pyrite by ferric iron was 5 to 15×10^{-9} mol FeS_2 pyrite m^{-2} s^{-1}. In the absence of dissolved oxygen, however, Fe^{3+} was rapidly consumed from the solution.

Rate laws – pH, oxygen, and iron dependence

Pyrite oxidation kinetics are strongly linked to Fe^{2+} oxidation (*i.e.* Fe^{3+} activity) and bacterial activity. While a number of rate laws have been developed for the abiotic oxidation of pyrite, biotic rate laws have been more difficult to derive due to the greater number of reaction parameters. Williamson and Rimstidt (1994) developed rate laws for the abiotic oxidation of pyrite by molecular oxygen and ferric iron that are applicable over a wide range of solution compositions. For the oxidation of pyrite by ferric iron in the presence of dissolved oxygen (DO), Williamson and Rimstidt (1994) produced the rate law:

$$r = 10^{-6.07}\,\frac{\left[Fe^{3+}\right]^{0.93}}{\left[Fe^{2+}\right]^{0.40}} \tag{16}$$

where r is the rate of pyrite destruction in mol m^{-2} s^{-1}. This rate is only appreciably affected by ferrous and ferric iron, and was in the range $0.2 - 6 \times 10^{-7}$ mol m^{-2} s^{-1} under the conditions in their experiment ($\log Fe^{3+} / Fe^{2+} = +0.5$ to 1.5 with $[Fe^{3+}] \approx 10^{-3}$ *M*). However, in tailings material where there is active sulfide oxidation, $\log Fe^{3+} / Fe^{2+}$ is less than -3 and $[Fe^{3+}] < 10^{-4.5}$ *M* (*e.g.* Blowes *et al*., 1991), much lower oxidation rates ($\leq 10^{-10}$ mol m^{-2} s^{-1}) would be expected, according to equation 16.

An interesting result of Williamson and Rimstidt's study was that the oxidation of pyrite by ferric iron *in the absence of DO* (N_2 purged solutions) showed distinctly different behavior from the experiments performed in the presence of DO. In the absence of DO, the rate law is:

$$r = 10^{-8.58}\,\frac{\left[Fe^{3+}\right]^{0.30}}{\left[Fe^{2+}\right]^{0.47}\left[H^{+}\right]^{0.32}} \tag{17}$$

As in the former experiment with DO present, the rate is dependent on Fe^{3+} and Fe^{2+} concentrations. However, in the absence of oxygen, the rate is significantly dependent on the pH.

The relationship between Fe^{3+}/Fe^{2+} and rate suggests a correlation of the rate with the E_H of the solutions. Multiple linear regression modeling showed that the kinetics of the reaction of pyrite with ferric iron were strongly influenced by the E_H of the solution:

$$\log r = -19.17 + 12.93E_H + pH \qquad \text{(for solutions with DO present)} \qquad (18)$$

$$\log r = -12.70 + 6.10E_H + 0.37pH \qquad \text{(for } N_2\text{-purged solutions)} \qquad (19)$$

where E_H is defined by the Nernst equation (25°C):

$$E_H\,(V) = 0.770 + 0.0591 \log (Fe^{3+})/(Fe^{2+}) \qquad (20)$$

The two rate laws (equations 16 and 17) indicate that the rate of reaction between Fe^{3+} and pyrite is enhanced by the presence of DO at high Fe^{3+} / Fe^{2+} ratios, but the rate is faster in the absence of DO when Fe^{3+} / Fe^{2+} is low. As the order with respect to ferric iron is greatly different in these two rate laws, there is probably a difference in reaction mechanisms. As shown in Figure 5, below, the rates are roughly equal at log Fe^{3+} / Fe^{2+} ~ 0. However, as mentioned above, such ratios are generally much lower in acid drainage environments (Fe^{3+} / $Fe^{2+} < 10^{-3}$); at this level, the reaction rate under anoxic conditions is almost two orders of magnitude greater than the rate under oxic conditions. The results of Williamson and Rimstidt (1994) are, however, difficult to interpret, since the rate laws were established from the regression of data from two different experimental techniques as well as data from different investigations.

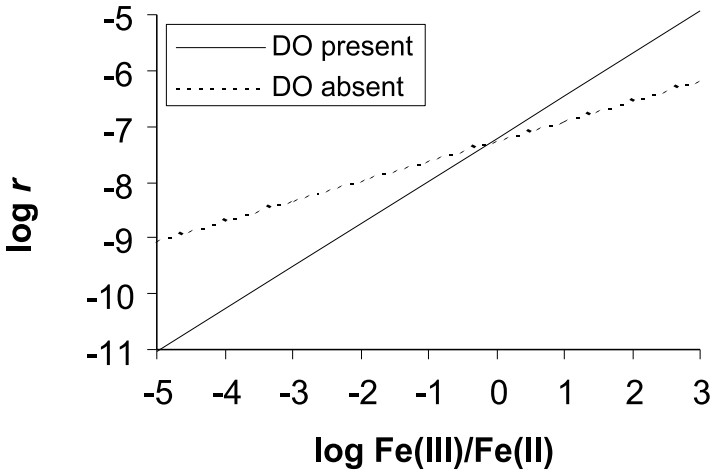

Figure 5: Oxidation of pyrite by ferric iron and in the presence and absence of dissolved oxygen (DO), at pH = 2. Adapted from Williamson and Rimstidt (1994).

As the rate of pyrite destruction was positively correlated with the concentration of the oxidant only, Williamson and Rimstidt (1994) concluded that electron transfer from the mineral to the aqueous oxidant is rate limiting (this is a chemical barrier to reaction, as opposed to a physical barrier such as molecular diffusion). Their study supported a reaction between pyrite and the aqueous oxidant as an electrochemical reaction involving nonsite-specific, multilayer adsorption of the oxidant for which the electron transfer is rate limiting.

The observations of Williamson and Rimstidt (1994) indicated that pyrite oxidation was not limited by the supply of O_2 in order to rejuvenate Fe^{2+} to Fe^{3+}, and that the faster pyrite destruction rate actually occurred in the absence of dissolved oxygen, albeit at low E_H.

Other studies of pyrite oxidation have revealed different rate laws. McKibben and Barnes (1986) demonstrated that the following rate laws could describe the rate of abiotic pyrite oxidation by ferric iron (r_{Fe3+}) and oxygen (r_{O2}):

$$r_{Fe3+} = -10^{-9.74}[Fe^{3+}]^{0.5}[H^+]^{-0.5} \qquad \text{(pH 1 – 2)} \qquad (21)$$

$$r_{O2} = -10^{-6.77}[O_2]^{0.5} \qquad \text{(pH 2 – 4)} \qquad (22)$$

where the reaction rate is in units of moles pyrite cm^{-2} min^{-1} and brackets denote molarity. For this study, $\log[Fe^{3+}]$ was varied from -0.3 to 0.5, $\log[H^+]$ from -1.0 to -2.0, and O_2 concentrations of 0.21 and 1.0 atm. were used. Otwinowski (1994) later remarked that the rate constants for equations 21 and 22 do not reproduce the results of McKibben and Barnes (1986), and that the rate constant should instead be $k_1 = 8.72 \times 10^{-7}$ $mol^{0.9}$ L m^{-2} s^{-1} and $k_2 = 2.83 \times 10^{-8}$ $M^{½}$ L m^{-2} s^{-1} at 30°C for the rate laws

$$d[Fe^{2+}]/dt = (15S/14V)\, k_1\, [Fe^{3+}]^{3/5}\, [H^+]^{-1/2} \qquad (23)$$

$$d[Fe^{2+}]/dt = (S/V)\, k_2\, [O_2]^{½} \qquad (24)$$

where S is pyrite surface area, V is solution volume, and $[O_2]$ is in *M*. For equations 23 and 24, the rates are given in terms of Fe^{2+} production (M s^{-1}).

Lowson (1982) reported that the abiotic oxidation of pyrite by O_2 can be described by the following equation:

$$-d[FeS_2]/dt = -4/15\, d[O_2]/dt = (S/V)\, k\, [O_{2(g)}]^n \qquad (25)$$

Nicholson *et al*. (1989) found that the oxidation reaction can be approximated by first-order kinetics (n=1) at low oxygen concentrations. Equation 25 was used by Elberling *et al*. (1994) to model abiotic pyrite oxidation using a shrinking core model, with $k = 5.9 \times 10^{-10}$ mol FeS_2 m^2 s^{-1}; and $[O_{2(g)}]$ initially at 9 mol m^{-2} (0.21 atm). Nicholson (1994) reported a linear decrease in the abiotic pyrite oxidation rate from ca. 5×10^{-9} mol m^{-2} s^{-1} when at equilibrium with atmospheric O_2, to less than 10^{-11} mol m^{-2} s^{-1} at O_2 concentrations of ~1 μM.

Nicholson *et al*. (1988) found the rate of abiotic pyrite oxidation at circumneutral pH to be non-linear with respect to oxygen concentration (see Figure 6). According to this study, the data were consistent with a mechanism involving equilibrium adsorption – desorption of O_2 on the pyrite surface, with a rate-limiting release of decomposition products from the pyrite surface. In a later study, Nicholson *et al*. (1990) demonstrated that the rate of pyrite oxidation at atmospheric O_2 levels in carbonate buffered media greatly decreased with time, because of the formation of ferric oxide surface precipitates.

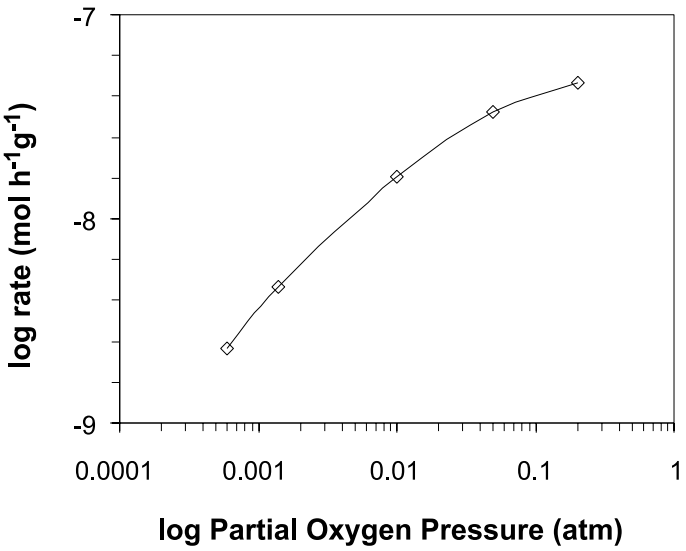

Figure 6: A log-log plot of the abiotic pyrite oxidation rate versus oxygen concentration from Nicholson et al. (1988), for circumneutral pH conditions.

