OXYGEN AND SULFUR ISOTOPE FRACTIONATION BY *ACIDITHIOBACILLUS FERROOXIDANS* DURING AEROBIC AND ANAEROBIC OXIDATION OF COPPER SULFIDE MINERALS UNDER SIMULATED ACID MINE DRAINAGE CONDITIONS

by

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A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science (Chemistry).

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ABSTRACT

Laboratory experiments were conducted to explore the reaction mechanisms and biogeochemical factors influencing chalcopyrite and covellite oxidation and how they affect the $\delta^{18}O$ and $\delta^{34}S$ signatures of the sulfate formed. Experiments were designed to determine how the oxidation environment, namely anaerobic oxidation via $Fe(III)_{(aq)}$ vs. aerobic oxidation via O_2, and the absence or presence of the bacterium *Acidithiobacillus ferrooxidans* may affect the process of chalcopyrite and covellite oxidation. The $\delta^{18}O_{H2O}$ values were varied in the experiments in order to determine the relative incorporation of molecular oxygen and water derived oxygen in the resulting sulfate and thus to help elucidate the reaction pathways. The anaerobic experiments were also conducted under varying $\delta^{18}O_{H2O}$ values in order to more accurately determine the sulfate water fractionation, $\varepsilon^{18}O_{SO4\text{-}H2O}$, as determined by the y-intercept value when $\delta^{18}O_{SO4}$ is plotted relative to $\delta^{18}O_{H2O}$. As expected, anaerobic oxidation of chalcopyrite showed ~100% water oxygen incorporation and $\varepsilon^{18}O_{SO4\text{-}H2O}$ was found to be ~ (3.8 ± 0.4)‰. Additionally, the $\delta^{34}S_{SO4}$ values revealed that all samples showed a relatively large depletion in $\delta^{34}S_{SO4}$ of ~ (-3.8 ± 1.0)‰ relative to the parent mineral during anaerobic oxidation of chalcopyrite, with no significant difference between abiotic and biological samples. The aerobic biological experiments were conducted as long and short term to explore how the extent of the reaction might also affect isotopic fractionation during the oxidation process. Aerobic oxidation of chalcopyrite showed (92 ± 1) and (94 ± 1) % incorporation of

water oxygen into the resulting sulfate during the short and long term biological experiments respectively. Although it is possible that the remainder of oxygen incorporation in the short and long term experiments (8 and 6%, respectively) was due to O_2 incorporation as observed in previous analogous studies of pyrite oxidation, due to inherent analytical uncertainties, it is difficult to say with absolute certainty. Nonetheless, using the measured $\varepsilon^{18}O_{SO4\text{-}H2O}$ value of 3.8‰ from the anaerobic chalcopyrite experiments, the $\varepsilon^{18}O_{SO4\text{-}O2}$ values of 11.7, 24.4, and -10.7 ‰ were estimated for the biological short and long term, and abiotic experiments, respectively. Relative to the $\delta^{34}S$ of the parent material, a small depletion of ~ (-1.5 ± 0.2)‰ in $\delta^{34}S_{SO4}$ was observed for the sulfate formed in the aerobic biological short and long term chalcopyrite experiments. In contrast, the depletion in $\delta^{34}S_{SO4}$ was ~ (-0.5 ± 0.7)‰ for the abiotic aerobic experiments. The $\delta^{18}O_{SO4}$ values measured from the aerobic oxidation of covellite showed 49, 62, and 71 % incorporation of water oxygen into the sulfate formed for the biological short and long term, and abiotic experiments, respectively. However, due to relatively low rates of sulfate production and a high initial concentration of sulfate, estimates of analytical error propagation revealed in fact that incorporation of O2 into the sulfate may not have actually occurred. All samples for the aerobic oxidation of covellite showed ~ 0.2-0.5‰ fractionation of sulfur relative to the parent mineral in the $\delta^{34}S_{SO4}$ values.

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ACKNOWLEDGMENTS

First and foremost I would like to thank my friends and family who have all been very supportive during the writing of this thesis. I would also like to thank my advisor Kevin Mandernack and fellow lab mates Chris Mills and Nurgul Balci for their helpful advice. Thanks also to Wayne C. (Pat) Shanks III for his support from the USGS. Furthermore, I would like to thank many of the USGS scientists and staff for their generous help and support: Rich Wanty for Ion Chromatography (IC) sulfate analyses; Paul Lamothe for ICP-MS; Pamela Gemery-Hill and Cayce Gulbransen for water oxygen, sulfate oxygen, and sulfur isotope analyses; and Mark Stanton for BET measurements, all from the USGS as well. Additionally, I wish to thank Bernhard Mayer for additional sulfate oxygen isotope analyses at the University of Calgary.

CHAPTER 1

INTRODUCTION

The oxidation of sulfide minerals in acid mine drainage (AMD) systems can result in lower pH and the release of transition metals into stream environments, which in turn can have drastic effects on organisms downstream. To provide improved remediation strategies for AMD sites requires an understanding of the various chemical (aerobic vs. anaerobic) and biological vs. abiotic pathways by which these minerals are oxidized. Certain bacteria have been shown to greatly increase the rate of sulfide oxidation in these systems (Wakao et al, 1982; Baldi et al, 1992; Gleisner and Herbert Jr., 2002; Simpson et al, 2005).

Pyrite hosts various metal sulfide minerals which can have an impact on AMD. Except for chalcopyrite, copper sulfide minerals have not received much attention even though high concentrations of copper are potentially threatening to the overall health of aquatic organisms. The oxidation of copper sulfide minerals is largely not understood in terms of the exact mechanisms involved, the concentrations of copper and other elements released, and whether or not acidity is affected (Rodríguez et al., 2003).

Over the past 75 years, consumption of copper in the U.S. alone has tripled (Hudson et al, 1999). The U.S. is the largest consumer and the second largest producer of copper in the world (www.copper.org). Even though about half of the copper used in the U.S. today is recycled, about one million tonnes of copper is mined each year (www.copper.org). Utah and Arizona are the two highest producing copper

mining states in the U.S. and Utah has the largest copper mine in the world. However, all mining operations in the U.S. only cover about 0.02% of the total U.S. land area (Hudson et al, 1999).

A typical metal mine can produce 136,000 metric tonnes of ore daily and for each tonne mined 2-3 tonnes of waste rock are produced (Hudson et al, 1999). In a coal mine up to 80% of the mined material ends up as waste in the form of waste rock, mill tailings, or smelter slag (Ripley et al, 1996). Although the rate at which these waste materials leach metals and acid into the environment varies, a defunct mine can continue to produce these harmful substances for fifty years or more (Sengupta, 1993). A single ton of coal containing only 1% pyritic material can produce over 60 lbs. of sulfuric acid and 33 lbs. of iron hydroxides, or yellow-boy (Sengupta, 1993). In the state of Colorado alone it is estimated that over 1300 miles of rivers and streams are affected with metal pollution, and the majority of these problems are associated with waters draining from mines, mine waste dumps, mill tailings piles, or from abandoned smelter sites (Plumlee et al, 2000).

Once metal sulfide minerals have been mined and become exposed to weathering, they undergo a variety of oxidation pathways that ultimately releases the potentially toxic metals and acid. Aerobic oxidation of chalcopyrite has been described by no fewer than eight equations and several more that describe the anaerobic oxidation of chalcopyrite (Nordstrom, 1977; Steger and Desjardins, 1978; Rimstidt et al., 1994; Schippers et al., 1996; Konishi, et al., 2001). The anaerobic oxidation of chalcopyrite by $Fe(III)_{(aq)}$ is displayed in Equations 1-1 and 1-2 (Nordstrom, 1977; Rimstidt et al, 1994). The stoichiometry of Equation 1-1 predicts

100% incorporation of water oxygen in the resulting sulfate that is formed. Because elemental sulfur is a product of Equation 1-2, the stoichiometry predicts that there should only be 25% water oxygen incorporation when elemental sulfur is further oxidized by O_2 in Equation 1-3. However, this result would not be expected in the absence of air. Both Equations 1-1 and 1-3 predict an increase in $[H^+]$ resulting in a decrease in pH.

$$CuFeS_2 + 16\ Fe^{3+} + 8\ H_2O \longrightarrow Cu^{2+} + 2\ SO_4^{2-} + 17\ Fe^{2+} + 16\ H^+ \quad (1\text{-}1)$$

$$CuFeS_2 + 4\ Fe^{3+} \longrightarrow Cu^{2+} + 5\ Fe^{2+} + 2\ S^0 \quad (1\text{-}2)$$

$$2\ S^0 + 3\ O_2 + 2\ H_2O \longrightarrow 2\ SO_4^{2-} + 4\ H^+ \quad (1\text{-}3)$$

The direct oxidation of chalcopyrite by O_2 can be described by Equations 1-4 and 1-5 (Steger and Desjardins, 1978; Konishi et al., 2001). Although there are several other equations that have been provided to describe the oxidation of chalcopyrite by O_2 (King et al, 1973; Nordstrom, 1977, Rimstidt et al., 1994), these were chosen because they illustrate the large range in water oxygen incorporation as predicted from reaction stoichiometries. In both equations elemental sulfur is initially produced, and then the sulfur is oxidized to sulfate as shown in Equation 1-3 above, and as previously mentioned, the stoichiometry of which predicts that the sulfate should derive 25% of its oxygen atoms from water. Equation 1-5 shows that the oxidation of chalcopyrite produces both sulfur and sulfate. Since the sulfate produced is mostly from oxidation with molecular oxygen the oxygen in sulfate is predicted to come almost exclusively, ~97%, from atmospheric O_2 as predicted by the combined stoichiometries of Equations 1-3 and 1-5. The main difference between Equations 1-4 and 1-5 is that Equation 1-4, combined with Equation 1-3, shows no change in $[H^+]$,

while Equation 1-5 predicts an increase in $[H^+]$ due to the formation of $[H^+]$ during the subsequent oxidation of elemental sulfur by Equation 1-3. All other equations showing the aerobic oxidation of chalcopyrite predict between 75-100% incorporation of atmospheric oxygen in the sulfate formed (King et al, 1973; Nordstrom, 1977, Rimstidt et al., 1994).

$$CuFeS_2 + O_2 + 4\,H^+ \longrightarrow Cu^{2+} + Fe^{2+} + 2\,S^0 + 2\,H_2O \qquad (1\text{-}4)$$

$$8\,CuFeS_2 + 31\,O_2 \longrightarrow 8\,Cu^{2+} + 4\,Fe^{3+} + 14\,SO_4^{2-} + 2\,S^0 + 2\,Fe_2O_3 \qquad (1\text{-}5)$$

The aerobic oxidation of covellite is shown in Equations 1-6 and 1-7 (Nordstrom and Southam 1997; Suzuki, 2001). The stoichiometry of Equation 1-6 predicts that 100% of the oxygen in sulfate is derived from incorporation of atmospheric oxygen. In Equation 1-7, elemental sulfur is the initial oxidation product. This elemental sulfur can be further oxidized by Equation 1-3 to produce sulfate, thus predicting that 75% incorporation of molecular oxygen into the sulfate formed from these coupled reactions. Neither Equation 1-6 nor the combined stoichiometry of Equations 1-3 and 1-7 predicts a change in pH during the course of the reaction.

$$CuS + 2\,O_2 \longrightarrow CuSO_4 \qquad (1\text{-}6)$$

$$2\,CuS + O_2 + 4\,H^+ \longrightarrow 2\,S^0 + 2\,Cu^{2+} + 2\,H_2O \qquad (1\text{-}7)$$

Since the mechanisms by which sulfide minerals become oxidized are poorly understood, one approach for understanding these mechanisms is to measure the stable oxygen isotopic composition of the sulfate ($\delta^{18}O_{SO4}$) formed during the oxidation process (Taylor et al, 1984a: Taylor et al, 1984b; Schippers, 2004; Balci et al., 2007). Because most sulfide oxidation reactions involve the incorporation of

either O_2 and/or water oxygen into the sulfate product and each of these oxygen sources typically have very different $\delta\ ^{18}O_{H2O}$ signatures, one can determine the relative contribution from each by measuring the $\delta\ ^{18}O_{SO4}$ values under varying $\delta\ ^{18}O_{H2O}$ values. Studying the extent to which each of these molecules donates oxygen atoms to sulfate can be extremely useful in determining the exact mechanism of the reactions, which in turn can assist with development of superior AMD mitigation strategies. In this study, replicate laboratory oxidation incubation experiments with chalcopyrite and covellite were made in the presence and absence of sulfide oxidizing bacteria (*Acidithiobacillus ferrooxidans*) under varying $\delta\ ^{18}O_{H2O}$ values used in the microbiological medium.

1.1 How Acid Mine Drainage (AMD) is Produced

AMD is mainly produced by the oxidation of pyrite, the most prevalent host mineral of metal sulfides, and is usually described by Equations 1-8 to 1-10 (Hiskey and Schlitt 1982). Equation 1-8 shows the oxidation of pyrite with ferric iron and Equation

1-9 shows the oxidation of pyrite with molecular oxygen, in both cases acid is produced. Generally speaking, the oxidation of pyrite with ferric iron is faster than with oxygen (Schippers et al, 1996; Nordstrom and Alpers, 1999; Schippers and Sand, 1999). Equation 1-10 shows how ferrous iron is oxidized to ferric iron, which can then re-enter Equation 1-8 to oxidize pyrite further. This process is known as the propagation cycle and is known to be catalyzed by acidiphilic sulfur-oxidizing bacteria (Singer and Stumm 1970).

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \longrightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+ \quad (1\text{-}8)$$

$$2\ FeS_2 + 7\ O_2 + 2\ H_2O \longrightarrow 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+ \quad (1\text{-}9)$$

$$4\ Fe^{2+} + O_2 + 4\ H^+ \longrightarrow 4\ Fe^{3+} + 2\ H_2O \quad (1\text{-}10)$$

1.2 Factors Controlling Oxidation of Sulfide Minerals and AMD Formation

There are a variety of factors that can influence the formation of AMD. Among these factors are temperature, microbes, various mineral influences, and pH effects. Another factor is the mechanism(s) involved in the oxidation process. This section describes each of these factors in detail.

1.2.1 Temperature Effects on Sulfide Mineral Oxidation

Temperature is a major factor that can influence the oxidation rate of sulfide minerals. Generally, higher temperatures lead to higher oxidation rates (Gould et al, 1994; Nordstrom and Southam, 1997; Stott et al, 2003). However, if bacteria are involved in the process, the temperature of the system needs to be tolerable for the bacteria present (Nordstrom and Southam, 1997). This is because bacteria fall into four categories in regards to their preferred temperature range for survival: psychrophiles, mesophiles, thermophiles, and hyperthermophiles. The psychrophiles endure the coldest temperatures, which range from about 0 to 30 °C (Nordstrom and Southam, 1997). Mesophiles have a temperature range of about 25 to 40 °C, thermophiles range from about 40 to 80 °C, and hyperthermophiles can tolerate the highest temperatures, in excess of 80 °C (Nordstrom and Southam, 1997). Therefore, the temperature that the experiment is conducted at is extremely important depending

on which bacteria, and even the particular strain that are incorporated. For instance, *T. ferrooxidans* is considered to be a mesophile, yet this microbe has been observed to oxidize sulfide minerals both below 25 °C and above 40 °C (Nordstrom and Southam, 1997).