4.3 Bacterially-mediated pyrite weathering

In natural systems, the direct bacterial oxidation of pyrite surfaces will compete with the abiotic oxidation by ferric iron (*cf.* Figure 2). The dominance of one respective process is governed by environmental conditions, as illustrated in studies of ^{18}O fractionation in atmospheric oxygen, meteoric water, and aqueous sulfate (Taylor *et al*., 1984a,b). These investigations indicated that in well-aerated environments, most of the ^{18}O in sulfate is derived from atmospheric oxygen. This demonstrated that microbial sulfide oxidation by oxygen was dominant, since 3½ of the 4 moles of oxygen in sulfate are derived from O_2 (see equation 1). In well-aerated systems, *T. ferrooxidans* oxidized pyrite by direct attack (equation 1) at a faster rate than via abiotic oxidation by Fe^{3+} (equation 4). However, in submersed anerobic environments or in aerobic sterile environments, the isotopic studies showed that H_2O was the principal source of ^{18}O in sulfate (Taylor *et al*., 1984a,b), and thus a different oxidation mechanism must dominate. As all of the oxygen in sulfate is derived from water during the chemical oxidation of pyrite by Fe^{3+} (equation 4), this was the dominating mechanism in the anaerobic systems.

Much of the debate on sulfide oxidation centers on the evidence for an indirect or direct oxidation mechanism (*e.g*. Arkestyn, 1979; Wakao *et al*., 1984; Sand *et al*., 1995; Schippers *et al*., 1996). Free-floating bacteria can catalyze the oxidation of ferrous to ferric iron in aqueous solution, and then ferric iron is the electron acceptor in the abiotic oxidation of pyrite (Figure 2). This is the 'indirect mechanism'. The 'direct contact mechanism' works by direct bacterial adhesion to the pyrite surface, where the sulfide surface is solubilized through hypothesized

enzymatic oxidation reactions (Ehrlich, 1981). Extracellular polymeric substances, composed primarily of lipopolysaccharides, mediate the contact between the bacterial cell and sulfide energy source, and play an important role in organic film formation and bacterium-substratum interactions.

Nordstrom and Alpers (1998) contend that the indirect mechanism is dominant, as abiotic Fe^{3+} oxidation rates generally exceed biotic rates (see following section), but there is evidence to the contrary. In a study by Bennett and Tributsch (1978), they confirmed that the chemical processes taking place on the pyrite surface due to oxidizing bacteria occur mainly in the region of contact between bacteria and pyrite; such phenomena were also noted in studies of the bacterial oxidation of sulfur and lead sulfide (Tributsch, 1976). In addition, they also suggested that the bacterial distribution on the surface is dependent on the crystal structure and the diversity in the crystal order (fracture lines, dislocations) of the pyrite. This suggests that it is more favourable for the bacteria to obtain their energy from solid surfaces that are characterized either by weaker chemical bonding or by an increased surface area along cracks or polycrystalline regions. It appears that *T. ferrooxidans* is capable of distinguishing between favourable and less favourable sites for energy extraction, selecting the site of attack according to the availability of nutrient.

The surface properties of *Thiobacilli* have a great bearing on their ability to mediate pyrite oxidation. This statement is not always applicable, however, as the surfaces are preconditioned by the media in which bacteria are cultured. *T. ferrooxidans* grown in ferrous-containing solutions exhibited different surface chemistry than those grown on minerals such as pyrite (Devasia *et al*., 1993) as indicated by hydrophobicity and electrophoretic mobility measurements. The bacilli grown on mineral sulfides developed a proteinaceous cell surface appendage that adhered to the solid surface whereas cells grown in ferrous iron solutions did not contain these characteristics. Furthermore, a study by Lizama and Suzuki (1991a) indicated a competitive inhibition of *T. thiooxidans* sulfur oxidation by other *T. thiooxidans* cells which compete with sulfur for the sulfur-binding sites of active cells. The nature of the cell-cell competitive inhibition is not clear but is probably related to the mechanism of sulfur oxidation which is thought to occur at the cell surface. Cell-sulfur adhesion has been shown to be essential for oxidation to take place, and is pH and energy dependent. Thus the surface of *T. thiooxidans* is especially suited for adhesion to sulfur and could possibly interact with the surface of another, similarly endowed cell, thus temporarily blocking sulfur-binding sites (Takakuwa *et al*., 1979).

In the discussion of direct or indirect sulfide oxidation mechanisms, it may be that both groups are correct, as both bacterial attachment and Fe^{3+} are required. A recent study by Gehrke *et al*. (1998) indicated that extracellular polymeric substances, which are apparently necessary for bacterial attachment to solid substrates, are important in the first steps in metal sulfide dissolution. Charge effects are involved in the attachment. Primary attachment to pyrite is mediated by Fe^{3+} ions complexed with the bacterial exopolymers, which allows for an electro-chemical interaction with a negatively charged pyrite surface ($pH_{i.e.p.}$ ~ 1.5, Bebie *et al*., 1998). *T. ferrooxidans* regenerates the Fe^{3+} ions and uses the energy for growth. Thus, although direct bacterial contact is required for this oxidation mechanism, the main bacterial contribution is to keep the iron ions in the oxidized state. The actual sulfide oxidation proceeds chemically. Consequently, the indirect leaching mechanism is supported by the data of Gehrke *et al*. (1998), in addition to other studies by Sand *et al*. (1995) and Schippers *et al*. (1996).

Rate laws for pyrite oxidation

Otwinowski (1994) derived rate laws for bacterial pyrite oxidation from the experimental data of Lizama and Suzuki (1989b). Under optimal conditions (T = 30°C, pH = 2.3), the production of ferrous iron from pyrite can be described as follows:

$$d[Fe^{2+}]/dt = (2S/7V)\ k\ [O_2]^{½} \quad (26)$$

where S is the pyrite surface area, V is the solution volume, and $[O_2]$ is given in *M*. The rate constant for the experimental conditions is $k = 8.49 \times 10^{-6}\ M^{½}\ L\ m^{-2}\ s^{-1}$ for a cell concentration of 1 mg cells / mL solution. Otwinowski (1994) also derived rate laws for the bacterial oxidation of pyrite by ferric iron, in the absence of oxygen, from the experimental data of Lizama and Suzuki (1989b). Under optimal conditions (T = 30°C, pH = 2.3), the production of ferrous iron from pyrite can be described as follows:

$$d[Fe^{2+}]/dt = (15S/14V)\ k\ [Fe^{3+}]^{3/5}\ [H^{+}]^{-1/2} \quad (27)$$

The rate constant for the experimental conditions is $k = 2.18 \times 10^{-4}\ M^{0.9}\ L\ m^{-2}\ s^{-1}$ for a cell concentration of 1 mg cells / mL solution.

4.4 Reaction rates

Abiotic, microbial, and field oxidation rates for similar geochemical conditions (pH ~ 2, T ~ 25°C) have been compiled by Nordstrom and Alpers (1998), and are presented in Table 2. Since the abiotic pyrite oxidation rate by Fe^{3+} is much faster than by O_2, the former reaction is also dependent on the rate for the oxidation of Fe^{2+} to Fe^{3+}. In natural systems containing iron-oxidizing bacteria, ferrous iron oxidation is rapid and is not a rate-limiting step in the overall oxidation of pyrite by ferric iron (equation 4); the rate of pyrite oxidation proceeds about as fast as the aqueous ferric iron can be produced from ferrous iron through microbial catalysis. However, as shown in Table 2, the microbial oxidation rate with oxygen appears to be almost an order of magnitude faster than the pyrite oxidation rate with ferric iron. As the uncertainties in these rates are not insignificant, current data suggests that there is no discernible difference between the oxidation rate of pyrite by ferric iron (abiotic) and by *T. ferrooxidans* utilizing O_2 as an electron acceptor (Nordstrom and Alpers, 1998).

Table 2: Comparison of abiotic, microbial, and field oxidation rates (pH ~ 2, T ~ 25°C). From Nordstrom and Alpers (1998).

Reaction/Process	Abiotic Rate	Microbial Rate	Field Rate
Fe^{2+} (aq) oxidation by O_2 [a]	3×10^{-12} mol L^{-1} s^{-1}	5×10^{-7} mol L^{-1} s^{-1}	5×10^{-7} mol L^{-1} s^{-1} [g] $<10^{-9}$ to 3×10^{-6} mol L^{-1} s^{-1} [h]
Pyrite oxidation by O_2 [b,d]	0.3 to 3×10^{-9} mol m^{-2} s^{-1}	8.8×10^{-8} mol m^{-2} s^{-1}	
Pyrite oxidation by Fe^{3+} [b,c]	1 to 2×10^{-8} mol m^{-2} s^{-1}		
Oxidation of Waste dump[e]			0.03×10^{-8} mol m^{-2} s^{-1}
Oxidation of Tailings[f]			20 to 60×10^{-8} mol m^{-2} s^{-1}

References:

[a] Singer and Stumm (1968, 1970), Lacey and Lawson (1977)

[b] McKibben and Barnes (1986), Moses and Herman (1991) (pH 7) Sasaki (1994)

[c] Rimstidt et al. (1994)

[d] Olson (1991)

[e] Ritchie (1994a,b)

[f] Elberling *et al*. (1993)

[g] Nordstrom (1985)

[h] Kirby and Elder Brady (1998)

Weathering rates, however, are scale dependent, such that rates measured under controlled laboratory conditions are often not equivalent to field rates (*c.f.* field rate vs. microbial rate, Table 2). Banwart *et al*. (1998) identified five parameters which contribute to the scale-dependence of sulfide oxidation rates: temperature, pore water pH, particle size distribution, mineral content, and water flow patterns. In their study, pyrite dissolution rates from batch tests and field observations at the Aitik mine site (Sweden) differed by approximately two orders of magnitude. It was found that a model including these five parameters could account for the upscaling from batch to field rates, with differences in temperature and particle size distribution accounting for most of the discrepancy.

The field oxidation rates for mine wastes presented in Table 2 are primarily based on the flux rates of oxygen depletion upon reaction with pyrite in mine waste (Ritchie, 1994a). There may be difficulties in translating oxygen or temperature profiles into flux rates, where the main problem is estimating the reactive surface area of the sulfides. For field systems, the microbial oxidation of aqueous ferrous iron is the fastest rate in the system and provides an upper limit to the pyrite oxidation rate; the lower limit is zero in the absence of water and oxygen. In the field, the pyrite oxidation rate is also dependent on reactive sulfide surface area, degree of crystallinity, and purity (*i.e*. solid solution substitutions), as well as air and water transport processes, microbial growth kinetics, microbial ecology, organic compounds, temperature gradients, secondary mineral formation, neutralization reactions, climatic patterns, and site-specific mine workings, waste dumps, and tailings (Nordstrom and Alpers, 1998). While bacterially-catalyzed pyrite oxidation proceeds relatively rapidly in the presence of oxygen, the overall rate of pyrite oxidation in a tailings pile is largely determined by the rate of oxygen transport to the sulfides (*i.e*. by advection, convection, and diffusion; Elberling *et al*., 1994; Nordstrom and Southam, 1997). For abiotic sulfide oxidation by ferric iron, the transport rate of ferric iron to sulfide surfaces is also an important component affecting the overall pyrite oxidation rate.