1.2.2 Microbial Influences

Equation 1-10 is generally recognized as the rate determining step in the oxidation of pyrite (Singer and Stumm 1970). Catalysis of this reaction by bacteria has been observed to be orders of magnitude faster than when oxidation occurs abiotically (McKibben and Barnes, 1986). At the low pH's of AMD systems, oxidation of Fe(II) and pyrite is very slow if the proper bacteria are absent (Ehrlich, 1996; Fowler et al, 2001). This is because oxygen does not abiotically oxidize Fe(II) rapidly under acidic conditions (<pH 4) (Schwertmann and Fitzpatrick, 1992). It is generally accepted that bacteria, especially *Acidithiobacillus ferrooxidans,* can also catalyze the oxidation of other sulfide minerals such as pyrite, marcasite, pyrrhotite, chalcopyrite, sphalerite, chalcocite, covellite, and molybdenite (Nordstrom, 1977). However, certain strains of a bacterium may not be able to oxidize all or most minerals. For instance, one strain of *A. ferrooxidans* was able to oxidize pyrite or chalcopyrite, but unable to oxidize sphalerite, and another strain could oxidize pyrite or sphalerite, but not chalcopyrite (Nordstrom and Southam, 1997). Tributsch (2001) found that the rate of bacterial metal sulfide dissolution is approximately proportional to the sulfide mineral's solubility product. Therefore, the presence or absence of

bacteria is one factor which controls the dissolution and oxidation of sulfide minerals, which in turn contributes to the problem of AMD (Nordstrom and Southam 1997).

Additionally, different bacteria oxidize sulfide minerals at different rates and in different ways. For instance, *A. ferrooxidans* and *Leptospirillum ferrooxidans* are both capable of oxidizing iron and sulfide minerals, and *A. ferrooxidans* is known to oxidize pyrite at one of the fastest rates of [8.8×10^{-8} (mol/(m^2*s))] (Nordstrom and Southam, 1997). Also, *A. ferrooxidans* is known to oxidize minerals at a lower temperature range (< 20 °C) than *L. ferrooxidans* (Gould et al 1994). Various strains of a bacterium are capable of oxidizing minerals at different maximum rates of oxidation at different temperatures. For instance, two strains of *A. ferrooxidans*, Hardanger and DSM 2705, display similar maximum pyrite oxidation rates, yet DSM 2705's maximum occurs at about 35 °C and strain Hardanger's maximum is at about 20 °C (Nordstrom and Southam, 1997). A third strain of *A. ferrooxidans*, DSM 583, has an even higher rate of oxidation than the other two strains that occurs at about 30 °C (Nordstrom and Southam, 1997). In the oxidation of chalcopyrite, Konishi et al (2001) discovered that *Acidianus brierleyi* oxidized the mineral at a much faster rate at a temperature of 60 °C, compared to *A. ferrooxidans* at 30 °C. Additional studies have shown that thermophilic, or heat loving, bacteria such as *Sulfolobus metallicus* and *Metallosphaera prunae* were also able to oxidize chalcopyrite faster than mesophilic bacteria. However it was shown that this difference is at least partly because chalcopyrite is abiotically oxidized faster at higher temperatures, making the role of bacteria harder to define in terms of optimal temperatures, (Stott et al., 2003).

This simply means that the bacteria chosen for an experiment will determine the temperature required for that experiment.

Other bacteria, such as *A. thiooxidans*, are capable of oxidizing only the sulfur, or intermediate sulfur compounds formed, during the oxidation of sulfide minerals (Nordstrom, 1982). Also, it has been discovered that mixed cultures of bacteria are often able to oxidize a given mineral at a faster rate than just one type of bacteria by itself (Nordstrom, 1982; McGuire et al, 2001). Additionally, not all microbes in a consortium need to be iron or sulfur oxidizers in order for the oxidation rate to be increased (Gould, 1994; Nordstrom and Southam, 1997). For instance, the heterotroph *Acidiphilium cryptum* utilizes pyruvate as its energy source, and since *A. ferrooxidans* excretes pyruvate as a by-product during oxidation, the presence of the heterotroph increases the rate of pyrite oxidation by *A. ferrooxidans* due to Le Chatlier's principle (Nordstrom and Southam, 1997). Therefore, any experiment designed to explore the oxidation of sulfide minerals in the presence of bacteria must incorporate the proper strain(s) of microbes to obtain useful new information.

Another factor controlling microbially mediated oxidation of sulfide minerals is the amount of bacteria present. Wakao et al (1982) found that the release of iron from pyrite oxidation was greatly enhanced in cultures containing 10^9 cells per experiment. This enhanced rate was not observed in samples inoculated with 10^8 cells or less. However, for mixed cultures of *A. thiooxidans* and *A. ferrooxidans*, the increased rate was observed in cultures containing 10^8 cells or less. Wakao et al (1982) also noticed that the lag phase, the time it takes for the inoculated bacteria to start to utilize the pyrite for energy, is dependent on the amount of bacteria

inoculated, and this effect has been noticed by other researchers as well (Rojas-Chapana and Tributsch, 2001; Sand et al, 2001; Gleisner and Herbert Jr., 2002). The fewer bacteria present in the sample, the longer the lag time required for growth. Additionally, Wakao et al (1982) found that the more time that passed between growing the bacteria and actually adding them to the samples also increased the lag time, with two day old cultures exhibiting the smallest lag time compared with 11 day old cultures. Sand et al (2001) found that the lag phase could also be shortened by adding Fe(III) to the solution, and Rojas-Chapana and Tributsch (2001) found that the addition of cysteine could also decrease the lag time. In both cases it was proposed that the Fe(III) and cysteine facilitated the binding of cells to the surface of pyrite. In the case of Fe(III), the added iron may have simply increased the rate of pyrite oxidation via Equation 1-8.

As well as reducing the lag time, the addition of cysteine to biological sulfide mineral oxidation experiments has been observed to increase the rate by a factor of three compared to experiments with no added cysteine (Rojas-Chapana and Tributsch, 2001; Tributsch, 2001). However, the addition of cysteine to abiotic controls also increases the rate of oxidation relative to an abiotic control sample with no added cysteine. Rojas-Chapana and Tributsch (2001) found that the rate for abiotic oxidation of pyrite with cysteine was roughly equal to the biological oxidation rate without added cysteine. Therefore it is thought that cysteine accelerates the oxidation rate by acting as a reducing agent and solubilizing the mineral thereby making the oxidation of the mineral easier (Rojas-Chapana and Tributsch, 2001). These results hint at the role of bacteria and whether or not they need to attach to the

mineral during oxidation. This leads into a discussion between the different proposed mechanisms by which sulfide minerals are oxidized, namely the direct oxidation, indirect oxidation, or the indirect contact oxidation, as outlined in section 1.2.7.

1.2.3 Mineral Type, Grain Size, and Surface effects on Reactivity

Studies have also shown that the type and reactivity of the mineral plays a vital role in determining the rate of oxidation in AMD systems (Olson, 1991). For instance, in the oxidation of pyrite, pure euhedral pyrite has a much slower oxidation rate than framboidal pyrite (Lowson, 1982). Accordingly, depending on the reactivity of the mineral, bacteria may not accelerate the reaction if the reactivity is too low (Baldi et al, 1992). Baldi et al (1992) found that euhedral pyrite remained non-reactive even after being finely ground. In addition to the mineral phase, the reactive surface area is also important (Gleisner and Herbert Jr., 2002). Cracks, pits, and fissures on the surface of the mineral provides more reactive surface area and both biological and abiotic oxidation can occur in these areas as opposed to unmarred surfaces on the mineral (Hiskey and Schlitt, 1982; Goldhaber, 1983; Sand et al, 2001). However, defects and surface debris on the mineral's surface can also impede oxidation of the mineral (Nicholson, 1994; Gleisner and Herbert Jr., 2002). In this case, defects refer to the presence of non-stoichiometric amounts of the elements composing the mineral, other compounds/elements either in the mineral structure, or adsorbed to the mineral's surface, or other minerals in the matrix of the sample. Therefore, researchers in this field suggest that prior to beginning an experiment, mineral samples should be cleaned to remove surface debris and homogenize the

remaining surface by removing air oxidation products (McKibben and Barnes, 1986; Olson, 1991; Nicholson, 1994).

1.2.3.1 Effect of Grain Size/Surface Area on Sulfide Mineral Oxidation Rates

Oxidation rates are often expressed either in terms of the formation of sulfate or the release of metal ions to solution. However, the rate of appearance of these elements is not necessarily equal to the rate of oxidation of the mineral itself or even to each other. Rates of pyrite oxidation based on iron released are typically higher than those based on sulfate production (Lowson, 1982; Janzen et al, 2000). Obviously grain size affects the total surface area of a mineral, so most studies use grain sizes on the order of 50-100 μm in diameter (Nicholson, 1994). It is for these reasons that oxidation rates are typically reported in terms of the surface area of the mineral and how it has changed over the course of the experiment due to sulfate formation, metal ions released, or oxygen consumption (Lowson, 1982, Nicholson, 1994; Janzen et al, 2000). Additionally, the rate of oxidation is often related to the porosity, or mineral surface, to the mass of solution, and not just the surface area alone (Nordstrom and Alpers, 1999). Therefore, researchers have insisted that surface areas of mineral samples be measured, and solution volumes reported for all experiments.

1.2.3.2 Purity of Mineral Phase

Another factor that governs the rate of sulfide mineral oxidation is the presence of impurities in or on the mineral surface (Nordstrom, 1982; Janzen et al,

2000). These impurities can be either non-stoichiometric amounts of other elements composing the mineral or simply another mineral that is co-mixed with the sample matrix. Generally, impurities tend to slow down the rate of mineral oxidation. However, co-existing secondary minerals can increase or decrease the rate of oxidation depending on which minerals are present (Nordstrom and Alpers, 1999). For instance, pyrite oxidizes more slowly than chalcopyrite, but will oxidize even more slowly if these two minerals co-exist. Conversely, chalcopyrite will oxidize at a faster rate when mixed with pyrite than when oxidized alone. This phenomenon is due to galvanic action (Lowson, 1982; Sato, 1992). The more electroconductive sulfide, or the one with the higher standard electrode potential, will oxidize more slowly, and the less electroconductive sulfide will oxidize faster (Sato, 1992; Nordstrom and Alpers, 1999). However, abiotic studies suggest that the oxidation rate of a mineral mixture is the same as the oxidation rate calculated from the sum of the respective mineral fractions combined with their respective rate constants (Nicholson, 1994).

1.2.3.3 Mineral Surface Effects

As oxidation occurs, the reactive surface area of the mineral actually decreases over time, especially in the presence of bacteria. This is because sulfide oxidizing bacteria can create iron hydroxide, or other mineral coatings which prevent the further oxidation of the mineral (Hiskey and Schlitt, 1982; Wakao et al, 1984; Morse, 1991; Moses and Herman, 1991). Additionally, iron hydroxide coatings can form faster than the mineral can be oxidized (Morse, 1991). The formation of iron

hydroxides is expressed in Equations 1-11 and 1-12, which occur after the oxidation of $Fe(II)_{(aq)}$ to $Fe(III)_{(aq)}$ (Nordstrom, 2000):

$$Fe^{3+} + H_2O \longrightarrow FeOH^{2+} + H^+ \quad (1\text{-}11)$$

$$FeOH^{2+} + 2\,H_2O \longrightarrow Fe(OH)_3 + 2\,H^+ \quad (1\text{-}12)$$

De Giudici et al (2002) suggested that the decrease in surface reactivity of sphalerite in their experiment was due to a change in the mechanism governing the dissolution rate. These authors further suggested a change in surface structure during oxidation to explain this finding. They used atomic force microscopy to investigate the mineral's surface during dissolution, and observed that oxidation was taking place on rough edges as opposed to flat surfaces. This resulted in the formation of step edges and nanometric protrusions during dissolution of the mineral. These investigators theorized that the protrusions were formed as a result of a reorganization of the surface at the nanometric scale, and therefore, the rate of oxidation is limited by the dissolution of these protrusions. Williamson and Rimstidt (1994) performed experiments in which the same pyrite sample was repeatedly reacted under the same sample conditions, with a washing step in between each re-exposure. They found that the original reactivity decreased, and could not be reached again even after repeated washings with nitric acid or ethylene-diamine-tetra-acetic acid (EDTA).

1.2.3.4 Mineral Surface Effects-Activation Energy

The dependence of sulfide mineral oxidation on surface reactions is also supported by experimentally derived activation energies (Ea) for the reaction in question. Diffusion controlled reactions typically have an Ea that is 20 (kJ/mol) or

less, while surface driven reactions typically have Ea's above this value. Depending on temperature and stir rates, Ea's estimated for sulfide mineral oxidation reactions ranged from 39 to 92 (kJ/mol) (Lowson, 1982; Goldhaber, 1983; Wiersma and Rimstidt, 1984; McKibben and Barnes, 1986; Williamson and Rimstidt, 1994; Janzen et al 2000). This suggests that the indirect mechanism is more likely than the direct mechanism during the oxidation of sulfide minerals. The direct and indirect mechanisms for sulfide mineral oxidation are detailed in section 1.2.7.

1.2.3.5 Mineral Surface Effects-Adsorbed Chemical Species

Many investigators have also noticed that the ratio of ferric to ferrous iron adsorbed on the surface of pyrite also affects the rate of oxidation (Lowson, 1982; Wiersma and Rimstidt, 1984; Baldi et al, 1992; Nicholson, 1994). When $Fe(III)_{(aq)}$ is the oxidant and the Fe(III)/Fe(II) ratio on the pyrite surface is high, a high concentration of oxygen accelerates the rate of oxidation. However, if oxygen is absent but the $iron(III)_{(aq)}/iron(II)_{(aq)}$ ratio is low, the rate of oxidation is even higher (Nicholson, 1994). These results imply that adsorption of $Fe(III)_{(aq)}$ onto the mineral surface is an important control on the rate of oxidation and that a site-specific adsorption model fits the data. However, results from Williamson and Rimstidt (1992, 1994) support a Freundlich adsorption mechanism that is non-site specific. Within this scenario the oxidation of pyrite is not limited by adsorption. Wiersma and Rimstidt (1984) calculated the rate constants for the oxidation of iron and determined that they were dependent upon the initial ferric iron concentration. The rate constants increased with decreasing initial ferric iron concentration, indicating

that the actual rate of oxidation is inversely related to the ferric iron concentration (Wiersma and Rimstidt, 1984). However, the ferric to ferrous iron ratio in solution can change reversibly depending on the Eh of the solution (Sato, 1992).

1.2.3.6 Oxidation Rates of Different Sulfide Minerals

Different sulfide minerals oxidize at different rates (Janzen et al, 2000; Gleisner and Herbert Jr., 2002). For instance, chalcopyrite abiotically oxidizes faster than sphalerite, which oxidizes faster than pyrite. Furthermore, arsenopyrite oxidizes faster than marcasite, which oxidizes faster than pyrite (McGuire et al, 2001; Gleisner and Herbert Jr., 2002). However, in the presence of bacteria, the rates of oxidation of chalcopyrite and sphalerite are reversed compared to abiotic oxidation (Gleisner and Herbert Jr., 2002). The reason why sulfide minerals oxidize at different rates may have to do with the classification of sulfide minerals into either acid soluble or acid insoluble, as described later in section 4.4.