4.5 Oxidation products

The complete bacterial oxidation of disulfide (S_2^{2-}) in pyrite to sulfate involves the transfer of 14 electrons per mole pyrite (equation 1). Since the oxidation of S in pyrite requires the transfer of many electrons, various S species with progressively greater oxidation states are produced

during the intermediate steps. Examples of intermediate species include elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), and tetrathionate ($S_4O_6^{2-}$; see Figure 7). Elemental sulfur has been identified in mine waste deposits, but is generally associated with the oxidation of monosulfides such as pyrrhotite ($Fe_{1-x}S$; Bhatti *et al*., 1993) and not with pyrite, which only produces high yields of elemental sulfur at elevated temperatures (100 – 150°C; Lowson, 1982). The persistence of elemental sulfur is of some significance, as certain studies have indicated that *T. ferrooxidans* can couple the oxidation of S^0 to the reduction of ferric iron, in the absence of dissolved oxygen (Brock and Gustafson, 1976; see later discussion).

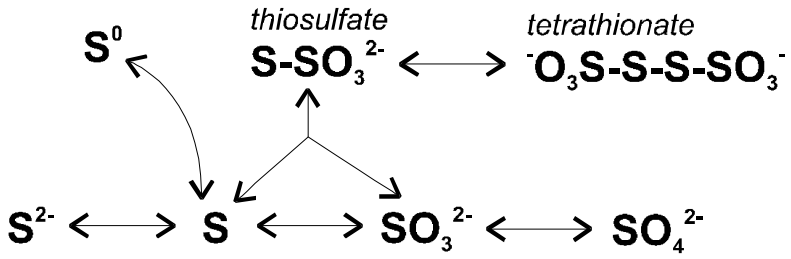

Figure 7: Bacterial oxidation of sulfide to higher oxidation states. Adapted from Suzuki *et al*. (1994).

In recent years, the production of oxidation products on pyrite and other sulfide surfaces has been investigated in a number of surface-sensitive studies, utilizing various methods such as X-ray photoelectron spectroscopy (XPS). Buckley and Woods (1987) concluded that iron tends to be easily leached from a pyrite surface producing an iron-deficient surface that does not have the properties of elemental sulfur unless prolonged strong acid attack is used. The presence of surficial elemental sulfur was confirmed in studies by Sasaki *et al*. (1995). Other studies (Sasaki, 1994; Nesbitt and Muir, 1994) have shown that initial dissolution under acidic conditions releases iron and produces a surface layer containing disulfide, monosulfide, and polysulfides. With increased oxidation, the levels of monosulfide decrease relative to disulfide and polysulfides, and thiosulfate and sulfate begin to form (Guevremont *et al*., 1998). This sulfur-rich surface should be energetically favorable for *T. thiooxidans*, and may explain their association with *T. ferrooxidans* (Norris, 1990; Sasaki *et al*., 1995).

The production of sulfur oxidation intermediates has been indicated by a number of studies (Moses *et al*., 1987; Suzuki *et al*., 1992, Chan and Suzuki, 1994; Schippers *et al*., 1999). For example, sulfur-grown *T. thiooxidans* cells have been shown to oxidize sulfide under acidic conditions, producing sulfuric acid with elemental sulfur and sulfite as possible intermediates. These intermediates accumulate when their oxidation is inhibited by NEM (*N*-ethylmaleimide; inhibits sulfur oxidation) and HQNO (2-*n*-heptyl-4-hydroxyquinoline *N*-oxide; inhibits sulfite oxidation). Suzuki *et al*. (1992) have presented direct evidence that sulfite is the oxidation product of sulfur in *T. thiooxidans* cells, when the further oxidation of sulfite is inhibited. Under certain conditions, sulfur is nearly stoichiometrically oxidized to sulfite ($S^0 + O_2 + H_2O \Rightarrow H_2SO_3$); i.e. the oxidation of sulfur to sulfite is totally dissociated from the oxidation of sulfite to sulfate. The accumulation of sulfite could lead to the formation of thiosulfate in the presence of sulfur ($S^0 + SO_3^{2-} \Rightarrow S_2O_3^{2-}$). The work of Chan and Suzuki (1994) showed that thiosulfate is oxidized by *T. thiooxidans* (S^0-grown) only at very acidic conditions with an optimum at pH 2.3. In the presence of HQNO, sulfite accumulated following oxidation of S^0 in thiosulfate ($S^0S^{IV}O_3^{2-}$), with a stoichiometry agreeing with $S_2O_3^{2-} + O_2 + H_2O \Rightarrow 2SO_3^{2-} + 2H^+$. NEM-treated cells oxidized thiosulfate to tetrathionate ($2S_2O_3^{2-} + ½O_2 + H_2O \Rightarrow S_4O_6^{2-} + 2OH^-$); sulfur oxidation was inhibited by NEM, so that S(IV) in one thiosulfate was oxidized to S(VI) forming tetrathionate ($2S_2O_3^{2-} \Leftrightarrow S^0{}_2S^{IV}S^{VI}O_6^{2-} + 2e^-$).

As shown in Figure 7, sulfur anions of higher oxidation state than S^0 (*e.g.* sulfite, thiosulfate, polythionates) may also be produced in intermediate oxidation steps (Steger and Desjardins, 1978; Moses *et al*., 1987), but these are metastable and will rapidly oxidize to sulfate in the presence of the Fe^{3+} (Luther, 1990). These compounds are thus considered short-lived in mine waste environments. In Fe-rich low pH environments, thiosulfate will readily form ferric thiosulfate complexes. Although uncomplexed thiosulfate is fairly stable at neutral pH, even in the presence of dissolved oxygen, the ferric thiosulfate complex is unstable at low pH. Ferric thiosulfate will decompose to sulfur and bisulfite upon reaction with H^+ at pH > ~ 1.7, or will oxidize to polythionate after electron transfer with Fe^{3+} at pH < ~ 1.7 (Williamson and Rimstidt, 1993). In addition to the above-described mechanism, S^0, sulfite, thiosulfate, and polythionates are not likely to form from disulfide oxidation because the autocatalytic oxidation on a semi-conducting surface such as pyrite is much too rapid (Nordstrom and Southam, 1997).

5 Oxidation of complex sulfide mixtures

In mine wastes containing a variety of sulfide minerals, the intimate association of different sulfides as, for example, intergrowths, may lead to the preferential dissolution of one sulfide relative to another. Sulfide minerals interact electrochemically with each other when they are in contact in aqueous medium, forming galvanic currents. Such currents flow from the mineral with the higher electrode potential (cathode) to the mineral with the lower potential. Thus, the mineral with the lowest potential (anode) in the electromotive series will corrode more rapidly (Mehta and Murr, 1982). Galvanic interactions have a significant effect in leaching by *T. ferrooxidans*; these bacteria presumably sustain these interactions not only by oxidation of ferrous iron to ferric iron but also by oxidizing the formed elemental sulfur to sulfuric acid, maintaining the surface of the minerals exposed (Lizama and Suzuki, 1991b). For the galvanic interaction of chalcopyrite and pyrite, the $CuFeS_2/FeS_2$ cell can be described by an anodic oxidation reaction on the chalcopyrite surface and a cathodic oxygen reduction on the FeS_2 surface:

$$CuFeS_2 \Rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 4e^- \quad (28)$$

$$O_2 + 4H^+ + 4e^- \Leftrightarrow 2H_2O \quad (29)$$

with the overall reaction summed as:

$$CuFeS_2 + O_2 + 4H^+ \Rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 2H_2O \quad (30)$$

Elemental sulfur and ferrous iron will subsequently be oxidized by bacteria. The galvanic interaction of chalcopyrite and pyrite is illustrated in Figure 8. This relationship has been observed in various studies (*e.g.* Mehta and Murr, 1983; Ahonen and Tuovinen, 1995), where chalcopyrite oxidized more rapidly than pyrite, yet both these minerals were more recalcitrant than sphalerite, pyrrhotite, and pentlandite, which have lower electrode potentials.

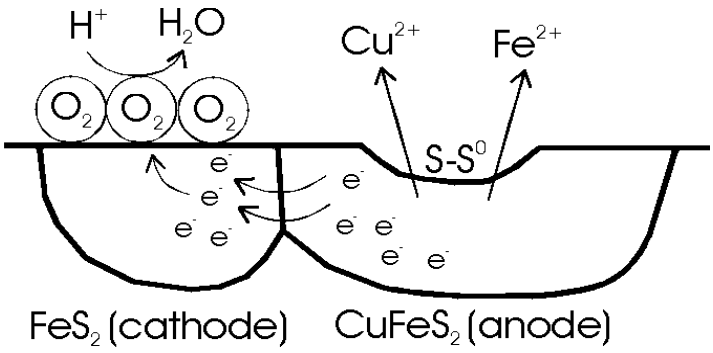

Figure 8: A simplified illustration showing the galvanic interaction of pyrite and chalcopyrite in the presence of oxygen.

In a study by Lizama and Suzuki (1991b), the interaction of the sulfide minerals chalcopyrite ($CuFeS_2$) and sphalerite (ZnS) with pyrite was studied for 13 days in (aerobic) shake-flask leaching experiments with and without the presence of *T. ferrooxidans* and *T. thiooxidans*, or both cultures. The results for the experiments are summarized in Table 3.

Table 3: Pyrite interaction with chalcopyrite or sphalerite during leaching, illustrating galvanic interaction. Data shows metal release (as percent of total concentration) after 13 days of leaching, summarized from Lizama and Suzuki (1991b). Two pyrite samples from different sampling locations (#1, #2) were used.

	FeS_2 (#1) alone	$CuFeS_2$ alone	$CuFeS_2$ + FeS_2 (#1)	$CuFeS_2$ + FeS_2 (#2)	ZnS alone	ZnS + FeS_2 (#1)	ZnS + FeS_2 (#2)
Chemical Control							
% Cu / Zn extraction	--	1.6	10.2	3.5	1.6	13.0	5.8
% Fe extraction	7.3	6.3	2.8	2.7	--	1.2	2.1
T. ferrooxidans							
% Cu / Zn extraction	--	11.1	14.5	12.2	36.0	61.7	65.8
% Fe extraction	29.9	6.4	22.6	32.2	--	12.8	39.7
T. thiooxidans							
% Cu / Zn extraction	--	0.8	10.3	5.8	8.3	22.4	14.7
% Fe extraction	9.5	5.5	1.7	5.1	--	7.1	9.2
Mixed Culture							
% Cu / Zn extraction	--	10.4	13.6	9.9	25.0	53.9	63.3
% Fe extraction	28.1	6.5	21.5	25.2	--	11.0	30.0

Note: Electrode potential values of 0.6 V, 0.5 V, and 0.23 V have been reported for pyrite, chalcopyrite, and sphalerite, respectively (Karavaiko, 1985).

As shown by the data, the abiotic interaction of pyrite with chalcopyrite and sphalerite resulted in an increased extraction of both Cu and Zn, relative to the leaching of chalcopyrite and sphalerite alone. The No. 1 pyrite was much more effective in increasing Cu extraction than the No. 2 pyrite, perhaps as a result of a stronger galvanic interaction with chalcopyrite. Similarly, the introduction of *T. ferrooxidans* had a very stimulatory effect on the rate of Cu extraction from chalcopyrite. Leaching of chalcopyrite alone by *T. thiooxidans* was less effective than the chemical control. In contrast to the chalcopyrite experiment, *T. thiooxidans* was effective in leaching sphalerite alone, increasing Zn extraction 5 times over the chemical rate. In addition, the two pyrite samples had different effects on sphalerite leaching, where *T. thiooxidans* appeared to be more effective in leaching Zn in the presence of pyrite No. 1, and *T. ferrooxidans* was more effective in the presence of pyrite No. 2. Pyrite samples can have different crystal and semiconductor properties, which influences bacterial oxidation (Ahonen *et al*., 1986). Mixed cultures were effective in leaching both chalcopyrite and sphalerite, but the metal extractions were higher with *T. ferrooxidans* alone. In summary, the paper by Lizama and Suzuki (1991b) demonstrated the positive effect of pyrite on Cu and Zn leaching from chalcopyrite and sphalerite, respectively. A stronger galvanic interaction was noted for sphalerite than chalcopyrite (as the result of the lower electrode potential for sphalerite relative to chalcopyrite), as well as a stronger enhancement of the galvanic effect by Fe^{2+}-grown *T. ferrooxidans* than by S^0-grown *T. thiooxidans*.

6 Sulfide oxidation under dysoxic / anoxic conditions

6.1 Oxygen limitation in mine drainage environments

Oxygen is the compound which drives sulfide oxidation in mine drainage deposits: oxygen rejuvenates ferric iron from ferrous iron, and is necessary for metabolism in aerobic iron-oxidizing bacteria such as *T. ferrooxidans*. Oxygen is consumed during microbial sulfide oxidation, such that in mine tailing deposits and rock dumps, oxygen is often depleted (< 0.001 atm) below the depth of active sulfide oxidation, which is often < 1 m depth (Goodman *et al*., 1983; Southam and Beveridge, 1992; Blowes *et al*., 1998). Rock dumps, however, are often unsaturated and have therefore a greater air permeability, and can contain detectable oxygen down to depths of > 10 m (Goodman *et al*., 1981).

The potential for anaerobic sulfide oxidation in mine waste deposits is dependent on the presence of oxidizing agents in the porewater. In mine wastes that are exposed to the atmosphere, the inward transport of oxygen for both the oxidation of sulfides and ferrous iron is controlled by gaseous diffusion, and to a lesser extent advection and convection. However, for wastes that have been covered with soil or inundated with water, the availability of oxygen will be limited. As illustrated in Figure 9, with a water-saturated cover emplaced over previously oxidized tailings, oxygen concentrations in the porewater will be limited to oxygen's solubility in water (~ 11.3 mg/L at 10°C). For an organic-rich cover (*e.g*. peat, sewage sludge), dissolved oxygen (DO) may be completely consumed within the cover by microbial respiration coupled with organic matter oxidation [oxygen profile (a), Figure 9].

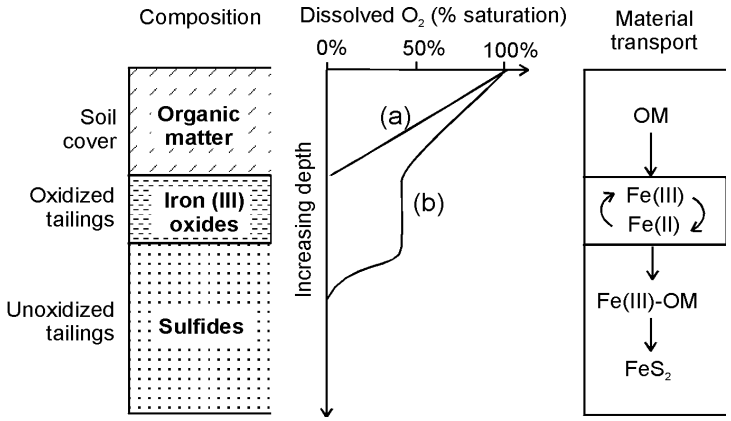

Figure 9: Schematic of oxygen profile and potential material transport in a covered mine waste deposit.

In covers with less organic matter (e.g. glacial till, clay) or in flooded deposits (*e.g*. Holmström, 1998), DO may only be partially consumed during transport through the barrier [oxygen profile (b), Figure 9], with low (oxic to dysoxic) concentrations of DO available for sulfide oxidation. In this latter case, sulfide oxidation may be mediated by *Thiobacilli* along aerobic pathways (see this report, sections 4.2 and 4.3). *T. ferrooxidans* are capable of growing under conditions of low oxygen tension (Goodman *et al*., 1981), and it has been generally accepted that *T. ferrooxidans* activity ceases when O_2 is depleted. However, even under extremely low $^{P}O_2$ bacterial activity can persist. Myerson (1981) demonstrated that dissolved oxygen concentrations are not limiting for the growth of *T. ferrooxidans* (pyrite substrate, pH = 2.5) until concentrations drop below 5% of saturation (DO ≈ 0.5 mg/L at 10°C or $^{P}O_2$ ≈ 0.01 atm).

If DO concentrations are maintained at dysoxic to anoxic levels in a covered deposit, there will be much competition among *Thiobacilli* and other iron-oxidizing bacteria for the use of DO as an electron acceptor in sulfide or ferrous iron oxidation. The oxidation potential in these environments is thus generally considered low. In the absence of DO, ferric iron may persist as a sulfide oxidant under anaerobic conditions, through the action of the following processes:

- *T. ferrooxidans* metabolization of ferric iron,
- ferric iron reduction in oxidized tailings, followed by reoxidation and transport to sulfide surfaces,
- the heterotrophic growth of iron-reducing bacteria.

These different processes will be discussed in the subsequent sections.

6.2 Anaerobic bacterial oxidation of sulfides

The studies by Taylor *et al*. (1984a,b) and the reaction rates presented in Table 2 indicate that in oxic environments, iron-oxidizing bacteria will use O_2 as an electron acceptor in sulfide oxidation. Thus, once O_2 is depleted from the mine waste porewater, other electron acceptors may react with sulfides (*e.g*. Fe^{3+}, NO_3^-). While the oxidation of pyrite by ferric iron can itself proceed anaerobically, the released Fe^{2+} would not be rejuvenated to ferric iron. Various studies have nevertheless shown that under anaerobic conditions, the oxidation of pyrite by Fe^{3+} is faster in the presence of *T. ferrooxidans* cells relative to the abiotic reaction. This may be due to the presence of Fe^{3+}-sulfur oxidoreductase in *T. ferrooxidans* which oxidizes sulfur or sulfide to sulfite by using Fe^{3+} (Sugio *et al*., 1989). This interpretation is significant as it implies that the bacteria can anaerobically oxidize the pyrite sulfur or its dissociated forms, using Fe^{3+} as an electron acceptor (Lizama and Suzuki, 1989b).

Recent studies have shown that *T. ferrooxidans* can couple ferric iron reduction to sulfide oxidation in the absence of oxygen. Although *T. ferrooxidans,* among other iron-oxidizing bacteria, has traditionally been considered to be an obligately aerobic organism, *T. ferrooxidans* is rather a facultive anaerobe and can survive anaerobically by using ferric iron as an electron acceptor (Lizama and Suzuki, 1989a; Suzuki *et al*., 1990; Pronk *et al*., 1992; Ledin and Pedersen, 1996).

The coupling of sulfide or sulfur oxidation with ferric iron reduction by acidophiles was first demonstrated by Brock and Gustafson (1976). These authors showed that cell suspensions of the obligately autotrophic acidophiles *T. ferrooxidans* and *T. thiooxidans* coupled the anaerobic oxidation of elemental sulfur to the reduction of ferric iron:

$$S^0 + 6Fe^{3+} + 4H_2O \Rightarrow HSO_4^- + 6Fe^{2+} + 7H^+ \qquad (31)$$

The studies of Brock and Gustafson (1976) have shown that ferric iron, in addition to oxygen, can act as an electron acceptor for the oxidation of elemental sulfur by *T. ferrooxidans* (and *T. thiooxidans*). In the presence of *T. ferrooxidans*, ferric iron is the initial electron acceptor for the oxidation of elemental sulfur and pyritic sulfide (Sugio *et al*., 1989). The ferrous iron produced by this reaction can subsequently be oxidized by the iron-oxidizing enzyme system [*i.e*. sulfur (sulfide):Fe^{3+} oxidoreductase] in *T. ferrooxidans*. Other studies have demonstrated that ferric iron respiration by *T. ferrooxidans* can, under anaerobic conditions, provide the organism with metabolic energy (Guay *et al*., 1992; Pronk and Johnson, 1992). Pronk *et al*.

(1992) demonstrated that the bacteria is indeed a facultive anaerobe. Anaerobic growth of the organism occurred in mineral media supplemented with both elemental sulfur and ferric iron.

Suzuki *et al*. (1990) studied the aerobic rates of Fe^{2+} and S^0 oxidation by O_2 and the anaerobic rates of S^0 oxidation by Fe^{3+} using *T. ferrooxidans* strains grown on ferrous iron, elemental sulfur, or complex sulfide ores. For Fe^{2+}-grown cells, the anaerobic S^0 oxidation rate with Fe^{3+} as an electron acceptor was often equal to the aerobic activity with O_2 in some strains. When Fe^{2+}-grown cells were transferred to media containing complex sulfides, various strains leached Cu and Zn efficiently from the complex sulfide ores. The ability of *T. ferrooxidans* resting cells to reduce Fe^{3+} with pyrite anaerobically suggests a possible role of the sulfur (sulfide):Fe^{3+} oxidoreductase which oxidizes sulfur or sulfide to sulfite by using Fe^{3+} (Sugio *et al*., 1989).