1.2.4 pH Effects on Sulfide Mineral Oxidation

The pH of the AMD system also influences the oxidation of sulfide minerals. Generally speaking, lowering the pH tends to increase the oxidation rate (Tributsch, 2001; Gleisner and Herbert Jr., 2002). However, several research groups have reported the reverse trend where increased pH increased the oxidation rate (Hiskey and Schlitt, 1982; Goldhaber, 1983; Moses et al, 1987). Others found no difference in the oxidation rates of experiments conducted at two different acidic pH's and with different media (Nordstrom and Southam, 1997). For pH's between 0 and 2, pH has

little effect on the oxidation rate of pyrite (Hiskey and Schlitt, 1982; Goldhaber, 1983). At more neutral pH, oxygen is more important than $Fe(III)_{(aq)}$ as an oxidant for pyrite oxidation, presumably because $Fe(III)_{(aq)}$ is insoluble at neutral pH. However, Moses and Herman (1991) found that $Fe(III)_{(aq)}$ at circumneutral pH could still oxidize pyrite, but the rate was determined by the levels of dissolved oxygen (DO), with faster rates occurring when DO was absent. Additionally, microbial oxidation of pyrite generally occurs only when the pH is less than 5 or 6 (Hiskey and Schlitt, 1982; Nicholson, 1994; Kirby et al, 1999). However, *T. ferrooxidans* has been known to oxidize pyrite up to a pH of ~ 8, with a subsequent reduction of pH to acidic conditions with time (Nordstrom and Southam, 1997).

1.2.5 Flow Rate on Sulfide Mineral Oxidation

Flow or stir rate of an experiment (Moses et al, 1987) is another factor that has been implicated as being important to sulfide mineral oxidation rates. Moses et al (1987) found that oxidation rates increased with increased stir rates, presumably because this increases the frequency by which the oxidant comes into contact with the mineral's surface. This in turn implies that a surface reaction controls the oxidation rates, which supports either the direct contact or indirect contact mechanisms of sulfide mineral oxidation, discussed in section 1.2.7 (Wiersma and Rimstidt, 1984). Additionally, Moses et al (1987) discovered that intermediate sulfur species, such as sulfite, thiosulfate, and polythionates, were only found in samples with high stirring rates.

1.2.6 Why AMD Formation is not a Simple Mechanism

Equation 1-8 shows the loss of one electron from iron, 14 electrons from sulfur, and the gain of 7.5 electrons by oxygen per mole of pyrite oxidized, for a total of 15 electrons transferred (Nicholson, 1994; Nordstrom, 1982). However, most redox reactions involve the movement of only one or two electrons per reaction (Basolo and Pearson, 1967; Luther III, 1987). Therefore Equations 1-8 and 1-9 represent the overall reaction, which must be composed of some or many intermediate reactions in which sulfate is not the first and only sulfur species produced. Instead sulfide ion is probably the initial sulfur product with dissolution of the sulfide mineral, and elemental sulfur and or sulfoxy anions such as thiosulfate and tetrathionate are intermediate species (Sato, 1992; Tributsch, 2001). However, this concept is not universally accepted as discussed in the next section.

1.2.6.1 Intermediate Sulfur Species

Many studies have shown the formation of intermediate sulfur species such as thiosulfate, sulfite, and polythionates during the oxidation of sulfide minerals (Steger and Desjardins, 1978; Goldhaber, 1983; McKibben and Barnes, 1986; Moses et al, 1987, Schippers et al, 1996; Tributsch 2001). However, these intermediate sulfur species only formed when O_2 served as the oxidant (Moses et al, 1987). Studies of pyrite oxidation by ferric iron did not show production of these intermediate species (McKibben and Barnes, 1986). However, it is known that ferric iron reacts rapidly with these sulfur intermediates to form sulfate, especially in the presence of sulfur oxidizing bacteria (Moses et al, 1987). Additionally, *T. ferrooxidans* has been known

to have sulfur globules, containing elemental sulfur and thiosulfate, in its periplasmic space. Therefore, these bacteria possibly absorb and store some of these species before they can react to form sulfate (Schippers et al, 1996).

Luther (1987) theoretically showed that the formation of intermediate sulfur species can easily be explained with molecular orbital theory, and that thiosulfate is almost certainly the first sulfur product produced during pyrite oxidation (Luther III, 1987). Schippers et al (1996) also proposed a reaction pathway that would explain the formation of sulfate through intermediate sulfur species, starting with thiosulfate (Equation 1-13) (Schippers et al, 1996):

$$FeS_2 + 6\ Fe(H_2O)_6^{3+} + 3\ H_2O \blacktriangleright Fe^{2+} + S_2O_3^{2-} + 6\ Fe(H_2O)_6^{2+} + 6\ H^+ \quad (1\text{-}13)$$

$$2\ Fe^{3+} + 2\ S_2O_3^{2-} \longrightarrow 2\ FeS_2O_3^{+} \longrightarrow 2\ Fe^{2+} + S_4O_6^{2-} \quad (1\text{-}14)$$

$$S_4O_6^{2-} + H_2O \longrightarrow HS_3O_3^{-} + SO_4^{2-} + H^+ \quad (1\text{-}15)$$

$$2\ S_3O_3^{2-} + 2\ S_2O_3^{2-} + O_2 + 4\ H^+ \longrightarrow 2\ S_5O_6^{2-} + 2\ H_2O \quad (1\text{-}16)$$

$$S_3O_3^{2-} + S_4O_6^{2-} \longrightarrow S_2O_3^{2-} + S_5O_6^{2-} \quad (1\text{-}17)$$

$$2\ S_3O_3^{2-} + 3\ O_2 \longrightarrow 2\ S_3O_6^{2-} \quad (1\text{-}18)$$

$$4\ S_3O_3^{2-} \longrightarrow S_8 + 4\ SO_3^{2-} \quad (1\text{-}19)$$

Schippers et al (1996) suggest that the sulfite produced in Equation 1-19 is unstable in acidic conditions and is therefore oxidized in the presence of heavy metal cations to sulfate. Although the authors did not report a specific equation to account for this, Equation 1-20 could be inferred:

$$2\ Fe^{3+} + SO_3^{2-} + H_2O \longrightarrow 2\ Fe^{2+} + SO_4^{2-} + 2\ H^+ \quad (1\text{-}20)$$

However, Balci et al (2007) suggested that O_2 may also serve as an oxidant during the oxidation of sulfite via Equation 1-20. Taylor et al (1984 a,b) also found

that there was essentially no sulfur isotope fractionation between the pyrite and the sulfate in solution. This suggests that there are no intermediate sulfur species, or that the intermediate species are so short lived and quantitatively convert to the final sulfate product, resulting in no measurable isotopic fractionation.

1.2.7 Proposed Mechanisms of Sulfide Mineral Oxidation

The actual mechanism by which bacteria oxidize sulfide minerals has been debated. Three general mechanisms have been postulated to explain the microbial influence during sulfide mineral oxidation, namely the direct, indirect, and indirect contact mechanisms (Fowler et al, 2001). Each of the first two mechanisms is supported with experimental results. However, some have postulated that the actual mechanism is a combination of these two, and this third mechanism is referred to as the indirect contact mechanism (Edwards et al, 1998). Additionally, Tributsch (2001) suggested that all three mechanisms may coexist. The following reaction schemes have been postulated for the direct and indirect mechanisms respectively (Sand et al, 2001):

Direct Mechanism

$$2\ FeS_2 + 7\ O_2 + 2\ H_2O \longrightarrow 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+ \quad (1\text{-}9)$$

$$4\ Fe^{2+} + O_2 + 4\ H^+ \longrightarrow 4\ Fe^{3+} + 2\ H_2O \quad (1\text{-}10)$$

Indirect Mechanism

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \longrightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+ \quad (1\text{-}8)$$

$$MS + 2\ Fe^{3+} \longrightarrow M^{2+} + S^0 + 2\ Fe^{2+} \quad (1\text{-}21)$$

$$2\ S^0 + 3\ O_2 + 2\ H_2O \longrightarrow 2\ SO_4^{2-} + 4\ H^+ \quad (1\text{-}3)$$

Where M in the above equations stands for a metal species.

The indirect contact mechanism is simply a merging of the two other mechanisms. The direct contact mechanism requires that the bacteria attach themselves to the surface of the mineral to oxidize it. However, the indirect mechanism does not require contact between the bacteria and the mineral, the bacteria simply oxidize ferrous iron to ferric iron in solution, and then the ferrous iron oxidizes the mineral. For the indirect contact mechanism, the bacteria attach themselves to the mineral yet still oxidize ferrous iron to ferric iron in solution, and then the ferric iron oxidizes the mineral.

1.2.7.1 Direct Oxidation

The direct mechanism involves the attachment of microbes directly to the mineral surface and subsequent enzymatic attack resulting in the oxidation of the mineral surface (Devasia et al, 1993). Devasia et al (1993) showed that the isoelectric point (IEP) of pyrite surfaces shifted significantly during oxidation in the presence of *A. ferrooxidans*, resulting in the bacterial cells exhibiting a greater hydrophobicity compared to cells grown only on ferrous ions. This implies that hydrophobic interactions are important in the adhesion of cells to a mineral surface for oxidation to occur. This conclusion is supported by the studies of Blake II et al (2001), who found that the strength of hydrophobic bonding was greater when the bacteria had been grown on sulfur rather than iron. Interestingly, anionic surfactants are known to be bactericidal under acidic conditions, and anionic surfactants would be hydrophilic in nature (Gould et al, 1994). This also seems to support the findings from Devasia et al (1993), because hydrophilic surfactants would adhere to a mineral's surface making it

hydrophilic, thus preventing the adhesion of hydrophobic bacteria to the mineral. Due to the change in the IEP, Devasia et al (1993) concluded that this represented a change in the surface of both the mineral and the attached cell. This was further supported by enzyme-linked immunosorbent assay (ELISA) experiments. In these experiments, an antiserum was developed that bound to all microbes grown on pyrite, chalcopyrite, and elemental sulfur (Devasia et al, 1993). However, cells grown on ferrous iron showed no immunoreactivity when exposed to this antiserum because these cells do not attach to a surface. This implies that cells grown on sulfur and sulfide mineral cultures form some bond not required by cells grown on ferrous ion (Devasia et al, 1993). Using Fourier transform infrared (FT-IR) spectroscopy, Devasia et al (1993) also observed a new proteinaceous cell surface in sulfur and sulfide grown cells formed, that was not found in cultures grown on ferrous iron. From this observation, this group of researchers postulated that this proteinaceous cellular material represented an appendage that allowed the microbes to attach to the sulfide mineral surface, which was unnecessary for the microbes when using aqueous ferrous ions (Devasia et al, 1993). The new surface was termed proteinaceous because the IR spectroscopy revealed bands displaying organic carbon compounds, including amine and carboxyl components, which were not present in ferrous iron grown cells. Sand et al (2001) suggested that the proteinaceous material was actually an extracellular polymeric substance (lipopolysaccharides).

Blake II et al (2001) found that aporusticyanin, a modified protein thought to enhance bacterial attachment to surfaces, influenced the adhesion of cells to the pyrite surface. Incubating pyrite with dissolved aporusticyanin prior to adding the bacteria

inhibited the adhesion of the cells to the mineral's surface compared to replicate experiments without added aporusticyanin. In contrast, when the bacteria were exposed to aporusticyanin before being added to the pyrite sample, the number of cells that adhered to the pyrite increased relative to replicate control experiments without aporusticyanin (Blake II et al, 2001). Conversely, when both the pyrite and the bacteria were exposed to aporusticyanin before being exposed to each other, relative to all of their other experiments, the smallest amount of cells adhered to the pyrite. Therefore, these authors postulated that soluble exogenous aporusticyanin worked as a stimulant for the adhesion of cells to pyrite, and at the same time, competed for available binding sites on the mineral's surface. Edwards et al (1998) also found that yeast extract added to microbial pyrite oxidation experiments increased the number of bacteria adsorbed to the surface of the mineral. Therefore, direct contact of the bacteria to the surface of the sulfide mineral is required for this mechanism to occur.

1.2.7.2 Indirect Oxidation

With the indirect mechanism, the microbes do not have to be in direct contact with the mineral. Instead the microbes simply oxidize soluble iron(II)$_{(aq)}$ near the mineral's surface, and the iron(III)$_{(aq)}$ formed then attacks the mineral resulting in its oxidation (Fowler et al, 2001; Sand et al, 2001). Sand et al (2001) further proposed that there were actually two different indirect mechanisms operating, depending on the presence of key intermediates, thiosulfate or polysulfide. The presence of these intermediates in turn depends on whether the parent mineral is either pyrite or another

metal sulfide with a similar or different electronic and crystal structure. They proposed two reaction mechanisms for thiosulfate and polysulfide (Sand et al, 2001):

Thiosulfate

$$FeS_2 + 6\ Fe^{3+} + 3\ H_2O \longrightarrow 7\ Fe^{2+} + S_2O_3^{2-} + 6\ H^+ \quad (1\text{-}22)$$

$$S_2O_3^{2-} + 8\ Fe^{3+} + 5\ H_2O \longrightarrow 8\ Fe^{2+} + 2\ SO_4^{2-} + 10\ H^+ \quad (1\text{-}23)$$

Polysulfide

$$MS + Fe^{3+} + H^+ \longrightarrow M^{2+} + 0.5\ H_2S_n + Fe^{2+} \quad (n \geq 2) \quad (1\text{-}24)$$

$$8\ H_2S_n + 16\ Fe^{3+} \longrightarrow n\ S_8 + 16\ Fe^{2+} + 16\ H^+ \quad (1\text{-}25)$$

$$S_8 + 12\ O_2 + 8\ H_2O \longrightarrow 8\ SO_4^{2-} + 16\ H^+ \quad (1\text{-}3)$$

1.2.7.3 Indirect Contact Oxidation

As the name implies, the indirect contact mechanism means that the oxidation of the mineral proceeds by reaction with the oxidized iron formed by the activity of the microbes which are attached to the surface of the mineral (Fowler et al, 2001; Sand et al, 2001). This mechanism seems to be the most accurate since the attachment of microbes to a mineral's surface is well known, microbes are known to catalyze the oxidation of iron(II)$_{(aq)}$ to iron(III)$_{(aq)}$, and activation energies imply a chemical reaction. Nonetheless, which of the three mechanisms is dominant is still not known. However, Fowler et al (2001) reported that an increase in the bioleaching rate after the addition of bacteria is likely due to the indirect contact mechanism. They further found that there was an increase in the pH at the mineral surface, which was due to the attachment of microbes via Equation 1-10 above. This can only be explained by the indirect contact mechanism (Fowler et al, 2001).

1.2.8 Comparison of Experimentally Derived Rate Laws and Expressions

Various researchers have reported different rates and rate laws for the oxidation of sulfide minerals. However, because various investigators conducted their experiments under different conditions, direct comparison of rates is extremely difficult. This is especially true because the rates are often reported in different units. Therefore, only a brief review of some of the more recent oxidation rates and rate laws will be discussed here, which also serves to show how these various rates differ from one another. For instance, McKibben and Barnes (1986) reported a rate law for the oxidation of pyrite by ferric iron and compared their results to those in the literature given in Table 1-1. Unfortunately, rate laws derived from ferric iron oxidation from other investigators did not agree well with their experiments (McKibben and Barnes, 1986). However, the rate laws derived for DO agreed fairly well. In all cases McKibben and Barnes (1986) reported only the dependence of the rate on molarity of the chemical species, and did not report a correction factor for these comparisons, or the units of the rate. Williamson and Rimstidt (1994) also reported a rate law for the oxidation of pyrite by dissolved oxygen and hydrogen peroxide in Table 1-1.