Field evidence for sulfide oxidation under anoxic conditions is provided in the study of Goodman *et al*. (1983). They indicated that even though O_2 was virtually absent below a depth of 0.5 m in an unsaturated rock dump, *T. ferrooxidans* levels were high and did not decrease with depth. In addition, Fe and Cu were being solubilized in significant amounts from the waste rock. High levels of CO_2 were present in the pore space (20 to 100 times atmospheric levels), and Goodman *et al*. (1983) concluded that *T. ferrooxidans* was capable of leaching a Zn-Fe sulfide in the absence of O_2, provided CO_2 is available, where higher CO_2 levels contribute to higher leaching rates. Ferric iron was presumably the oxidant in this case. Other studies have also shown that bacterial growth increases with CO_2 concentration (Otwinowski, 1994), suggesting that controlling the levels of CO_2 in waste piles may be almost as important as controlling the levels of oxygen.

6.3 Ferric iron reduction by Fe(II) complexes

In the tailings cover, or in organic bottom sediments accumulating in a flooded deposit, simple and complex carboxylic acids and hydroxylated organic compounds may be present, as these compounds are common by-products of plant exudates, sulfate reduction, and fermentation processes. These ligands complex both Fe(II) and Fe(III), although Fe(III) complexes are thermodynamically stronger (Hider, 1984; Luther *et al*., 1992).

A mechanism for the dysoxic weathering of sulfides has been proposed by Luther *et al*. (1992) for iron cycling in salt-marsh sediments. With this model, Fe(III) in ferric oxides may be dissolved by siderophores such as catechol ($C_6H_4(OH)_2$) (Hider, 1984):

$$FeOOH(s) + C_6H_4(OH)_2 \rightarrow \{Fe^{3+}[C_6H_4(O)_2]^{2-}\}^+ + OH^- + H_2O \qquad (32)$$

Organic compounds can contribute to the reductive dissolution of ferric oxides, yielding ferrous iron to solution (LaKind and Stone, 1990):

$$2FeOOH(s) + C_6H_4(OH)_2 \rightarrow 2Fe^{2+} + 4OH^- + C_6H_4(O)_2 \qquad (33)$$

Alternatively, Fe^{2+} may complexed by an hydroxyl- or carboxyl-containing, ‘weak field ligand’ (LL) such as as simple carboxylic acids (e.g. oxalate, malonate, citrate), and reductively dissolve ferric oxides:

$$FeOOH(s) + H^+ + **Fe^{2+}(LL) \xrightarrow{LL} **Fe^{3+}(LL) + Fe^{2+}(LL) + 2OH^- \quad (34)$$

where '**' indicates the same iron atom. This reaction has been demonstrated by Sulzberger *et al.* (1989) and Wehrli *et al*. (1989) for the dissolution of hematite (α - Fe_2O_3) by Fe(II) complexed with oxalate, malonate, and citrate. This reaction is analogous to the reduction of O_2 by Fe^{2+} (Luther, 1990). Note that the cycle of iron solubilization will cease when sulfate reduction rates are high and organic ligand production low, resulting in the precipitation of iron sulfides.

Reactions 32 and 34 result in the direct formation of aqueous Fe(III) complexes. Thus, as illustrated in Figure 9, the downward mobilization of organic matter (OM) from a cover may lead to both the dissolution of ferric oxides and the reduction of Fe^{3+}. In either case, Fe^{3+}-OM complexes may result, which have a relatively high stability. These may be further transported down the profile, to react with sulfides in the unoxidized tailings (see Figure 9). *Thiobacilli* may couple iron reduction with sulfide oxidation in these anoxic zones. Note that a continual supply of organic ligands is needed to perpetuate this cycle.

6.4 Bacterially-mediated iron reduction

Iron reduction, as presented in the preceeding section, is generally mediated by heterotrophic organisms. A wide range of fungi and bacteria reduce Fe(III) under various conditions. Fermentative bacteria are known to reduce Fe(III) under anaerobic conditions while metabolizing fermentable sugars or amino acids, but this is only a minor pathway for electron flow in the microorganisms. Sulfur-oxidizing bacteria such as *T. ferrooxidans* and *T. thiooxidans* reduced Fe(III) to Fe(II) with sulfur as the electron donor (Brock and Gustafson, 1976), but subsequent studies have shown that neither of these bacteria were capable of obtaining energy to support growth from this reaction (Sugio *et al*., 1988; Sand, 1989).

Various Fe(III)-reducing bacteria can effectively couple the oxidation of organic matter to Fe(III) reduction. The only well-documented pure cultures of organic acid-consuming, Fe(III)-reducing microorganisms are the so-called GS-15 isolate and *Shewanella putrefaciens* (Lovley, 1991). The former isolate oxidized acetate to carbon dioxide under strictly anaerobic conditions. *S. putrifaciens* is a facultive anaerobe, and is known to completely oxidize formate or incompletely oxidize lactate and pyruvate to acetate and CO_2, and may also oxidize H_2. Sulfate reduction and methane production are generally inhibited in environments in which organic matter oxidation is being coupled to Fe(III) reduction. This can be primarily attributed to Fe(III)-reducing microorganisms maintaining the concentration of electron donors at levels too low for sulfate reducers or methanogens to metabolize them (Lovley, 1991).

The presence of three strains of Fe-reducing bacteria were reported from a flooded open pit mine and in alum shale tailings from Ranstad, Sweden (Nejad, 1998). The strains were related to *Shewanella putrefaciens, S. alga* and *Aeromonos salmonicida.* The presence of iron-reducing bacteria in anoxic bottom sediments in the open pit lake and in the tailings indicates that there is organic matter available for metabolism by microorganisms. As chemical data from the Ranstad uranium mine showed that iron concentrations were increasing around the open pit lake (Nejad, 1998), the ability of the bacteria isolated from this lake to reduce Fe(III) may partly explain these observed increases.

Mixotrophic strains of *T. ferrooxidans* have been identified (Pronk and Johnson, 1992) which can grow autotrophically on ferrous iron but was also capable of heterotrophic growth on a

yeast extract/ferric iron medium. Certain obligately heterotrophic bacteria are also capable of oxidizing reduced sulfur compounds (Mason and Kelly, 1988). Bacterial isolates ('T-21') from a sulfur mine in north Wales catalyzed ferrous iron oxidation, but the rate of oxidation was limited to the availability of organic carbon (Pronk and Johnson, 1992). Although this isolate was incapable of oxidizing reduced forms of sulfur, pyrite oxidation was enhanced in solutions containing 'T-21' with yeast extract and ferrous iron. This isolate apparently oxidized ferrous to ferric iron, which in turn oxidized pyrite according to the reaction shown in equation 4.

While many iron-reducing bacteria are neutrophilic, acidophilic growth is required in acidic mine tailings deposits. Heterotrophic acidophiles capable of catalyzing dissimilitory ferric iron reduction include species belonging to the genus *Acidiphilium*, which grow under aerobic or microaerophilic conditions, and also *T. acidophilus* (Pronk and Johnson, 1992). As noted above, iron-oxidizing chemolithotrophic mesophiles can also reduce ferric iron under appropriate conditions. Mixed cultures of either *T. ferrooxidans* or *L. ferrooxidans* and an *Acidiphilium*-like isolate displayed alternating iron oxidation / reduction in shake flask cultures with varying oxygen input (Johnson *et al*., 1993). In these systems, iron oxidation proceeded in aerated solutions. However, ferric iron reduction by the *Acidiphilium*-like isolates began to occur when dissolved oxygen concentrations dropped to < 10% of saturation. It appears that some acidophilic heterotrophs have the ability to use ferric iron as a direct electron acceptor, and are facultive anaerobes rather than obligate aerobes, as was earlier thought.

7 Conclusions – Iron cycling in mine wastes

Iron- and sulfide-oxidizing bacteria are indisputably linked to the process of sulfide oxidation in mine waste deposits. In acidic, aerated deposits, the presence of high levels of dissolved oxygen and ferric iron result in the relatively rapid oxidation of sulfides. In order to prevent sulfide oxidation, traditional remedial efforts minimize oxygen diffusion to sulfide surfaces through the installation of water-saturated sealing covers on the deposits. A critical assumption in this practice is that by limiting the supply of dissolved oxygen, the rate of sulfide oxidation would be minimal because of the lack of electron acceptors for microbial catalysis. However, the complete inhibition of pyrite oxidation by flooding or covering may never be possible because of the difficulty in the total exclusion of O_2, thereby maintaining the availability of Fe^{3+} as an oxidant. In addition, even under anoxic conditions, sulfide oxidation may be perpetuated by 1) the ability of *T. ferrooxidans* to couple ferric iron reduction to sulfide oxidation in the absence of oxygen, and 2) the oxidation of sulfides by organically-complexed ferric iron. while the latter case requires a continuous supply of weak field ligands.

There is increasing evidence that *T. ferrooxidans* is capable of growth on reduced sulfur in dysoxic – anoxic environments. Various studies (Brock and Gustafson, 1976; Lizama and Suzuki, 1989a,b; Sugio *et al*., 1989; Suzuki *et al*., 1990; Pronk *et al*., 1992) have shown that under anaerobic conditions, the oxidation of pyrite and other reduced sulfur compounds by Fe^{3+} is faster in the presence of *T. ferrooxidans* cells relative to the abiotic reaction. This may be due to the presence of Fe^{3+}-sulfur oxidoreductase in *T. ferrooxidans* which oxidizes sulfur or sulfide to sulfite by using Fe^{3+} (Sugio *et al*., 1989). The anaerobic growth of *Thiobacilli* on reduced sulfur, using Fe^{3+} as an electron acceptor, requires the continual supply of ferric iron by either the dissolution of ferric oxides, or by the oxidation of ferrous to ferric iron in aerated regions of the deposit.

In accordance with the previous discussions, the cycling of iron through the ferrous and ferric oxidation states may potentially be a key process in anaerobic mine deposits. As shown in Figure 10, ferric iron may be reduced by either autotrophic acidophiles (*e.g. T. ferrooxidans*) or by heterotrophic acidophiles (*e.g. Acidiphilium*), where the reaction is coupled with the oxidation of sulfides and organic compounds, respectively. Mixotrophic strains of *T. ferrooxidans* have been identified (Pronk and Johnson, 1992) which can grow autotrophically on ferrous iron but was also capable of heterotrophic growth on a yeast extract/ferric iron medium. Certain obligately heterotrophic bacteria are also capable of oxidizing reduced sulfur compounds (Mason and Kelly, 1988).

Organic carboxylic acids and phenolic compounds, derived from either organic matter (OM) degradation in a cover or bacterial exudates, can facilitate electron transfer between Fe^{2+} - OM complexes and solid phase iron(III) oxides, releasing soluble Fe^{3+} - OM complexes to solution (Figure 10). The ferric complex may then oxidize reduced sulfur in a bacterially-mediated reaction.