Table 1-1: Rate laws for the oxidation of pyrite by ferric iron, dissolved oxygen, and hydrogen peroxide

Fe(III)

Rate	Temp. (°C)	pH	Reference
$R(\text{mol of pyrite/cm}^2\text{*min}) = (-10^{-9.74})*(M_{Fe3+})^{0.58}*(M_{H+})^{-0.5}$	30	1-2	McKibben and Barnes, 1986
$R(\text{mol of pyrite/m}^2\text{*s}) = (10^{-8.58})*(m_{Fe3+})^{0.3}*(m_{H+})^{-0.32}*(m_{Fe2+})^{-}$	25	0.5-3	Williamson and Rimstidt, 1994
$R(\text{mol of pyrite/m}^2\text{*s}) = (10^{-6.07})*(m_{Fe3+})^{0.93}*(m_{Fe2+})^{-0.4}$	25	0.5-3	Williamson and Rimstidt, 1994
$R = (M_{Fe3+})$	25-50	2	Wiersma and Rimstidt, 1984
$R = (M_{Fe3+})*(M_{Fe3+} + M_{Fe2+})^{-1.0}*(M_{H+})^{-0.44}$	30-70	0-1.5	Mathews and Robins, 1972
$R = (M_{Fe3+})*(M_{Fe3+} + M_{Fe2+})^{-1.0}$	30	0-2	Garrels and Thompson, 1960

Dissolved O_2

Rate	Temp. (°C)	pH	Reference
$R(\text{mol of pyrite/cm}^2\text{*min}) = (-10^{-6.77})*(M_{O2})^{0.5}$	30	2-4	McKibben and Barnes, 1986
$R(\text{mol of pyrite/m}^2\text{*s}) = (10^{-8.19})*(m_{DO})^{0.5}*(m_{H+})^{-0.11}$	25	2-10	Williamson and Rimstidt, 1994
$R = (M_{O2})^{0.81}$	30-70	-0.1-1.2	Mathews and Robins, 1972
$R = (M_{O2})^{0.7}*(M_{H+})^{-0.1}$	20-35	2-10	Smith and Shumate, 1970

H_2O_2

Rate	Temp. (°C)	pH	Reference
$R(\text{mol of pyrite/cm}^2\text{*min}) = (-10^{-1.43})*(M_{H2O2})$	30	2-4	McKibben and Barnes, 1986

Experimentally determined rates for abiotic and biological pyrite oxidation of $1.3*10^{-6} - 2.8*10^{-7}$ (mol of pyrite/m^2*s) and $1*10^{-5} - 1.4*10^{-6}$ (mol of pyrite/m^2*s), respectively, were reported by Edwards et al (1998). The biological rate was measured in the presence of a mixed culture of bacteria, and the temperature of the experiment was 42 °C with a pH of 0.7. These results were compared by Edwards et al (1998) with an abiotic oxidation rate at 30 °C and pH of 2-4 of $3*10^{-6} - 1.7*10^{-8}$ (mol of pyrite/m^2*s) by McKibbens and Barnes (1986) and abiotic and biological rates of $2.5*10^{-9}$ (mol of pyrite/m^2*s) and $8.6*10^{-8}$ (mol of pyrite/m^2*s), respectively, by Olson (1991). The experiments of Olson (1991) were conducted at 25 °C and a pH of 2-3, and the biological experiments were in the presence of *A. ferrooxidans*. The mixed culture used by Edwards et al (1998) did not contain *A. ferrooxidans*.

Janzen et al (2000) reported a mean rate for pyrrhotite oxidation by DO as ($4*10^{-9}$ (mol of iron/m^2*s)), at 25 °C and pH of 2.75. They also reported oxidation rates for pyrrhotite by ferric iron under three different conditions (Janzen et al 2000):

$3.5*10^{-8}$ at 25 °C, pH 2.75, and initial [ferric iron] as $2*10^{-4}$ mol/L

$3.1*10^{-8}$ at 25 °C, pH 2.5, and initial [ferric iron] as $2*10^{-4}$ mol/L

$6.8*10^{-8}$ at 25 °C, pH 2.5, and initial [ferric iron] as 10^{-3} mol/L

Additionally, Janzen et al (2000) reported a non-oxidative pyrrhotite dissolution rate of $5*10^{-10}$ (mol/m^2*s) at a temperature of 25 °C and a pH of 2.75.

McGuire et al (2001) found the following dissolution rates for pyrite, marcasite, and arsenopyrite in the presence and absence of various bacteria as summarized in Table 1-2. These results also serve to show how a mixture of bacteria

can greatly increase oxidation rates compared to abiotic oxidation rates, and oxidation rates observed when only one species of bacteria is present.

Table 1-2: Dissolution rates (μmol/m^2*day) of pyrite, marcasite, and arsenopyrite with calculated errors, at 37 °C and pH of 1.5

Culture	Mineral		
	Pyrite	Marcasite	Arsenopyrite
T. caldus	100 ± 20	200 ± 30	380 ± 120
Ferroplasma acidarmanus	150 ± 10	320 ± 100	980 ± 150
Enrichment culture	230 ± 30	1000 ± 330	1330 ± 360
Abiotic control	100 ± 20	250 ± 80	510 ± 110

Note: The enrichment culture came from a mine at Iron Mountain, California, and was suspected of containing organisms closely related to *L. ferrooxidans*, *F. acidarmanus*, and *T. caldus*. From McGuirre et al, 2001.

As can be seen from the above rate equations, rate laws, and conditions, it can be quite difficult to directly compare the results from various studies. This underscores the need for experiments to be conducted under known conditions so that accurate comparisons between different studies can be made. For instance, when Edwards et al (1998) compared their results to Olson's (1991) results, they had to make corrections for different units, from (mg of Fe/L*h) to (mol of pyrite/m^2*s), even though BET measurements were not provided by Olson (1991). Therefore, Edwards et al (1998) had to make an estimated guess using the weight of pyrite and the grain sizes given by Olson.

CHAPTER 2

MATERIALS AND METHODS

2.1 Mineral Preparation

Chalcopyrite ($CuFeS_2$) and covellite (CuS) samples were both obtained from Ward's Natural Science and are research grade (>90% pure). The composition of the chalcopyrite sample was independently measured at the U.S. Geological Survey (USGS) in Denver, CO by inductively coupled mass spectrometry (ICP-MS) to be 27.5% Fe, 27.2% Cu, 44.9% S, and 0.3% Zn. Similarly, the covellite sample was determined to be 43% S, 56.2% Cu, and 0.7% Fe. Prior to the experiments all minerals were ground and sieved to a grain size between 5 and 63 μm. Surface area measurements were determined by Brunauer-Emmett-Teller, (BET), adsorption isotherms at the USGS in Denver Colorado, and were 0.9051 and 0.3202 (m^2/g) for chalcopyrite and covellite respectively. Insufficient chalcopyrite remained after these measurements for the anaerobic experiments, so additional chalcopyrite was ground and sieved. The BET measurement for these samples was <0.10 (m^2/g). Noticeable quartz crystals were removed from the covellite during crushing. The mineral samples were sterilized by spreading the mineral in a thin layer and liberally spraying with 70% ethanol followed by a 30 minute exposure to ultraviolet (UV) light in a sterile flow hood. The minerals were then mixed with a sterile spatula and the process was repeated with liberal ethanol spraying and another 30 minute exposure to

the UV light. After treatment the minerals were either used immediately or stored in sterile containers.

2.2 Bacterial Culture Preparation

A. ferrooxidans (23270) is an acidiphilic Fe(II) and sulfur oxidizing bacteria used in all experiments, which was obtained from the American Type Culture Collection (ATCC). The bacteria were maintained in a modified 2039-ATCC medium containing the following per liter: 0.6 g NH_4Cl, 0.2 g $MgCl_2 \cdot 6H_2O$, 0.5 g K_2HPO_4, 0.54 g $MgSO_4 \cdot 7H_2O$, 5 ml of a modified Wolfe's mineral salt solution (1.5 g nitrilotriacetic acid, 2.5 g $MgCl_2 \cdot 6\ H_2O$, 0.59 g $MnCl_2 \cdot 4\ H_2O$, 1 g NaCl, 0.07 g $FeCl_2 \cdot 4\ H_2O$, 0.1 g $CoCl_2 \cdot 6\ H_2O$, 0.1 g $CaCl_2$, 0.09 g $ZnCl_2 \cdot 7\ H_2O$, 0.01 g $CuSO_4 \cdot 5\ H_2O$, 0.01 g $AlK(SO)_4 \cdot 12\ H_2O$, 0.01 g H_3BO_3, 0.01 g $Na_2MoO_4 \cdot 2\ H_2O$), and 1 g of either elemental sulfur or chalcopyrite. The bacteria were subcultured three times before inoculation into the final experiments with chalcopyrite or elemental sulfur. Because the bacteria grow well on elemental sulfur and not covellite, elemental sulfur was used for maintenance of the covellite experimental cultures and was sterilized in the same fashion as described above for the minerals. The final pH was adjusted to ~2.8 with hydrochloric acid. All media solutions were filter sterilized (0.1 μm Pall life Sciences Supor-450 membrane filters) prior to use.

After 1-2 months the bacteria were harvested by first filtering through sterile Whatman #1 filter papers to remove the mineral still present and then the cultures were centrifuged at 9000 RPM to obtain a bacterial pellet. For the aerobic and anaerobic experiments the pellet was then washed with 1 M HCl and re-dissolved in

100 or 60 ml of media, respectively, containing no mineral nor $MgSO_4 \cdot 7H_2O$ as energy or nutrient sources (blank media) (Balci et al, 2007). Five ml of cell suspension was used per 250 ml of media in each of the biological experiments.

The cell densities used for the experiments were estimated using both a Petroff-Hausser counting chamber and the Most Probable Number (MPN) methods. MPN counts were conducted using the same media described above except that 7.1 g of $FeCl_2 \cdot 2H_2O$ was used as an electron donor instead of chalcopyrite or elemental sulfur to allow for production of iron oxyhydroxides, the appearance of which is used as a proxy for determining bacterial growth. For both the covellite and chalcopyrite experiments the MPN determination gave a value of ~1.1 X 10^8 (cells/ml) and 1.9 X 10^8 (cells/ml) for the aerobic and anaerobic experiments, respectively, which was used as the initial value to inoculate the biological experiments. All Petroff-Hausser counts were consistently slightly lower than the MPN counts, ~7.0 X 10^7 (cells/ml) and 1.2 X 10^8 (cells/ml) for the aerobic and anaerobic experiments, respectively.

2.3 Anaerobic Biological and Abiotic Experiments

All glassware and equipment used in the experiments were acid washed and autoclaved. The anaerobic experiments were setup in an anaerobic chamber with a gas mixture composed of 5 % CO_2, 5 % H_2, and 90 % N_2. The chamber was equipped with a UV lamp which, after spraying the working surface with 70 % ethanol, was turned on for 25 minutes to sterilize the inside of the anaerobic chamber. The anaerobic experiments were setup using the same ratio of liquid media (ml) to

mass of mineral (g) as in the aerobic experiments, which is 0.3 g of chalcopyrite per 100 ml of media.

To determine the relative percent incorporation of H_2O derived oxygen into the sulfate produced, microbiological media was prepared using waters with three different oxygen isotopic values: -15.8 ‰ for normal laboratory DI water (Light water), -2.8 ‰ (Medium water), and 14.6 ‰ (Heavy water). The media waters also all had a starting pH value of ~2.8.

All abiotic and biological anaerobic experiments incubated for only a single ~8 week period. The biological experiment was conducted with only one light $\delta^{18}O_{H2O}$ value because a previous report for Fe(III) mediated pyrite oxidation indicated no distinguishable difference between biological and abiotic experiments (Balci et al, 2007). An additional incubation flask was used solely for monitoring pH and chemistry during the oxidation process. Additional vials were used for $\delta^{18}O_{SO4}$ and $\delta^{34}S_{SO4}$ analysis. All incubations for isotopic analysis were conducted in 1 L sealed media bottles with 500 ml of media. Monitoring experiments were setup in 200 ml serum vials with 100 ml of media, with each serum vial being sacrificed at different time points. In addition to the media, 2 ml of concentrated bacteria or blank media was added per 100 ml of media.

Anhydrous $FeCl_3$ was added as the oxidant for the anaerobic experiments. A 1 M solution of anhydrous $FeCl_3$ was first prepared by adding the $FeCl_3$ to acidified DI water. This solution was then filter sterilized. The $FeCl_3$ and all solutions used in the anaerobic experiments were first purged with nitrogen for 25 minutes and then left to equilibrate in the anaerobic chamber for at least one day. The $FeCl_3$ stock

solution was added to the media to a final concentration of 10 mM. After sealing the serum vials inside the chamber, the vials were removed from it and placed on a shaker table (150 RPM) in a 25 °C environmental room for the duration of the experiment. All experiments were run in duplicate.

At each time point, replicate serum vials from each experiment were opened in the anaerobic chamber and filtered (0.1 μm filters). The filtrates were measured for pH with a Denver Instruments AP-25 pH/ion/FET meter with an Accumet electrode and copper and iron concentrations were determined by a DR-700 Colorimeter Hach field kit. Sulfate concentrations were also analyzed by ion chromatography, (IC), using a Dionex-500 instrument running with an AG-17 guard column, AS-17 analytical column, and KOH as the eluent. IC analyses were made at the USGS in Denver, CO. At the end of each incubation experiment all excess filtrate from samples designated for isotopic analysis were treated with 10 ml of a 10 % wt./wt. $BaCl_2*2H_2O$ solution to precipitate $BaSO_4$ for $\delta^{18}O_{SO4}$ and $\delta^{34}S_{SO4}$ analysis via isotope ratio mass spectrometry (IRMS).

2.4 Aerobic Biological and Abiotic Experiments

Similar sterile technique and experimental design as described above was also used in the aerobic incubation experiments, except that no $FeCl_3$ was added as an oxidant. However, all aerobic experiments were designed as abiotic long term and biological short and long term incubations to additionally investigate the effects of prolonged incubation and decreased pH on the final $\delta^{18}O_{SO4}$ values. As for the anaerobic experiments, to determine the relative percent incorporation of H_2O derived

oxygen into the sulfate produced, the microbiological media was prepared using the same waters with different $\delta^{18}O_{H2O}$ values: -15.8 ‰, -2.8 ‰, and 14.6 ‰. The short and long term biological and abiotic controls were prepared in 500 ml Erlenmeyer flasks and each had 250 ml of blank media. To each biological experiment 5 ml of the concentrated bacterial culture was added, or 5 ml of additional blank media for the abiotic controls. The pH in all flasks was adjusted to ~2.8 with HCl. To each flask 0.75 g of chalcopyrite or covellite was added. At the end of each experiment, all monitoring and experimental flasks were sampled, and $BaSO_4$ was again precipitated from the excess filtrate for $\delta^{18}O_{SO4}$ and $\delta^{34}S_{SO4}$ analysis as previously described. All incubation flasks were fitted with rubber stoppers fitted with inflow and outflow tubes to allow continuous sparging with air (~1 bubble/s). The inflow and outflow tubes were fitted with 0.2 μm sterile hydrophobic membrane filters to prevent both bacterial contamination and evaporation. Once assembled the experiments were removed from the sterile flow hood and placed on shaker tables (150 RPM) in a 25 °C temperature-controlled room for the duration of the experiment. All experiments were run in duplicate.

As in the anaerobic experiments, monitoring flasks were again prepared to periodically measure pH and chemistry to asses the extent of the reaction. The monitoring flasks had twice the amounts of media, (+/-) bacteria, and minerals as listed above and were prepared in 1 L flasks. At various time points, ~30 mL samples were removed during the course of the experiment and filtered through 0.1 μm filters in the sterile flow hood. A ~10 ml portion of this filtrate was acidified with 1 M HCl for Fe and Cu analysis by a Hach field kit and inductively coupled plasma mass

spectrometry (ICP-MS) using a Perkin-Elmer ELAN model 6000 equipped with a pneumatic nebulizer, cyclonic spray chamber, and a CETAC aerosol desolvation system that attenuates the formation of element oxides in the plasma at the USGS in Denver, CO. Sulfate concentrations were also measured by IC throughout the course of the experiments. A water sample was also removed at the end of the experiment to monitor for possible changes in $\delta^{18}O_{H2O}$ due to evaporation.

2.5 Isotopic Analysis

The $BaSO_4$ collected from each incubation was washed with 100 ml of 1 M HCl and 500 ml DI water. The filter cake was then baked at 100 °C for 24 hrs. for abiotic samples or 500 °C for 2 hrs. for biological samples prior to analysis to more effectively remove cellular material according to methods described elsewhere (Mandernack et al, 2000). Sulfur isotope ratios were performed at the USGS in Denver, CO by combustion using continuous flow methods described by Giesemann et al. (1994) using a Costech Instruments elemental combustion system with a zero-blank autosampler coupled to a Finnigan Delta Plus XP mass spectrometer. The sulfur isotope measurements have a deviation of ± 0.2‰. Oxygen isotope ratios for sulfate collected from the anaerobic experiments were performed at the University of Calgary using a Thermo-Finnigan TC/EA at 1450 °C coupled to a gas source mass spectrometer with a deviation of ± 0.5‰. Oxygen isotope ratios for the aerobic experiments were performed at the USGS in Denver, CO by pyrolysis with a Finnigan TC/EA coupled to a Finnigan Delta Plus XL mass spectrometer using

continuous flow methods modified from Hilkert et al. (1999) and Kornexl et al. (1999), respectively. The $\delta^{18}O_{SO4}$ measurements have a deviation of ± 0.4‰.

The $\delta^{18}O$ of the waters used in the media were analyzed at the beginning and end of the experiment to assess whether evaporation had occurred during the incubations. These average $\delta^{18}O_{H2O}$ values were used for graphing the $\delta^{18}O_{SO4}$ vs. $\delta^{18}O_{H2O}$ plots. The $\delta^{18}O$ of the media waters were measured by IRMS at the USGS in Denver, CO. These analyses were conducted by analyzing $CO_2(g)$ that had equilibrated with 200 µl aliquots of the media water at 40 °C in septum capped vials. Then the raw data was corrected for isotopic fractionation between H_2O and CO_2, and then further corrected for small instrumental effects using results from water standards that were previously calibrated against both Vienna Standard Mean Ocean Water (VSMOW) and Standard Light Antarctic Precipitation (SLAP). Standard deviation for this method is ± 0.1‰.

The oxygen isotope results are reported relative to Standard Mean Ocean Water (SMOW) and the sulfur isotopes are reported relative to Canyon Diablo Troilite (CDT). The $\delta^{18}O$ and $\delta^{34}S$ notations are expressed in Equations 2-1 and 2-2:

$$\delta^{18}O_{(SMOW)}\text{sample} = [(^{18}O/^{16}O\text{sample})/(^{18}O/^{16}O_{(SMOW)}) - 1)] * 10^3 \quad (2\text{-}1)$$

$$\delta^{34}S_{(CDT)}\text{sample} = [(^{34}S/^{32}S\text{sample})/(^{34}S/^{32}S_{(CDT)}) - 1)] * 10^3 \quad (2\text{-}2)$$

2.6 Calculation of Oxygen Source and Isotopic Fractionation in Sulfate

The $\delta^{18}O_{SO4}$ value from the anaerobic oxidation of chalcopyrite will directly reflect the $\delta^{18}O_{H2O}$ value and the extent to which water oxygen atoms are isotopically fractionated during incorporation into sulfate, $\varepsilon_{SO4\text{-}H2O}$. Because the oxygen in sulfate

formed during aerobic oxidation of metal sulfides can come from two different sources, either water or molecular oxygen, this can complicate the interpretation of $\delta^{18}O_{SO4}$ values. When dealing with a dual source system, there are certain variables that become important as outlined in Equation 2-3 below. The $\delta^{18}O_{SO4}$ value is dependent on the fraction of oxygen atoms coming from water, (X), the $\delta^{18}O_{H2O}$ value, $\varepsilon_{SO4\text{-}H2O}$, the fraction of oxygen atoms derived from molecular O_2, (1-X), the $\delta^{18}O_{O2}$ value (~23.5-24‰), and the isotopic fractionation of O_2 during incorporation into sulfate, $\varepsilon_{SO4\text{-}O2}$ (Mandernack et al, 1995). Equation 2-3 assumes that any kinetic isotope fractionation effects are relatively small.

$$\delta^{18}O_{SO4} = X*(\delta^{18}O_{H2O} + \varepsilon_{SO4\text{-}H20}) + (1\text{-}X)*(\delta^{18}O_{O2} + \varepsilon_{SO4\text{-}O2}) \quad (2\text{-}3)$$

By graphing $\delta^{18}O_{SO4}$ vs. $\delta^{18}O_{H2O}$ used in the microbiological media the slope of the resulting plot equals the percentage of water-derived oxygen in the sulfate formed, also equal to X in equation 2-3. A slope of one would indicate 100% water oxygen incorporation and a slope of 0 would indicate 100% atmospheric oxygen incorporation as shown in Figure 2-1. Equation 2-3 can be rearranged as follows:

$$\delta^{18}O_{SO4} = X*(\delta^{18}O_{H2O}) + ((1\text{-}X)*(\delta^{18}O_{O2} + \varepsilon_{SO4\text{-}O2}) + X*\varepsilon_{SO4\text{-}H20}) \quad (2\text{-}4)$$

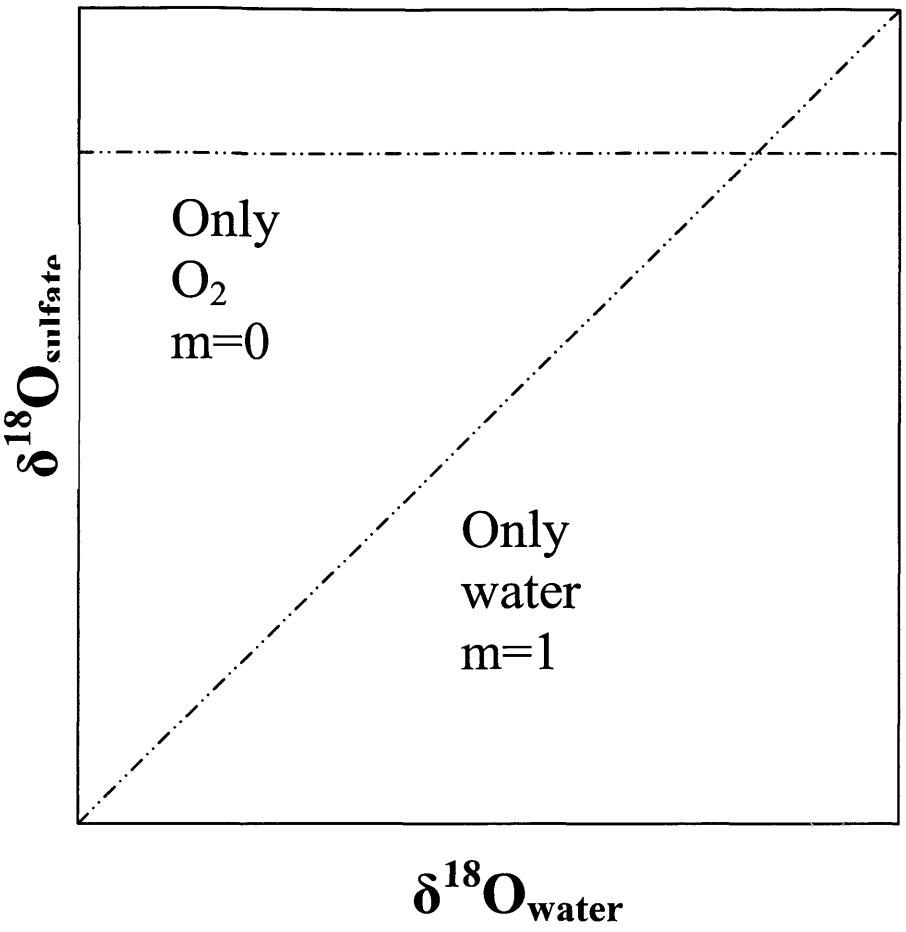

Figure 2-1: Theoretical Plot of $\delta^{18}O_{SO4}$ vs. $\delta^{18}O_{H2O}$

From Equation 2-4 a direct relationship exists between the y-intercept, b, and $\varepsilon_{SO4\text{-}H2O}$, which can simply be estimated for the anaerobic experiments as $b = (\delta^{18}O_{SO4} - X*\delta^{18}O_{H2O})$. To then estimate $\varepsilon_{SO4\text{-}O2}$, the derived value for $\varepsilon_{SO4\text{-}H20}$ from the anaerobic experiments must be used. Therefore, the estimated value for $\varepsilon_{SO4\text{-}O2}$ is given by Equation 2-5.

$$\varepsilon_{SO4\text{-}O2} = (b\text{-}X^* \varepsilon_{SO4\text{-}H2O})/(1\text{-}X) - \delta^{18}O_{O2} \quad (2\text{-}5)$$

2.7 Error Propagation Analysis for Oxygen and Sulfur Isotope Measurements

The presence of substantial sulfate in the initial inoculum made it necessary to make corrections for the initial sulfate when calculating the final $\delta^{18}O_{SO4}$ values. Estimates were also made of error propagation, which is dependent on the initial or average $\delta^{18}O_{H2O}$ values, the measured $\delta^{18}O_{SO4}$ values, and also on the IC measurements for sulfate. The total error propagation associated with determination of the $\delta^{18}O_{SO4}$ values was calculated as follows starting with Equation 2-6.

$$\delta^{18}O_{measured} = [(1-X)*\delta^{18}O_{calculated}] + [X* \delta^{18}O_{initial}] \quad (2\text{-}6)$$

From this equation we see that the measured $\delta^{18}O_{SO4}$ values are partly dependent on some contribution, X, from the sulfate in the media water, $\delta^{18}O_{initial}$, plus some portion, 1-X, of the sulfate actually produced, which is the calculated value, $\delta^{18}O_{calculated}$. Rearranging Equation 2-6 yields Equation 2-7.

$$\delta^{18}O_{calculated} = [(\delta^{18}O_{measured}) - X*(\delta^{18}O_{initial})]/(1-X) \quad (2\text{-}7)$$

The total error, E_c, involved with the determination of $\delta^{18}O_{calculated}$ is estimated by Equation 2-8. From Equation 2-8 we see that the error in $\delta^{18}O_{calculated}$ depends on the error, E_m, in calculating the $\delta^{18}O_{measured}$ value times the partial derivative of Equation 2-7 with respect to $\delta^{18}O_{measured}$, $\delta(\delta^{18}O_c)/\delta(\delta^{18}O_m)$. Similarly, E_c also depends on the following: the error in calculating $\delta^{18}O_{initial}$, E_i; the partial derivative of Equation 2-7 with respect to $\delta^{18}O_{initial}$, $\delta(\delta^{18}O_c)/\delta(\delta^{18}O_i)$; the error in calculating the sulfate concentrations, E_s; and finally the partial derivative of Equation 2-7 with respect to the relative amount of sulfate X, $\delta(\delta^{18}O_c)/\delta(X)$. Solving for Equation 2-8 yields Equation 2-9.

$$E_c = SQRT\{(E_m*[\delta(\delta^{18}O_c)/\delta(\delta^{18}O_m)])^2 + (E_i*[\delta(\delta^{18}O_c)/\delta(\delta^{18}O_i)])^2 + (E_s*[\delta(\delta^{18}O_c)/\delta(X)])^2\} \quad (2\text{-}8)$$

$$E_c = SQRT\{(0.4*(1/(1-X)))^2 + (0.4*(X/(X-1)))^2 + (0.1*((\delta^{18}O_m - \delta^{18}O_i)/(1-X)^2))^2\} \quad (2\text{-}9)$$

This error propagation was applied to all of the $\delta^{18}O_{SO4}$ vs. $\delta^{18}O_{H2O}$ plots as shown in Figures 3-3, 3-6, and 3-9. The $\delta^{18}O_{SO4}$ values measured for the anaerobic experiments were run on a different IRMS with an error of 0.5‰, compared to the error of 0.4‰ with the IRMS used for the aerobic experiments, and this was accounted for.

CHAPTER 3

RESULTS

3.1 Anaerobic Chalcopyrite Experiment

Table 3-1 shows the estimated extent of the reaction for the anaerobic oxidation of chalcopyrite by $Fe(III)_{(aq)}$ based on measured amounts of accumulated $Cu_{(aq)}$, $Fe(II)_{(aq)}$, and SO_4 concentrations. Although the biological experiment shows a slightly higher extent of reaction than the abiotic experiment in terms of both copper and sulfur, the difference is not significant.

Table 3-1. Average % Cu and S reacted and Fe(II) produced during oxidation of chalcopyrite by $Fe(III)_{(aq)}$

Experiment	% Cu Reacted[a]	% S Reacted[b]	% Fe(II) Produced[a]
Biological	16.8 ± 1.1[c]	5.1 ± 0.6[c]	12.3 ± 1.5[c]
Abiotic	12.3 ± 0.41[c]	4.4 ± 0.7[c]	12.8 ± 0.6[c]

[a]Based on Hach kit measurements
[b]Based on sulfate accumulation from IC measurements
[c]n = 4

Changes in pH and sulfate concentrations did not reveal major differences between the biological and abiotic anaerobic chalcopyrite oxidation experiments as seen in Table 3-2. Both experiments show a dramatic and almost instantaneous decrease in pH from the initial value of ~2.8 to ~2, while the sulfate concentration steadily increased until a maximum is reached (Figure 3-1). For the biological samples this maximum is reached at about day 60, whereas for the abiotic samples a maximum is reached between days 6 and 25.