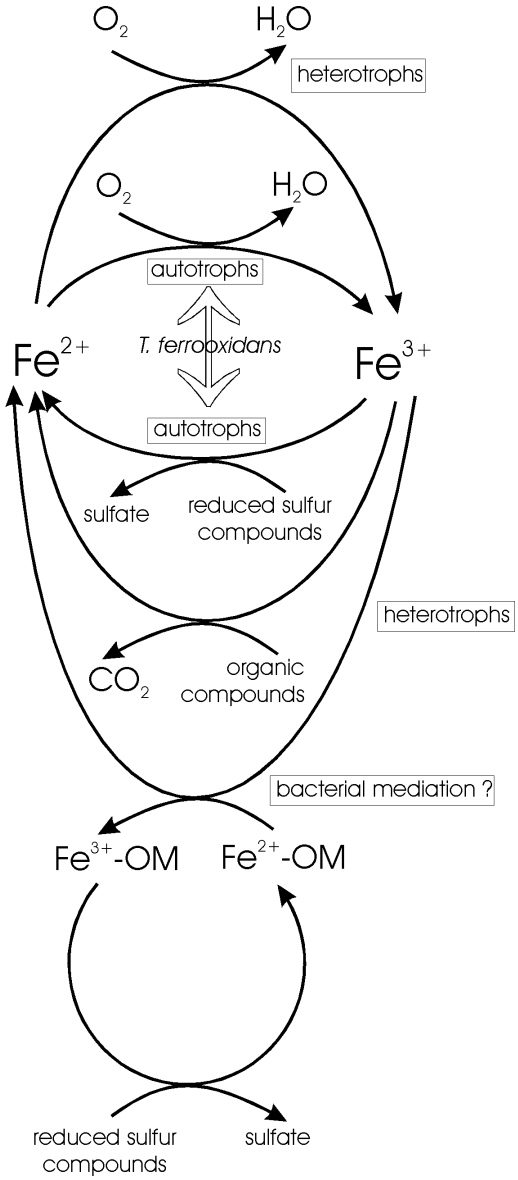

Figure 10: Iron cycling in acidic environments, showing involvement of heterotrophic and autotrophic acidophilic bacteria. Adapted from Pronk and Johnson (1992).

The involvement of acidophilic microorganisms in iron-cycling in mine waste deposits is likely quite important. Two recent developments emphasize this point: 1) the identification of new acidophilic species (*e.g.* heterotrophic iron-oxidizers), and 2) the isolation of strains with new physiological properties (*e.g.* the ability to grow anaerobically) (Pronk and Johnson, 1992). As shown in Figure 10, autotrophic and heterotrophic acidophiles can be involved in both iron oxidation and reduction.

In conclusion, the existence of facultively anaerobic acidophiles, which are capable of coupling the oxidation of organic or inorganic electron donors to the reduction of ferric iron (and hence sulfide oxidation in the latter case), indicates that microbial activity in acidic mine environments is not dependent on the presence of oxygen. The ability of at least some bacteria to use solid-phase iron compounds as electron acceptors (*e.g.* goethite) and possibly as electron donors (*e.g.* pyrite) vastly increases the amount of iron susceptible to microbial reduction (Pronk and Johnson, 1992), and thus potentially available for sulfide oxidation under dysoxic / anoxic conditions.

8 References

Ahonen L. and Tuovinen O.H. 1989. Microbiological oxidation of ferrous iron at low temperatures. *Appl. Environ. Microbiol*. **55**, 312 - 316.

Ahonen L. and Tuovinen O.H. 1991. Tempurature effects on bacterial leaching of sulfide minerals in shake flask experiments. *Appl. Environ. Microbiol*. **57**, 138 - 145.

Ahonen L. and Tuovinen O.H. 1992. Bacterial oxidation of sulfide minerals in column leaching experiemnts at suboptimal temnperatures. *Appl. Environ. Microbiol*. **58**, 600 - 606.

Ahonen L. and Tuovinen O.H. 1993. Alteration of surfaces and textures of minerals during the bacterial leaching of a complex sulfide ore. *Geomicrobiol. J*. **10** 207 - 217.

Ahonen L. and Tuovinen O.H. 1995. Bacterial leaching of complex sulfide ore samples in bench-scale column reactors, *Hydrometallurgy* **37**, 1 - 21.

Ahonen L., Hiltunen P. and Tuovinen O.H. 1986. The role of pyrrhotite and pyrite in the bacterial leaching of chalcopyrite. In: *Fundamentals and Applied Biohydrometallurgy* (R.W. Lawrence, R.M.R. Branion, and H.G. Ebner, Eds.), p. 13 - 22. Elsevier, Amsterdam.

Banwart S.A., Destouni G. and Maltström M. 1998. Assessing the scale-dependence of mineral weathering rates at the Aitik waste rock deposit in northern Sweden. *Mineral. Mag*. **62A**, 108 - 109.

Bebie J., Schoonen M.A.A., Fuhrmann M. and Strongin D.R. 1998. Surface charge development on transition metal sulfides: An electrokinetic study. *Geochim. Cosmochim. Acta* **62**, 633 - 642.

Bennett J. and Tributsch H. 1978. Bacterial leaching patterns on pyrite crystal surfaces. *J. Bacteriol*. **134**, 310–317.

Bhatti T., Bigham J., Carlson L. and Tuovinen, O. 1993. Mineral products of pyrrhotite oxidation by Thiobacillus ferrooxidans. *Appl. Environ. Microbiol*. **59**, 1984 - 1990.

Blowes D.W. and Jambor J.L. 1990. The pore-water geochemistry and the mineralogy of the vadose zone of sulfide tailings, Waite Amulet, Quebec, Canada, *Appl. Geochem.* **5**, 327 - 346.

Blowes D.W., Reardon D.J., Jambor J.L. and Cherry J.A. 1991. The formation and potential importance of cemented layers in inactive sulfide mine tailings. *Geochim. Cosmochim. Acta* **55**, 965 - 978.

Blowes D.W., Jambor J.L., Hanton-Fong C.J., Lortie L. and Gould W.D. 1998. Geochemical, mineralogical and microbiological characterization of a sulphide-bearing carbonate-rock gold-mine tailings impoundment, Joutel, Québec. *Appl. Geoch*. **13**, 687 - 705.

Brock T.D. and Gustafson J. 1976. Ferric iron reduction by sulfur- and iron-oxidizing bacteria. *Appl. Environ. Microbiol*. **32**, 567 - 571.

Brown A.D. and Jurinak J.J. 1989. Mechanisms of pyrite oxidation in aqueous mixtures. *J. Environ. Qual*. **18**, 545.

Buckley A.N. and Woods R.W. 1987. The surface oxidation of pyrite. *Appl. Surf. Sci*. **27**, 437 - 452.

Chan C.W. and Suzuki I. 1994. Thiosulfate oxidation by sulfur-grown Thiobacillus thiooxidans cells, sell-free extracts, and thiosulfate-oxidizing enzyme. *Can. J. Microbiol*. **40**, 816 - 822.

Cornell R.M. and Schwertmann U. 1996. *The Iron Oxides*. VCH, Weinheim, 573 p.

Devasia P., Natarajan K.A., Sathayanarayana D.N. and Rao, G.R. 1993. Surface chemistry of Thiobacillus ferrooxidans relevant to adhesion on mineral surfaces. *Appl. Environ. Microbiol*. **59**, 4051 - 4155.

Eggleston C.M., Ehrhardt J.-J. and Stumm W. 1996. Surface structural controls on pyrite oxidation kinetics: An XPS-UPS, STM, and modeling study. *Am. Mineral*. **81**, 1036 - 1056.

Ehrlich H.L. 1981. *Geomicrobiology*, Dekker, New York.

Elberling B., Nicholson R.V. and David D.J. 1993. Field evaluation of sulphide oxidation rates. *Nord. Hydrol*. **24**, 323 - 338.

Elberling B., Nicholson R.V. and Scharer J.M. 1994. A combined kinetic and diffusion model for pyrite oxidation in tailings: A change in controls with time. *J. Hydrol*. **157**, 47 - 60.

Evangelou V.P. 1994. Potential microencapsulation of pyrite by artificial inducement of ferric phosphate coatings. *J. Environ. Qual*. 24, 535 - 542.

Evangelou V.P. and Huang X. 1994. Infrared spectroscopic evidence of an ironII-carbonate complex on the surface of pyrite. *Spectrochim. Acta* **50A**, 1333 - 1340.

Evangelou V.P. and Zhang Y.L. 1995. A review: Pyrite oxidation mechanisms and acid drainage prevention. *Crit. Rev. Environ. Sci. Technol*. **25**, 141 - 199.

Evangelou V.P., McDonald L.M. Jr. and Seta A.K. 1998a. Acid mine drainage, In: *Encyclopedia of Environmental Analysis and Remediation* (R.A. Meyer, Ed.), pp. 1 - 17. John Wiley and Sons, Inc., New York.

Evangelou V.P., Seta A.K., and Holt A. 1998. Potential role of bicarbonate during pyrite oxidation. *Environ. Sci. Technol.* 32, 2084 - 2091.

Fortin D. and Beveridge T.J. 1997. Microbial sulfate reduction within sulfidic mine tailings: Formation of diagenetic Fe sulphides. *Geomicrobiol. J*. **14**, 1 - 21.

Fortin D., Davis B. and Beveridge T.J. 1996. Role of Thiobacillus and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings. *FEMS Microbiol. Ecol*. **21**, 11 - 24.

Fortin D., Davis B., Southam G. and Beveridge T.J. 1995. Biogeochemical phenomena induced by bacteria within sulfidic mine tailings. *J. Ind. Microbiol*. **14**, 178 - 185.

Gehrke T., Telegdi J., Thierry D. and Sand W. 1998. Importance of extracellular polymeric substances from Thiobacillus ferrooxidans for bioleaching. *Appl. Environ. Microbiol*. **64**, 2743 - 2747.

Goldhaber M.B. 1983. Experimental study of metastable sulfur oxyanion formation during pyrite oxidation at pH 6–9 and 30^{o}C. *Am. J. Sci*. **283**, 193 - 217.

Goodman A.E., Babij T. and Ritchie A.I.M. 1983. Leaching of a sulphide ore by Thiobacillus ferrooxidans under anaerobic conditions. *Progress in Biohydrometallurgy* (Cagliari), 361 - 376.

Goodman A.E., Khalid A.M. and Ralph B.J. 1981. Microbial ecology of Rum Jungle. Part I. Environmental study of sulphidic overburden dumps, experimental heap leach piles and tailings dam area. Report AAEC/E531, Australian Atomic Energy Commission, 170 p.

Groudev S.N., Genchev F.N., and Gaidarjiev S.S. 1978. Observations on the microflora in an industrial copper dump leaching operation. In: *Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena* (L.E. Murr, A.E. Torma and J.A. Brierly, Eds.), pp. 253 - 274. Academic Press, New York.

Guay R., Dufresne S., Desjardins E.-R., and Gelinas P. 1992. Microbiological modeling of acid mine drainage production in pyritic waste rock piles. Abstr. 92nd General Meeting, American Society for Microbiology, New Orleans.