Table 3-2. Chemical composition of experimental solutions during oxidation of chalcopyrite by $Fe(III)_{(aq)}$

Biological						
Day	**pH**	**Cu (mmol/L)[a]**	**Fe(II) (mmol/L)[a]**	**Fe(Total) (mmol/L)[a]**	**SO_4^{2-} (mmol/L)[b]**	**SO_4^{2-} Based Oxidation Rate mol/(m^2*sec)[c]**
0	2.2	0.2	0.5	17.2	2.4	
8	2.2	1.0	0.8	19.4	3.2	
25	2.0	1.5	1.7	18.8	3.8	
46	1.8	1.9	1.9	20.3	3.9	
60	2.0	2.2	2.2	23.9	4.2	
67	1.9	2.9	1.8	20.9	3.7	3.5×10^{-9}
Abiotic						
Day	**pH**	**Cu (mmol/L)[a]**	**Fe(II) (mmol/L)[a]**	**Fe(Total) (mmol/L)[a]**	**SO_4^{2-} (mmol/L)[b]**	**SO_4^{2-} Based Oxidation Rate mol/(m^2*sec)[c]**
0	2.2	0.3	0.5	19.9	2.5	
2	2.4	0.6	0.8	18.7	2.9	
6	2.1	0.9	1.0	19.9	3.2	
25	2.2	1.5	1.5	20.4	3.5	
44	1.9	1.7	1.8	20.2	3.4	
48	1.8	1.8	1.9	20.7	3.7	
58	2.0	2.3	2.3	20.6	3.5	
66	2.0	2.1	2.2	20.7	3.4	2.2×10^{-9}

[a]Based on Hach kit measurements

[b]Based on IC measurements ± 10%

[c]Biological rates are calculated based on the slope from days 8-60, abiotic rates from days 2-48

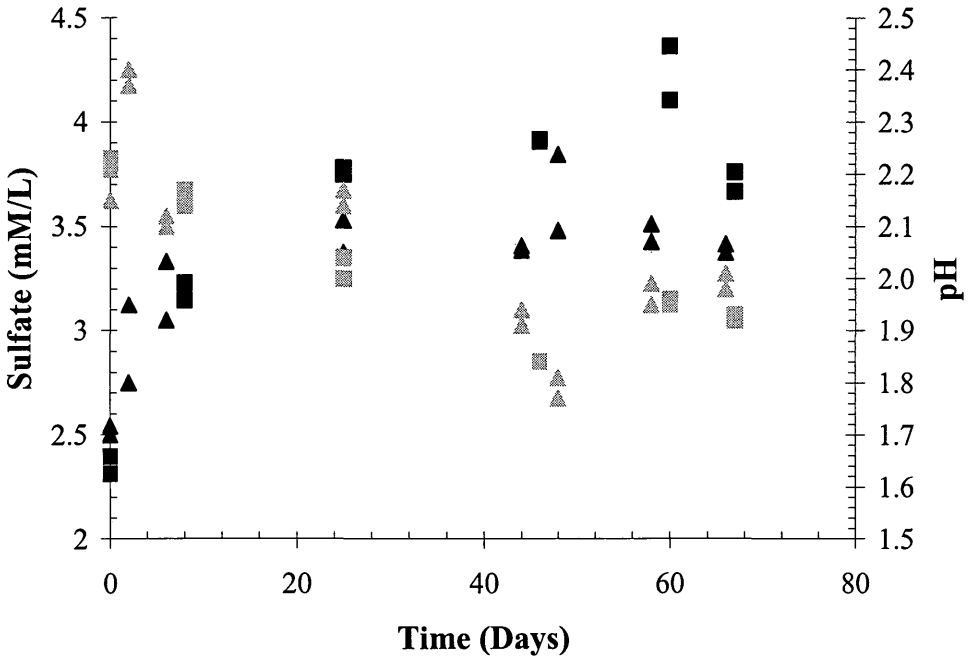

Figure 3-1: Change in solution chemistry with time for oxidation of chalcopyrite by $Fe(III)_{(aq)}$, ■ biological, ▲ abiotic, black = sulfate, gray = pH

Copper and ferrous iron concentrations increased at a steady rate throughout the anaerobic experiment. However, in the abiotic experiment the copper and $Fe(II)_{(aq)}$ concentrations both plateau at ~ day 58 (Figures 3-1 and 3-2). However, in the biological experiment copper continuously increases and ferrous iron only plateaus after day 60 (Figures 3-1 and 3-2).

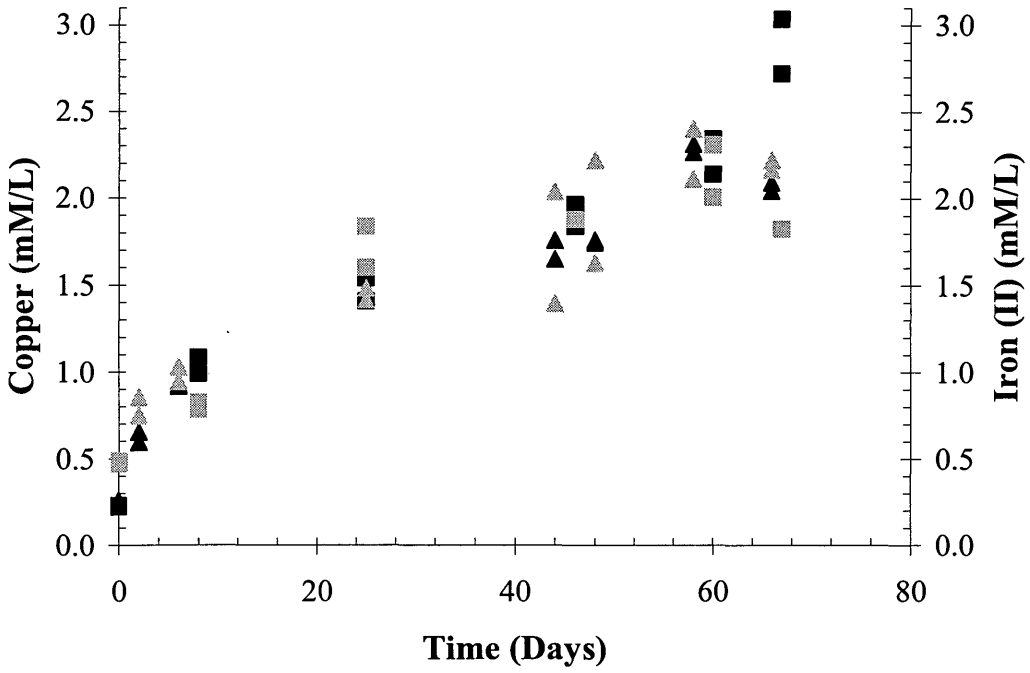

Figure 3-2: Change in solution chemistry with time for oxidation of chalcopyrite by $Fe(III)_{(aq)}$,,■ biological,▲ abiotic, black = copper, gray = $Fe(III)_{(aq)}$

Rates of chalcopyrite leaching and oxidation for the anaerobic experiments were calculated based on Equation 3-1 below, where a is the slope from Figure 3-1 for sulfate in (mol/day). A_{BET} is the surface area in (m^2/g), m is the mass of the mineral in grams, and c is a stoichiometric factor (Gleisner et al, 2006). In this case, c is equal to two for chalcopyrite and one for covellite oxidation. Additionally, a factor of (day/86400 sec.) must be multiplied to Equation 3-1 to give the proper units of ($mol/(m^2*sec)$).

$$R_{sulfide} = a/(A_{BET}*m*c), \text{ in } (mol/(m^2*sec)) \quad (3\text{-}1)$$

Based on sulfate production, the rate of anaerobic chalcopyrite oxidation in the biological experiments, [3.5×10^{-9} ($mol/(m^2*sec)$)], is slightly faster compared to the abiotic rate of [2.2×10^{-9} ($mol/(m^2*sec)$)] (Table 3-2). However for both the

abiotic and biological experiments, copper and iron were leached to a similar extent, approximately 2.5 times more than sulfate production.

Figure 3-3 plots the $\delta^{18}O_{SO4}$ values vs. $\delta^{18}O_{H2O}$ values for the anaerobic chalcopyrite oxidation experiments. As expected the slope of the graph of 0.92 is not significantly different from one when accounting for error propagation (Figure 3-1), which yields a slope of 1.2 when the maximum and minimum $\delta^{18}O_{SO4}$ values for the most $\delta^{18}O_{SO4}$ enriched and depleted experiments, respectively, are used. This suggests that all of the oxygen in the sulfate is derived from water. If it is then assumed that all of the oxygen in the sulfate comes from water, the y-intercept of 3.8‰ will closely approximate the fractionation effect between sulfate and water, $\varepsilon^{18}O_{SO4\text{-}H2O}$.

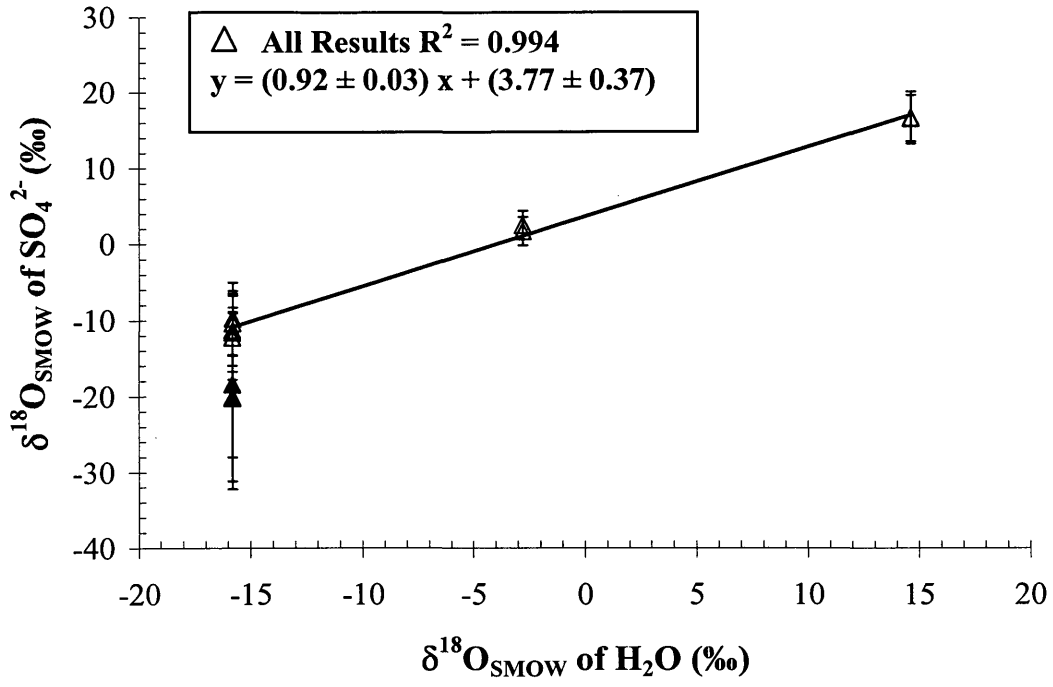

Figure 3-3: Plot of the $\delta^{18}O_{SO4}$ produced during Fe(III) oxidation of chalcopyrite by $Fe(III)_{(aq)}$ vs. $\delta^{18}O_{H2O}$ of water used in all experiments, ▲ outliers not used for regression

The $\delta^{34}S_{SO4}$ values from the anaerobic chalcopyrite oxidation experiments are presented in Table 3-3. The $\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ values for both the biological and abiotic experiments are -3.8 ± 1.0‰, n = 14, which is very distinct from the $\delta^{34}S$ value of 0.2 ‰ for the chalcopyrite mineral. There is no significant difference in $\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ values between the biological and abiotic experiments.

Table 3-3. Oxygen and sulfur isotope composition of sulfate from oxidation of chalcopyrite by $Fe(III)_{(aq)}$

		$\delta^{18}O_{H2Oinitial}$ (‰)	$\delta^{18}O_{SO4}$ (‰)	$\delta^{34}S_{SO4}$ (‰)	$\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ (‰)[a]
Biological	**Light**	-15.8	-10.6 ± 0.9[b]	-3.8 ± 0.4[b]	-4.0 ± 0.4[b]
Abiotic	**Light**	-15.8	-17.6 ± 3.8[b]	-3.4 ± 1.8[b]	-3.6 ± 1.8[b]
	Medium	-2.8	1.8, 2.6	-3.5, -3.8	-3.7, -4.0
	Heavy	14.6	16.8, 16.9	-3.3, -3.8	-3.5, -4.0

[a] $\delta^{34}S$ of chalcopyrite used in experiments was 0.2 ± 0.5 ‰
[b] n = 4

3.2 Aerobic Chalcopyrite Experiment

The biological aerobic chalcopyrite oxidation experiments showed faster rates of sulfide oxidation than the abiotic control experiments as evidenced by changes in both pH and sulfate concentrations (Figure 3-4). Biological and abiotic experiments each showed an initial small increase in pH. Following this, the abiotic experiments leveled off at a pH of ~3.5, whereas the biological experiments show a sharp decrease in pH to a value of two over the remainder of the experiment. Coincident with the drop in pH, the sulfate concentration increases sharply in the biological samples. The abiotic controls show very little increase in sulfate during the course of the experiment. By the end of the long term experiment, on average 36% of the sulfur in chalcopyrite was oxidized to sulfate in the biological experiments (Table 3-4). In the abiotic experiments only ~ 5% of the sulfur had been oxidized.

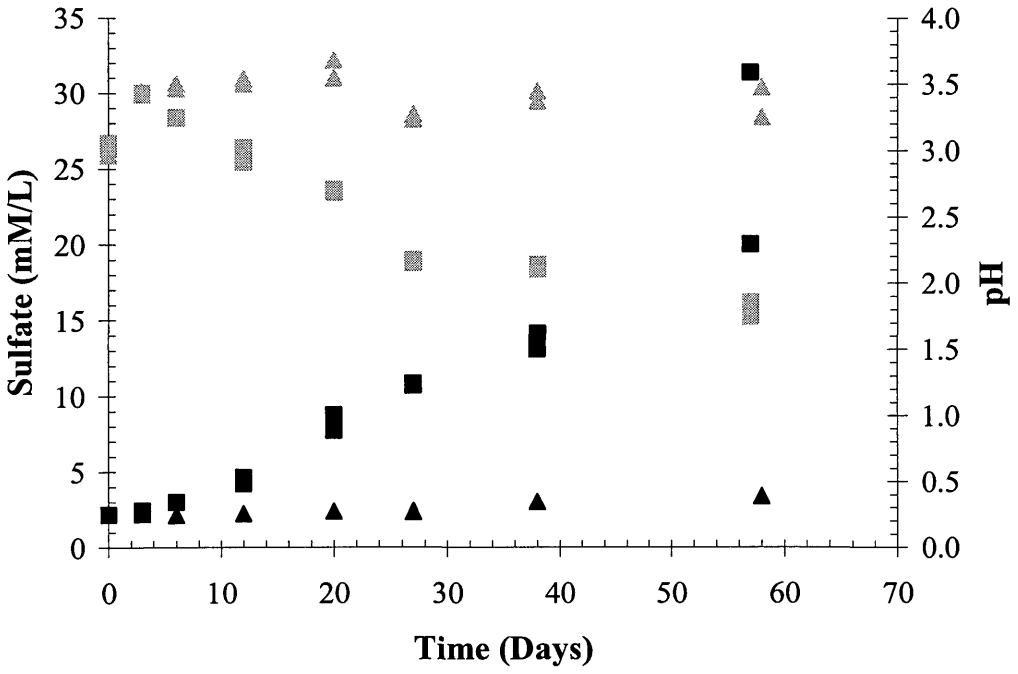

Figure 3-4: Change in solution chemistry with time for oxidation of chalcopyrite by O_2, ■ biological, ▲ abiotic, black = sulfate, gray = pH, high sulfate value at day 60 not used in rate calculations

Table 3-4. Average % Cu, S, and total Fe reacted during oxidation of chalcopyrite by O_2

Expt.	% of Cu Reacted[a]	n	% of Cu Reacted[b]	n	% of S Reacted[c]	n	% of S Reacted[b]	n	% of Fe Reacted[a]	n	% of Fe Reacted[b]	n
Biotic Long Term	28.6 ± 16.4	10	21.4 ± 7.3	10	36.4 ± 22.8	10	19.7 ± 3.2	10	19.7 ± 15.3	10	12.7 ± 5.0	10
Biotic Short Term	11.1 ± 1.4	6	9.7 ± 0.9	6	14.6 ± 2.2	6	11.5 ± 1.1	6	7.0 ± 2.2	6	4.9 ± 1.1	6
Abiotic	2.9 ± 0.5	5	2.9 ± 0.4	5	5.1 ± 3.9	8	3.4 ± 0.9	5	2.0 ± 1.1	5	1.2 ± 0.4	5

[a]Based on Hach kit measurements
[b]Based on ICP measurements
[c]Based on sulfate accumulation from IC measurements ± 10%

The biological experiments also leached more copper and iron than the abiotic control experiments (Figure 3-5). In all samples copper showed higher concentrations than Fe(II) (Table 3-5). The biological experiments released an average of 28% and 19% of copper and iron, respectively. In contrast, the abiotic samples had leached ~3% of the copper and 1-2% of the iron from the mineral. Figures 3-4 and 3-5 suggest that chalcopyrite is appreciably leached only in the presence of bacteria under my experimental conditions.