Guevremont J.M., Bebie J., Elsetinow A.R., Strongin D.R. and Schoonen M.A.A. 1998. Reactivity of the (100) plane of pyrite in oxidizing gaseous and aqueous environments: Effects of surface imperfections. *Environ. Sci. Technol*. **32**, 3743 - 3748.

de Haan S.B. 1991. A review of the rate of pyrite oxidation in aqueous systems at low temperature. *Earth-Sci. Rev*. **31**, 1 - 10.

Haddock B.A. and Jones C.W. 1977. Bacterial respiration. *Bacteriol. Rev*. **41**, 47 - 99.

Hallmann R., Friedrich A., Koops H., Pommerening-Röser A., Rohde K., Zenneck C. and Sand, W. 1992. Physiological characteristics of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* and physicochemical factors influence microbial metal leaching. *Geomicrobiol. J*. **10**, 193 - 206.

Hider R.C. 1984. Siderophore mediated adsorption of iron. *Struct. Bonding* Berlin, **58**, 26 - 76.

Holmström H. 1998. *Geochemical field and laboratory investigations of the sulphide tailings impoundments at Laver and Stekenjokk, Northern sweden*. Licentiate thesis, Department of Environmental Planning and Design, Luleå University of Technology.

Jaynes D.B., Rogowski A.S. and Pionke H.B. 1984. Acid mine drainage from reclaimed coal strip mines. I. Model description. *Water Resourc. Res*. **20**, 233.

Johnson D.B., Ghauri M.A. and McGinness S. 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiol. Rev*. **11**, 63–70.

Kalin M., Smith M.P., and Fyson A. 1997. The use of natural phosphate rock to reduce acidic drainage from pyritic waste rock. In: *Proceedings of the ICARD conference*, Vancouver, May 30 – June 6, 1997.

Karavaiko G.I. 1985. Microbiological processes for the leaching of metals from ores, State-of-the-art review. United Nations Environment Programme, USSR commission for UNEP (A.E. Torma, Ed.). Center of International Projects, GKNT, Moscow.

Kirby C.S. and Elder Brady J.A. 1998. Field determination of Fe^{2+} oxidation rates in acid mine drainage using a continuously-stirred tank reactor. *Appl. Geochem*. **13**, 509 - 520.

Lacey D.T. and Lawson F. 1977. Kinetics of the liquid-phase oxidation of acid ferrous sulfate by the bacterium Thiobacillus ferrooxidans. *Biotechnol. and Bioengin*. **12**, 29 - 50.

LaKind J.S. and Stone A.T. 1989. Reductve dissolution of geothite by phenolic reductants. *Geochim. Cosmochim. Acta* **53**, 961 - 971.

Ledin M. and Pedersen K. 1996. The environmental impact of mine wastes – Roles of microorganisms and their significance in treatment of mine wastes. *Earth-Sci. Rev*. **41**, 67 - 108.

Lizama K.M. and Suzuki I. 1989a. Bacterial leaching of a sulfide ore by Thiobacillus ferrooxidans and Thiobacillus thiooxidans Part II: Column leaching studies. *Hydrometallurgy* **22**, 301 - 310.

Lizama H.M. and Suzuki I. 1989b. Rate equations and kinetic parameters of the reactions involved in pyrite oxidation by Thiobacillus ferrooxidans. *Appl. Environ. Microbiol*. **55**, 2918 - 2923.

Lizama H.M. and Suzuki I. 1991a. Kinetics of sulfur and pyrite oxidation by Thiobacillus ferrooxidans. Competitive inhibition by increasing concentration of cells. *Can. J. Microbiol*. **37**, 182 - 187.

Lizama H.M. and Suzuki I. 1991b. Interaction of chalcopyrite and sphalerite with pyrite during leaching by Thiobacillus ferrooxidans and Thiobacillus thiooxidans. *Can. J. Microbiol*. **37**, 304 - 311.

Lovely D.R. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev*. **55**, 259 - 287.

Lowson R.T. 1982. Aqueous oxidation of pyrite by molecular oxygen. *Chem. Rev*. **82**, 461 - 497.

Ljungberg J., Lindvall M., Holmström H. and Öhlander H. 1997. Geochemical field study of flooded mine tailings at Stekenjokk, Northern Sweden. *Proceedings of Fourth International Conference on Acid Rock Drainage,* Vol. III, pp. 1401 - 1417. May 31 – June 6, 1997, Vancouver, B.C., Canada.

Luther G.W. III. 1987. Pyrite oxidation and reduction: Molecular orbital theory considerations. *Geochim. Cosmochim. Acta* **51**, 3193 - 3199.

Luther, G.W. III. 1990. The frontier-molcular-orbital theory approach in geotechnical processes, In: *Aquatic Chemical Kinetics* (W. Stumm, Ed.) pp. 173–198. John Wiley & Sons, New York.

Luther G.W. III, Kostka J.E., Church T.M., Sulzberger B. and Stumm W. 1992. Seasonal iron cycling in the salt-marsh sedimentary environment: the importance of ligand complexes with Fe(II) and Fe(III) in the dissolution of Fe(III) minerals and pyrite, respectively. *Mar. Chem*. **40**, 81 - 103.

Machemer S.D. and Wildeman T.R. 1992. Adsorption compared with sulfide precipitation as metal removal process from acid mine drainage in a constructed wetland, *J. Contam. Hydrol*. **9**, 115 - 131.

Mason J. and Kelly D.P. 1988. Thiosulfate oxidation by obligately heterotrophic bacteria. *Microb. Ecol*. **15**, 123 - 134.

Matthews C.T. and Robins R.G. 1972. *Proc. Australias Inst. Mining Metall*. **242**, 47.

McKibben M.A. and Barnes H.L. 1986. Oxidation of pyrite in low temperature acidic solutions: Rate laws and surface textures. *Geochim. Cosmochim. Acta* **50**, 1509 - 1520.

Mehta A.P. and Murr L.E. 1982. Kinetic study of sulfide leaching by galvanic interaction between chalcopyrite, pyrite, and sphalerite in the presence of Thiobacillus ferrooxidans 30^{o}C and a thermophilic microorganism 55^{o}C. *Biotechnol. Bioeng*. **24**, 919 - 940.

Mehta A.P. and Murr L.E. 1983. Fundamental studies of the contribution of galvanic interaction to acid-bacterial leaching of mixed metal sulfides. *Hydrometallurgy* **9**, 235 - 256.

Meyerson A.S. 1981. Oxygen mass transfer requirements during the growth of *Thiobacillus ferrooxidans* on iron pyrite. *Biotechnol. Bioeng*. **23**, 1413 - 1416.

Moses C.O. and Herman J.S. 1991. Pyrite oxidation at circumneutral pH. *Geochim. Cosmochim. Acta* **55**, 471 - 482.

Moses C.O., Nordstrom D.K., Herman J.S. and Mills A. 1987. Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. *Geochim. Cosmochim. Acta* **51**, 1561 - 1571.

Nejad F. T. 1998. *Microbial iron reduction in mine waste at the former uranium mine in Ranstad*, Licentiate Thesis, General and Marine Microbiology, Göteborg University.

Nesbitt H.W. and Muir I.J. 1994. X-ray photoelectron spectroscopic study of a pristine pyrite surface reacted with water vapour and air. *Geochim. Cosmochom. Acta* **58**, 4667 - 4679.

Nicholson R.V., Gillham R.W. and Reardan E.J. 1988. Pyrite oxidation in carbonate-buffered solutions: 1. Experimental kinetics. *Geochim. Cosmochim. Acta* **52**, 1077 - 1085.

Nicholson R.V., Gillham R.W., Cherry J.A. and Reardon, E.J. 1989. Reduction of acid generation in mine tailings through the use of moisture-retarding cover layers as oxygen barriers. *Can. Geotech. J.* **26**, 1 - 8.

Nicholson R.V., Gillham R.W. and Reardan E.J. 1990. Pyrite oxidation in carbonate-buffered solution: 2. Rate controls by oxide coatings. *Geochim. Cosmochim. Acta* **54**, 395 - 402.

Nicholson R.V. 1994. Iron-sulfide oxidation mechanisms: Laboratory studies, In: *Environmental Geochemistry of sulfide mine wastes* (J.L. Jambor and D.W. Blowes, Eds.), pp. 163 - 183. Mineral Association of Canada, Short course handbook Vol. 22.

Nordstrom D.K. 1982. Aqueous pyrite oxidation and the consequent formation of secondary iron minerals, In: *Acid Sulfate Weathering* (L. R. Hossner, Ed.), pp. 95 - 108. *SSSA Publication* **10;** Soil Science Society of America, Madison.

Nordstrom, D.K. 1985. The rate of ferrous iron oxidation in a astream receiving acid mine effluent. In: *Selected Papers in the Hydrological Sciences*, pp. 113 - 119. US Geological Survey water-Supply Paper 2270.

Nordstrom D.K. and Alpers C.N. 1998. Geochemistry of acid mine waters, In: *The Environmental Geochemistry of Mineral Deposits* (G.S Plumlee and M.J. Logsdon, Eds.). *Rev. Econ. Geol*. Vol. 7A (in press).

Nordstrom D.K. and Southam G. 1997. Geomicrobiology of sulfide mineral oxidation, In: *Geomicrobiology: Interactions between microbes and minerals* (J.F. Banfield and K.H. Nealson, Eds.), pp. 361 - 390. *Rev. Mineral*. **35**.

Norris P.R. 1990. Acidophilic bacteria and their activity in mineral sulfide oxidation, In: *Microbial Mineral Recovery* (H.L. Ehrlich and C.L Brierly, Eds.), pp. 2 - 7. McGraw-Hill, New York.

Olson G.J. 1991. Rate of pyrite bioleaching by Thiobacillus ferrooxidans: Results of an interlaboratory comparison. *Appl. Environ. Microbiol*. **57**, 642 - 644.

Otwinowski M. 1994. Quantitative analysis of chemical and biological kinetics for the acid mine drainage problem, MEND project report 1.51.1.

Parizi D., Horneman J. and Rastogi V. 1994. Use of bactericides to control acid mine draiange from surface operations, In: *Proceedings, International Land Reclamation and Mine Drainage Conference*, USDI, Bureau of Mines SP 06B-94, Pittsburgh, PA, 319 - 325.

Pesic B., Oliver D.J. and Wichlacz P. 1989. An electro-chemical method of measuring the oxidation rate of ferrous to ferric iron with oxygen in the presence of Thiobacillus ferrooxidans. *Biotech. Bioeng*. **33**, 428 - 439.

Postma D., Boesen C, Krinstansen H. and Larsen F. 1991. Nitrate reduction in an unconfined sandy aquifer: Water chemistry, reduction processes, and geochemical modeling. *Water Resourc. Res*. **27**, 2027 - 2045.

Pronk J. and Johnson D. 1992. Oxidation and reduction of iron by acidophilic bacteria. *Geomicrobiol. J*. **10**, 153 - 171.