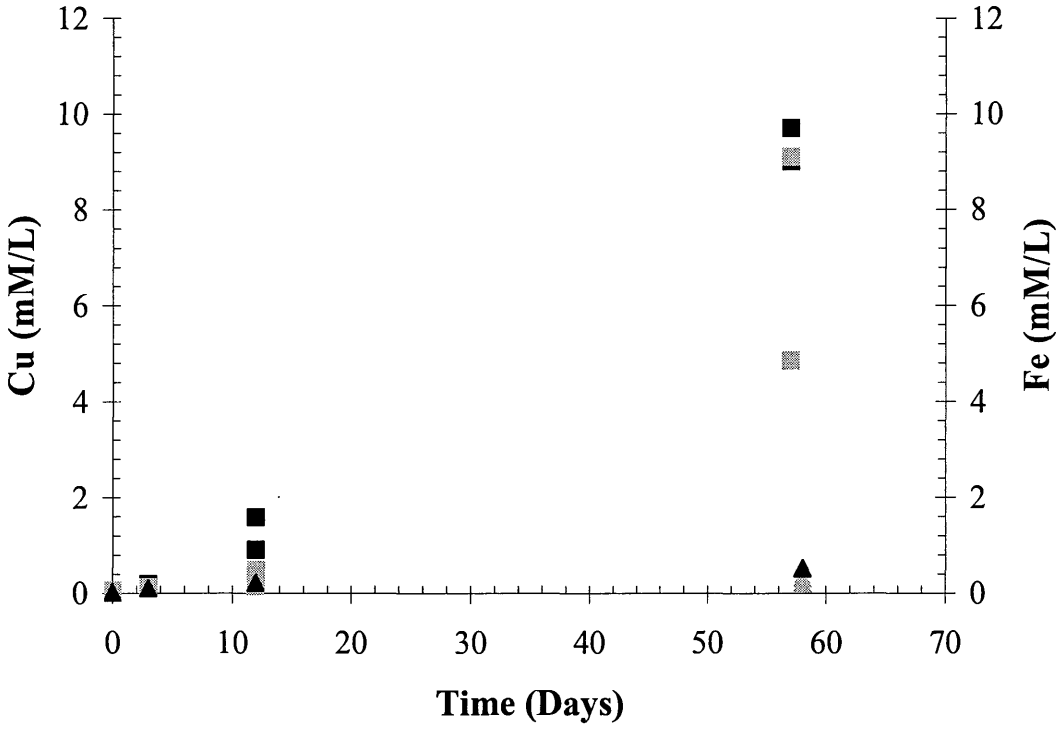

Figure 3-5: Change in solution chemistry with time for oxidation of chalcopyrite by O_2, ■ biological, ▲ abiotic, black = copper, gray = $Fe(III)_{(aq)}$

Table 3-5. Chemical composition of experimental solutions during oxidation of chalcopyrite by O_2

Biological								
Days	**pH**	**Cu (mmol/L)[a]**	**Cu (mmol/L)[b]**	**Fe (mmol/L)[a]**	**Fe (mmol/L)[b]**	**S (mmol/L)[b]**	**SO_4^{2-} (mmol/L)[c]**	**SO_4^{2-} Based Oxidation Rate mol/(m^2*sec)[d]**
0	3.1	0.02	0.02	0.05	0.02	2.2	2.2	
3	3.4	0.2	0.1	0.1	0.05	2.3	2.4	
6	3.3	N.D.	N.D.	N.D.	N.D.	N.D.	3.0	
12	3.0	5.4	N.D.	0.3	N.D.	N.D.	4.4	
20	2.7	N.D.	N.D.	N.D.	N.D.	N.D.	8.3	
27	2.2	N.D.	2.5	N.D.	1.5	6.7	10.8	
38[e]	2.1	N.D.	N.D.	N.D.	N.D.	N.D.	13.7	
57	1.8	9.4	5.4	7.0	3.2	7.6	25.7	4.1×10^{-9}
Abiotic								
Days	**pH**	**Cu (mmol/L)[a]**	**Cu (mmol/L)[b]**	**Fe (mmol/L)[a]**	**Fe (mmol/L)[b]**	**S (mmol/L)[b]**	**SO_4^{2-} (mmol/L)[c]**	**SO_4^{2-} Based Oxidation Rate mol/(m^2*sec)[d]**
0	3.0	0.02	0.02	0.04	0.03	2.5	2.2	
3	3.4	0.1	0.1	0.1	0.1	2.5	2.2	
6	3.5	N.D.	N.D.	N.D.	N.D.	N.D.	2.2	
12	3.5	0.2	N.D.	0.2	N.D.	N.D.	2.3	
20	3.7	N.D.	N.D.	N.D.	N.D.	N.D.	2.5	
27	3.3	N.D.	0.3	N.D.	0.6	2.8	2.5	
38[e]	3.4	N.D.	N.D.	N.D.	N.D.	N.D.	3.1	
58	3.4	0.5	0.5	0.3	0.2	3.3	3.3	2.6×10^{-10}

[a]Based on Hach kit measurement
[b]Based on ICP measurements
[c]Based on accumulated sulfate from IC measurements ± 10%
[d]Biological rates are calculated based on the slope from days 6-57, abiotic rates from days 0-58
[e]End of short term experiments

With respect to sulfate production, the measured biological and abiotic rates of chalcopyrite oxidation by O_2 were [4.0 X 10^{-9} (mol/(m^2*sec))] and [2.6 X 10^{-10} (mol/(m^2*sec))], respectively.

The $\delta^{18}O_{SO4}$ and $\delta^{18}O_{H2O}$ values measured from the biological and abiotic aerobic chalcopyrite oxidation experiments are presented in Table 3-6. Figure 3-6 plots the $\delta^{18}O_{SO4}$ values vs. $\delta^{18}O_{H2O}$ values during oxidation of chalcopyrite by O_2. The average $\delta^{18}O_{SO4}$ and $\delta^{18}O_{H2O}$ values used to construct Figure 3-6 are shown in Table 3-6. The short and long term biological experiments, respectively, showed 92 and 94% incorporation of water-derived oxygen into the sulfate formed. In contrast, the abiotic experiments show only ~ 51% incorporation of water oxygen into the sulfate (Figure 3-6). However, the abiotic experiments showed tremendous scatter in the data due largely to the much lower rates of sulfate production and the presence of a relatively large background concentration of sulfate in the initial media. Therefore, these results contain significant analytical error and may be suspect.

Table 3-6. Oxygen and sulfur isotope composition of sulfate produced from oxidation of chalcopyrite by O_2

		$\delta^{18}O_{H2Oinitial}$ (‰)	$\delta^{18}O_{H2Ofinal}$ (‰)	$\delta^{18}O_{H2Oave.}$ (‰)	$\delta^{18}O_{SO4}$ (‰)	n	$\delta^{34}S_{SO4}$ (‰)[a]	n
Biological Short term	**Light**	-15.8	-15.2	-15.5	-7.7, -8.2		-1.0, -1.2	
	Medium	-2.8	-2.4	-2.6	4.0, 4.0		-1.3, -1.4	
	Heavy	14.6	14.1	14.3	19.4, 19.6		-1.1, -1.3	
Biological Long term	**Light**	-15.8	-11.4	-13.6	-6.2 ± 1.2	4	-1.3 ± 0.1	4
	Medium	-2.8	-1.3	-2.1	4.1, 4.6		-1.2, -1.3	
	Heavy	14.6	14.8	14.7	20.2, 20.6		-1.6, -1.7	
Abiotic	**Light**	-15.8	-13.8	-15.1	-1.4 ± 1.6	3	-0.4 ± 0.4	4
	Medium	-2.8	-2.5	-2.6	7.3, 8.1		0.5, 0.5	
	Heavy	14.6	14.8	14.6	13.8, 17.5		-0.3, -1.5	

[a] $\delta^{34}S$ of chalcopyrite used in experiments was 0.2 ± 0.4 ‰

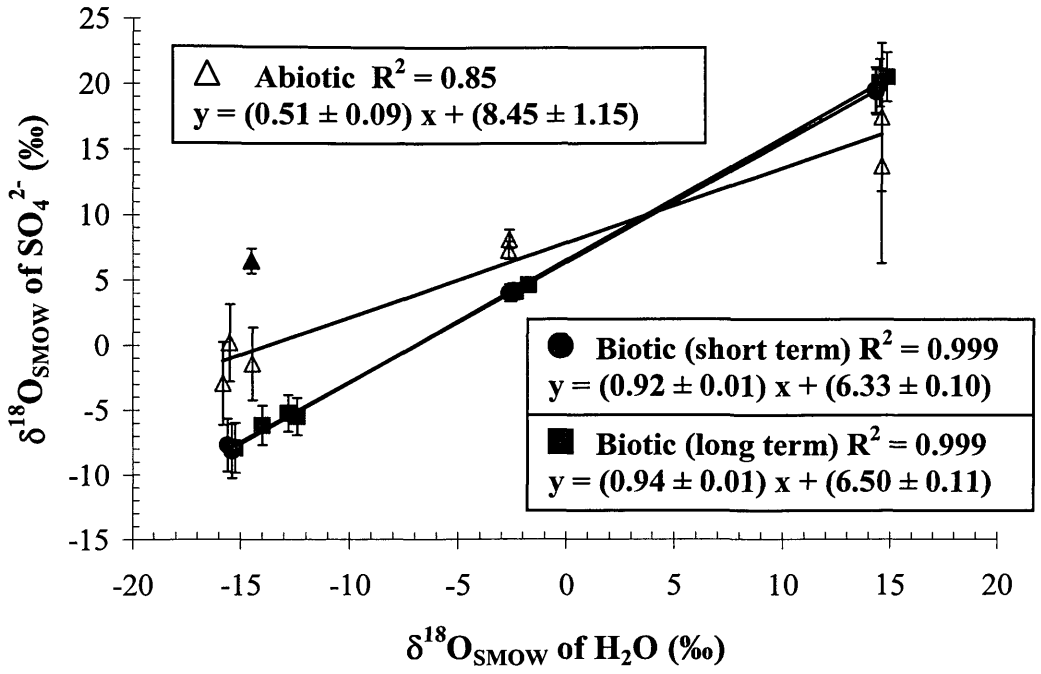

Figure 3-6: Plot of the $\delta^{18}O_{SO4}$ produced during oxidation of chalcopyrite by O_2 vs. $\delta^{18}O_{H2O}$ of water used in all experiments, ▲ represents outlier

The $\delta^{34}S_{SO4}$ values from the aerobic chalcopyrite oxidation experiments indicate small $\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ values of -1.5 ± 0.2 ‰ and -0.5 ± 0.7 ‰ for the biological and abiotic experiments, respectively (Table 3-7).

Table 3-7. Summary of oxygen and sulfur isotope fractionation during oxidation of chalcopyrite by O_2

Experiment		% H_2O Oxygen[a]	$\varepsilon^{18}O_{SO4\text{-}H2O}$ (‰)	n	$\varepsilon^{18}O_{SO4\text{-}H2O}$ (‰)[b]	n	$\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ (‰)[c]	n
Biological Short Term	**Light**		7.5		7.4 ± 0.4	6	-1.5 ± 0.2	14
	Medium	92	6.6					
	Heavy		5.2		6.5 ± 0.1	4		
Biological Long Term	**Light**		7.4 ± 0.4	4				
	Medium	94	6.4		5.4 ± 0.3	4		
	Heavy		5.7					
Abiotic	**Light**		15.6 ±3.8	4			-0.5 ± 0.7	8
	Medium	51	10.3					
	Heavy		1.0					

[a] % H_2O oxygen estimated from linear regressions between SO_4^{2-} and H_2O
[b] Average values for the biological short and long term experiments combined for each of the three initial waters
[c] $\delta^{34}S$ of chalcopyrite used in experiments was 0.2 ± 0.4 ‰

3.3 Aerobic Covellite Experiment

The abiotic and biological aerobic covellite oxidation experiments showed no significant difference with respect to sulfate production and change in pH (Figure 3-7). In the first four days the pH increased dramatically followed by a leveling off near pH ~5.8. Sulfate concentrations follow the opposite trend of increasing very slowly at first followed by a fairly rapid increase near the end of the experiment. However, there was no more than a 50-90% increase in sulfate concentrations above the initial value in any of the biological or abiotic experiments. By the end of the experiment all samples showed that only ~5% of the sulfur from the parent mineral was converted to sulfate (Table 3-8). The apparent lack of effectiveness by the

bacteria to aerobically oxidize covellite may be due to the initial increase in pH to a neutral pH. Since *A. ferrooxidans* is acidiphillic the oxidation of the mineral by the bacteria may have been inhibited by this increase in pH as seen in other studies of *A. ferrooxidans* at higher pH's (Hiskey and Schlitt, 1982; Nicholson, 1994; Nordstrom and Southam, 1997; Kirby et al, 1999).

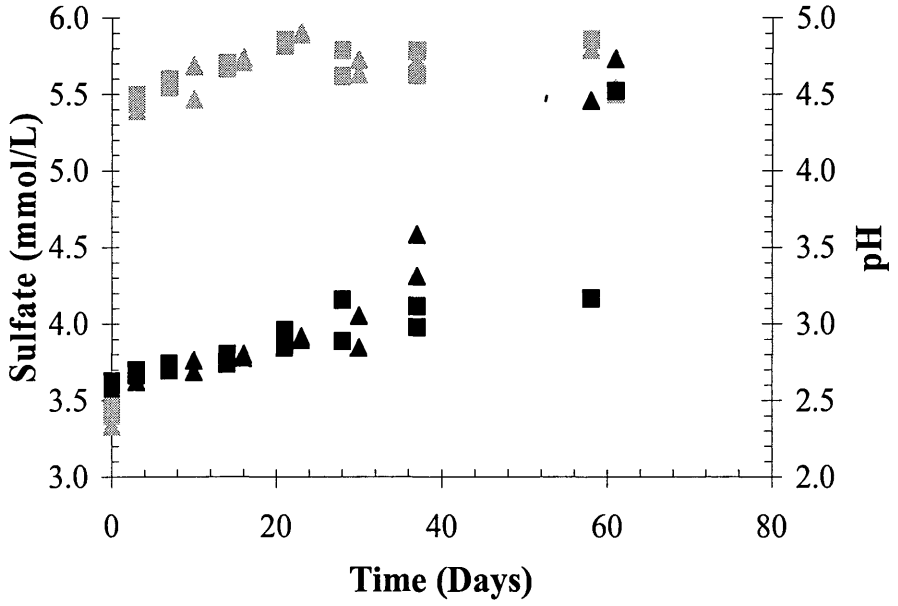

Figure 3-7: Change in solution chemistry with time for oxidation of covellite by O_2, ■ biological, ▲ abiotic, black = sulfate, gray = pH

Table 3-8. Average % Cu and S reacted during oxidation of covellite by O_2

Experiment	% of Cu Reacted[a]	n	% of Cu Reacted[b]	n	% of SO_4 Reacted[c]	n	% of S Reacted[b]	n
Biological Long Term	6.5 ± 1.5	5	5.6 ± 1.8[e]	7	4.9 ± 1.7[f]	8	6.3 ± 3.3[e]	7
Biological Short Term	3.3 ± 0.6	6	3.5 ± 0.5[g]	6	3.5 ± 0.5[g]	6	2.6 ± 0.6[g]	6
Abiotic	4.2 ± 0.4	8	5.1 ± 0.9[f]	8	5.4 ± 1.5[f]	8	4.4 ± 1.1[f]	8
All Long Term Expts.	6.0 ± 1.1	6	5.3 ± 1.4[i]	15	5.2 ± 1.6[j]	16	5.3 ± 2.5i[g]	6

[a]Based on Hach kit measurements
[b]Based on ICP measurements
[c]Based on sulfate accumulation from IC measurements ± 10%

In terms of leaching rates for copper, which were steady over time, there was also no difference between the biological and abiotic experiments (Figure 3-8). All samples show a steady increase in copper concentration over time. Similar to the results for sulfur, all samples showed that ~6% of the copper from the mineral was leached by the end of the experiment (Table 3-8). Table 3-9 summarizes the chemistry data for the aerobic biological and abiotic covellite oxidation experiments. The sulfate concentrations determined by IC agree well with the ICP measurements, suggesting most of the sulfur exists as sulfate. There is also good agreement between the ICP and Hach kit determinations for copper concentrations. The average rates for biological and abiotic aerobic oxidation of covellite were [1.5 X 10^{-10} (mol/(m^2*sec))] and [2.6 X 10^{-10} (mol/(m^2*sec))], respectively (Table 3-9).