Pronk J.T., de Bruyn J.C., Bos P. and Kuenen J.G. 1992. Anaeroic growth of *Thiobacillus ferrooxidans*. *Appl. Environ. Microbiol*. **58**, 2227 - 2230.

Rimstidt J.D., Chermak J.A. and Gagen P.M. 1992. Rates of reaction of galena, sphalerite, chalcopyrite, and arsenopyrite with Fe(III) in acidic solutions, In: *Environmental Geochemistry of Sulfide Oxidation* (C.N. Alpers and D.W. Blowes, Eds.), pp. 2 - 13. American Chemical Society symposium series 550, Washington, D.C.

Raiswell R. and Berner R.A. 1985. Pyrite formation in euxinic and semi-exeunic sediments. *Am. J. Sci*. **285**, 710 - 724.

Ritchie A.I.K. 1994a. Sulfide oxidation mechanisms – Control and rates of oxygen transport, In: *The Environmental Geochemistry of sulfide Mine-Wastes* (J.L. Jambor and D.W. Blowes, Eds.), pp. 210 - 245. Mineralogical Association of Canada, short Course Handbook, 22.

Ritchie A.I.K. 1994b. Rates of mechanisms that govern pollutant generation from pyrite wastes, In: *Environmental Geochemistry of Sulfide Oxidation* (C.N. Alpers and D.W. Blowes, Eds.), pp. 108 - 122. American Chemical Society symposium series 550, Washington, D.C.

Ritchie A.I.M. 1998. Lessons to be learned from rehabilitation of the waste rock dumps at the Rum Jungle uranium-copper mine site. Paper presented at workshop "Environmental remediation of waste rock rock piles", Nov. 6–8, 1995, Chemnitz, Germany.Rhoads D.C. and Morse J.W. 1971. Evolution and ecologic significance of oxygen-depleted marine basins. *Lethaia* **4**, 413 - 428.

Sand W. 1989. Ferric iron reduction by Thiobacillus ferrooxidans at extremely low pH-values. *Biogeochemistry* **7**, 195 - 201.

Sand W., Gerke T., Hallmann R. and Schippers A. 1995. Sulfur chemistry, biofilm, and the indirect attack mechamism – a critical evaluation of bacterial leaching. *Appl. Microbiol. Biotechnol*. **43**, 961 - 966.

Sasaki K. 1994. Effect of grinding on the rate of oxidation of pyrite by oxygen in acid solutions. *Geochim. Cosmochim. Acta* **58**, 4649 - 4655.

Sasaki K., Tsunekawa M., Ohtsuku T. and Konno H. 1998. The role of sulfur-oxidizing bacteria *Thiobacillus ferrooxidans* in pyrite weathering. *Colloids Surf. A* **133**, 269 - 278.

Sasaki K., Tsunekawa M., Ohysuku T. and Konno H. 1995. Confirmation of a sulfur-rich layer on pyrite after oxidative dissolution by Fe(III) ions around pH 2. *Geochim. Cosmochim. Acta* **59**, 3155 - 3158.

Sato M. 1960. Oxidation of sulfide ore bodies; II. Oxidation mechanisms of sulfide minerals at 25^{o}C. *Econ. Geol*. **55**, 1202 - 1231.

Sato M. 1992. Persistency-field Eh-pH diagrams for sulfides and their application to supergene oxidation and enrichment of sulfide ore bodies. *Geochim. Cosmochim Acta* **56**, 3133 - 3156.

Schippers A., Peter-Georg J. and Sand W. 1996. Sulfur chemistry in bacterial leaching of pyrite. *Appl. Environ. Microbiol*. **62**, 3424 - 3431.

Schippers A., Jozsa P.-G. and Sand W. 1998. Evaluation of the efficiency of measures for sulphidic mine wastes mitigation. *Appl. Microbiol. Biotechnol*. **49**, 698 - 701.

Schippers A., Rohwerder T. and Sand W. 1999. Intermediary sulfur compounds in pyrite oxidation : implications for bioleaching and biodepyritization of coal. *Appl. Microbiol. Biotechnol*. **52**, 104 - 110.

Schrenk M.O., Edwards K.J., Goodman R.M., Hamers R.J. and Banfield J.F. 1998. Distribution of Thiobacillus ferrooxidans and Leptospirillum ferrooxidans: Implications for generation of acid mine drainage. *Science* **279**, 1519 - 1522.

Schultze-Lam S., Fortin D., Davis, B.S. and Beveridge T.J. 1996. Mineralization of bacterial surfaces. *Chem. Geol*. **132**, 171 - 181.

Silverman M.P. and Lundgren D.G. 1959. Studies of the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. II. Manometric studies. *J. Bacteriol*. **94**, 1046 - 1051.

Singer P.C. and Stumm W. 1968. Kinetics of the oxidation of ferrous iron. In: *2nd Symposium on Coal Mine Drainage Research*, pp. 12 - 34. National Coal Association/Bituminous Coal Research.

Singer P.C. and Stumm W. 1970. Acidic mine drainage: The rate-determining step. *Science* **167**, 1121 - 1123.

Southam G. and Beveridge T.J. 1992. Enumeration of Thiobacilli within pH-neutral and acidic mine tailings and their role in the development of secondary mineral soil. *Appl. Environ. Microbiol.* **58**, 1904 - 1912.

Southam G. and Beveridge T.J. 1993. Examination of lipipolysaccharide O-antigen populations of Thiobacillus ferrooxidans from two mine tailings. *Appl. Environ. Microbiol.* **59**, 1283 - 1288.

Sposito G. 1989. *The Chemistry of Soils.* Oxford University Press, New York, 277 p.

Steger H.F. and Desjardins L.E. 1978. Oxidation of sulfide minerals, 4. Pyrite, chalcopyrite and pyrrhotite. *Chem. Geol*. **23**, 225 - 237.

Stumm W. and Lee G.F. 1961. Oxygenation of ferrous iron. *Indust. Eng. Chem*. **53**, 143 - 146.

Sugio T., Wada K., More M., Inagaki K. and Tano T. 1988. Synthesis of an iron-oxidizing system during growth of *Thiobacillus ferrooxidans* on sulfur-basal salts medium. *Appl. Environ. Microbiol*. **54**, 150 - 152.

Sugio T., Katagiri T., Inagaki K. and Tano T. 1989. Actual substrate for elemental sulfur oxidation by sulfur:ferric iron oxidoreductase purified from *Thiobacillus ferrooxidans*, *Biochim. Biophys. Acta* **973**, 250 - 256.

Suzuki I., Chan C.W. and Takeuchi T.L. 1992. Oxidation of elemental sulfur to sulfite by Thiobacillus thiooxidans cells. *Appl. Environ. Microbiol*. **58**, 3767 - 3769.

Suzuki I., Chan C.W. and Takeuchi T.L. 1994. Oxidation on inorganic sulfur compounds by *Thiobacilli*, In: *Environmental Geochemistry of Sulfide Oxidation* (C.N. Alpers and D.W. Blowes, Eds.), pp. 60 - 67. American Chemical Society symposium series 550, Washington, D.C.

Suzuki I., Takeuchi T.L., Yuthasastrakosol T.D. and Oh J.K. 1990. Ferrous iron and sulfur oxidation and ferric iron reduction activities on *Thiobacillus ferrooxidans* are affected by growth on ferrous iron, sulfur, or a sulfide ore. *Appl. Environ. Microbiol*. **56**, 1620 - 1626.

Sulzberger B., Sauter D., Siffert C., Banwart S. and Stumm W. 1989. Dissolution of Fe(III) hydroxides in natural waters: laboratory assessment of the kinetics controlled by surface coordination. *Mar. Chem*. **28**, 127 - 144.

Takakuwa S., Fujimori T. and Iwaskai H. 1979. Some properties of cell-sulfur adhesion in *Thiobacillus thiooxidans*. *J. Gen. Appl. Microbiol*. **25**, 21 - 29.

Taylor B.E, Wheeler M.C. and Nordstrom D.K. 1984. Stable isotope geochemistry of acid mine drainage: Experimental oxidation of pyrite. *Geochim. Cosmochim. Acta* **48**, 2669 - 2678.

Taylor B.E., Wheeler M.C. and Nordstrom D.K. 1984. Isotope composition of sulphate in acid mine drainage as measure of bacterial oxidation. *Nature* **308**, 538 - 541.

Tributsch H. 1976. The oxidative disintegration of sulfide crystals by *Thiobacillus ferrooxidans*. *Naturwissenschaften* **63**, 88.

Wakao N., Mishina M., Sakuri H. and Shiota H. 1982. Bacterial pyrite oxidation, I. The effect of pure and mixed cultures of *Thiobacillus ferrooxidans* and *Thiobaccilus thiooxidans* on release of iron. *J. Gen. Appl. Microbiol*. **28**, 331 - 343.

Wakao N., Mishina M., Sakuri H. and Shiota H. 1984. Bacterial pyrite oxidation, III. Adsorption of *Thiobacillus ferrooxidans* cells on solid surfaces and its effect on iron release from pyrite. *J. Gen. Appl. Microbiol*. **30**, 63 - 77.

Wehrli B., Sulzberger B., and Stumm W. 1989. Redox processes catalyzed by hydrous oxide surfaces. *Chem. Geol*. **78**, 167 - 179.

Wiersma C.L. and Rimstidt J.D. 1984. Rates of reaction of pyrite and marcasite with ferric iron at pH 2. *Geochim. Cosmochim. Acta* **48**, 85 - 92.

Williamson M.A. and Rimstidt J.D. 1993. The rate of decomposition of the ferric-thiosulfate complex in acidic aqueous solutions. *Geochim. Cosmochim. Acta* **57**, 3555 - 3561.

Williamsom M.A. and Rimstidt J.D. 1994. The kinetics and electrochemical rate-determining step of aqueous pyrite oxidation. *Geochim. Cosmochim. Acta* **58**, 5443 - 5454.

The vision of the MiMi-programme

Twenty years from today the mining industry in Sweden is still strong and flourishing, using technologies that are internationally competitive and environmentally acceptable. The environmental standards are set high, since most of the ore deposits and mining activities are situated in sparsely populated areas with a very sensitive nature of high ecological and recreational value. Applying economic methods for processing and reuse of waste products, the release of heavy metals from waste deposits is kept low, the impact on the environment is small and restricted to the close vicinity of the mining areas. Methods used for waste disposal and remediation are efficient, robust and reliable so that, when any remediation is completed, a deposit can be left without the need for supervision or maintenance.

The MiMi programme has made it possible to predict the extent of environmental impact and has provided tools and methods to control and design processes and waste treatment systems already from investigation of the mineralogical and chemical composition of the ore and the wall rocks, and the local hydrology and topography. Furthermore, it is possible to design cost-efficient treatment systems for existing deposits of mining waste.

MiMi
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