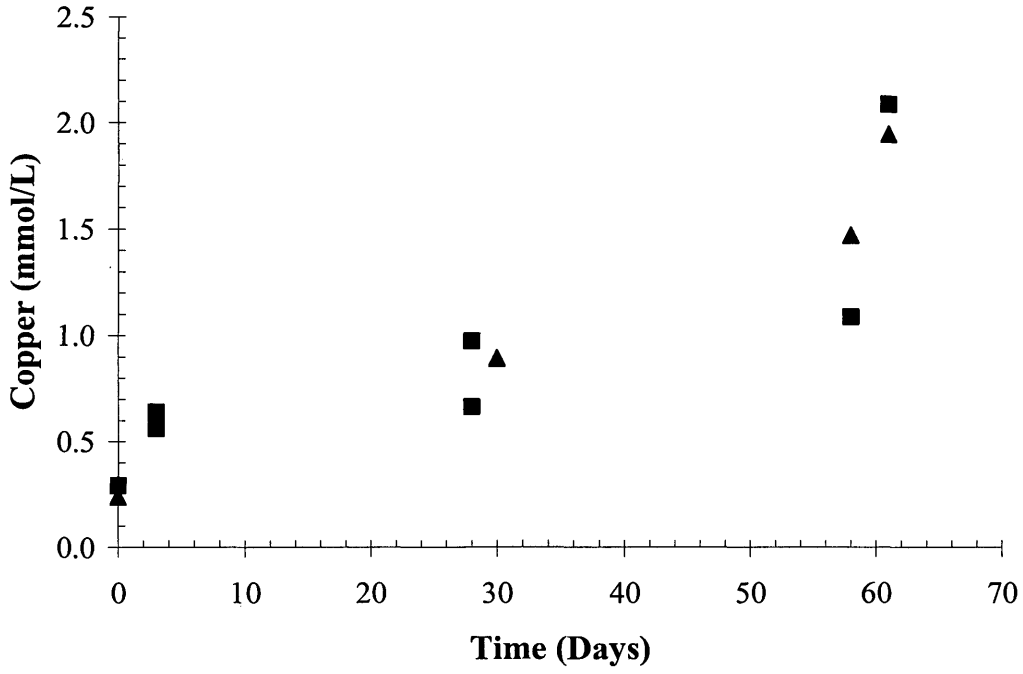

Figure 3-8: Change in solution chemistry with time for oxidation of covellite by O_2, ■ biological, ▲ abiotic

Table 3-9. Chemical composition of experimental solutions during oxidation of covellite by O_2

Biological							
Expt.	**Days**	**pH**	**SO_4^{2-} (mmol/L)[a]**	**SO_4^{2-} (mmol/L)[b]**	**Cu (mmol/L)[c]**	**Cu (mmol/L)[b]**	**SO_4^{2-} Based Oxidation Rate mol/(m^2*sec)[d]**
	0	3.5	2.6	2.5	N.D.	0.3	
	3	5.5	2.7	2.6	N.D.	0.6	
	7	5.6	2.7	N.D.	N.D.	N.D.	
	14	5.7	2.8	N.D.	N.D.	N.D.	
	21	5.9	2.9	N.D.	N.D.	N.D.	
	28	5.7	3.0	3.0	N.D.	0.9	
Short term	37	5.7	3.0	N.D.	N.D.	N.D.	
Long term	58	5.7	3.8	3.8	2.3	1.6	1.5×10^{-10}
Abiotic							
Expt.	**Days**	**pH**	**SO_4^{2-} (mmol/L)[a]**	**SO_4^{2-} (mmol/L)[b]**	**Cu (mmol/L)[c]**	**Cu (mmol/L)[b]**	**SO_4^{2-} Based Oxidation Rate mol/(m^2*sec)[d]**
	0	3.4	2.6	2.4	N.D.	0.3	
	3	5.5	2.7	2.5	N.D.	0.6	
	7	5.6	2.7	N.D.	N.D.	N.D.	
	14	5.7	2.8	N.D.	N.D.	N.D.	
	21	5.9	2.9	N.D.	N.D.	N.D.	
	28	5.7	2.9	3.0	N.D.	0.9	
Short term	37	5.7	3.4	N.D.	N.D.	N.D.	
Long term	58	5.7	4.6	4.1	1.7	1.8	2.6×10^{-10}

[a]Based on sulfate accumulated from IC measurements ± 10%
[b]Based on ICP measurements
[c]Based on Hach kit measurement
[d]All oxidation rates are calculated based on the slope from days 0-60

Table 3-10 summarizes the oxygen isotope data for the aerobic covellite experiment. Some evaporation did occur over the course of the experiment as the $\delta^{18}O$ of the media waters became enriched in ^{18}O compared to their starting values. Although this enrichment was relatively small for the biological long term and abiotic

experiments, enrichments of 3 and 5‰ occurred for the 'light' and 'medium' waters of the biological short term experiment. The $\varepsilon^{18}O_{SO4\text{-}H2O}$ values provided in Table 3-11 show that the results from the biological short term experiment are different from those of the biological long term and abiotic experiments and therefore could be suspect. However, the similarity of the biological long term and abiotic experiments suggest that bacteria have little effect on the final $\delta^{18}O_{SO4}$ values.

Table 3-10. Oxygen and sulfur isotope composition of sulfate produced during oxidation of covellite by O_2

		$\delta^{18}O_{H2Oinitial}$ (‰)	$\delta^{18}O_{H2Ofinal}$ (‰)	$\delta^{18}O_{H2Oave.}$ (‰)	$\delta^{18}O_{SO4}$ (‰)	n	$\delta^{34}S_{SO4}$ (‰)[a]	n
Biological	**Light**	-15.8	-12.8	-15.1	-6.1, -6.5		1.2, 1.4	
Short	**Medium**	-2.8	2.1	-2.4	-0.1, 3.4		1.2, 1.3	
Term	**Heavy**	14.6	14.3	14.5	6.9, 9.6		1.0, 1.6	
Biological	**Light**	-15.8	-14.5	-14.3	-14.5 ±3.1	3	1.4 ± 0.6	4
Long	**Medium**	-2.8	-2.0	-0.4	-0.8, -3.7		0.9, 0.9	
Term	**Heavy**	14.6	14.5	14.5	1.1, 4.4		1.1, 1.8	
Abiotic	**Light**	-15.8	-13.6	-14.7	-16.0 ±2.2	4	1.0 ± 0.2	4
	Medium	-2.8	-1.3	-2.0	-2.9, -8.5		0.9, 1.1	
	Heavy	14.6	15.1	14.8	4.3, 5.6		0.7, 1.2	

[a]$\delta^{34}S$ of covellite used in experiments was 0.75 ± 0.40

Table 3-11. Summary of oxygen and sulfur isotope fractionation during oxidation of covellite by O_2

Experiment		% H_2O Oxygen[a]	$\varepsilon^{18}O_{SO4\text{-}H2O}$ (‰)	n	$\varepsilon^{34}S_{SO4\text{-}CuS}$ (‰)[b]	n
Biological Short Term	**Light**		8.9		0.5 ± 0.4	14
	Medium	49	4.1			
	Heavy		-6.3			
Biological Long Term	**Light**		-0.1 ± 2.1	4		
	Medium	62	-1.9			
	Heavy		-11.7			
Abiotic	**Light**		-1.3 ± 2.2	4	0.2 ± 0.2	8
	Medium	71	-3.7			
	Heavy		-9.9			

[a] % H_2O oxygen estimated from linear regressions between SO_4^{2-} and H_2O
[b] $\delta^{34}S$ of covellite used in experiments was 0.75 ± 0.4 ‰

According to Equation 1-7 there should be a 25% incorporation of water oxygen into the sulfate formed from the oxidation of covellite under aerobic conditions. Table 3-11 and Figure 3-9 show that the sulfate produced in our experiments incorporated 49, 62, and 71% water oxygen for the biological short term, biological long term, and abiotic portions of the experiment, respectively, which is not in agreement with Equation 1-7. However, an analysis of error propagation due to isotopic plus concentration measurements of sulfate shows that these three results are statistically non-distinguishable. Therefore, $\delta^{18}O_{SO4}$ values from the covellite experiments were not examined any further.

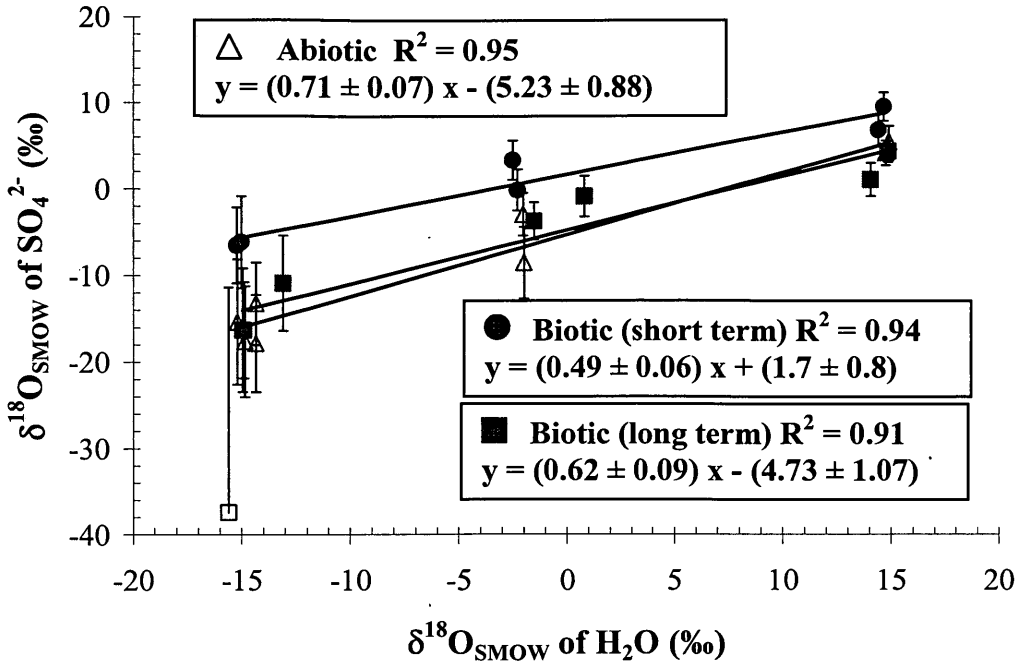

Figure 3-9: Plot of the $\delta^{18}O_{SO4}$ produced during O_2 oxidation of covellite vs. $\delta^{18}O_{H2O}$ of water used in all experiments, □ represents outlier

The $\varepsilon S_{SO4\text{-}CuS}$ values measured for the biological and abiotic experiments consistently showed relatively small fractionation effects of 0.5 ± 0.4‰ and 0.2 ± 0.2‰, respectively, with no apparent biological influence (Table 3-11).

CHAPTER 4

DISCUSSION

4.1 Rates of Chalcopyrite Oxidation

The biological and abiotic anaerobic experiments show virtually the same trend with sulfate, copper, and iron concentrations leveling off over the course of the experiments. However, the bacteria may slightly increase the oxidation rate as evidenced by rates of [3.5 X 10^{-9} (mol/(m^2*sec))] and [2.2 X 10^{-9} (mol/(m^2*sec))] for the biological and abiotic experiments, respectively (Table 3-1). Even though the biological and abiotic experiments showed a leveling off of sulfur, copper, and iron, the abiotic samples began to plateau sooner than the biological experiments (Figures 3-1 and 3-2).

Previous studies suggest that elemental sulfur, chalcocite, disulfide, jarosite and polysulfides accumulate on the surface of chalcopyrite and any of these species could be precipitating during this experiment (Yin et al., 1995; Yuehua et al., 2002; Rodríguez et al., 2003; Stott et al., 2003; Todd et al., 2003; Harmer et al., 2006). Since the bacteria are capable of oxidizing some of these species, the bacteria may be preventing the layer from building up as quickly as under abiotic conditions. However, no detailed analyses were conducted to explore the formation of intermediate products in this study. If any intermediate sulfur species are being precipitated, then this would lead to an underestimation of the oxidation rate.

Both iron and copper were leached at rates 2-3 times that of the sulfur oxidized under anaerobic conditions. All previously reported results for the oxidation

of chalcopyrite by O_2 with *A. ferrooxidans* suggested that ~17-25% of the copper had been leached from their experiments in ~30 days (Konishi et al., 2001; Yuehua et al., 2002; Stott et al., 2003; Mathur et al., 2005), which is in good agreement with the results of this study. However, according to stoichiometry iron and copper should be, at most, one half of the amount of sulfate oxidized. As previously noted, this suggests that some undetermined sulfur species may have precipitated. This is especially true when the estimated amounts of reacted iron and copper are compared between the aerobic and anaerobic experiments (Tables 3-1 and 3-4). Although the % of iron and copper reacted from the anaerobic experiments is only slightly lower than the amount reacted from the aerobic experiments, the amount of sulfur reacted for the anaerobic experiments is 4-7 times lower than that in the aerobic experiments (Tables 3-1 and 3-4). This result again suggests that under anaerobic conditions a passivating layer of intermediate sulfur compounds could be precipitating on the chalcopyrite surface which prevents further oxidation of the mineral. Because the anaerobic experiments involved $Fe(III)_{(aq)}$ as the oxidant and sulfate did not accumulate in solution to an appreciable degree, the precipitation of either jarosite ($KFe_3(SO_4)_2(OH)_6$) or some other iron sulfate is also a distinct possibility as jarosite has previously been observed or suspected of precipitating under high $Fe(III)_{(aq)}$ concentrations (Yuehua et al., 2002; Rodríguez et al., 2003; Stott et al., 2003; Todd et al., 2003; Balci et al, 2007). Similar results in experiments of pyrite oxidation by $Fe(III)_{(aq)}$ also showed a much greater release of iron relative to sulfate production, which seems to support the argument that some intermediate sulfur species are forming (Balci et al, 2007). Under aerobic conditions iron-oxyhydroxides have also

been shown to form during oxidation of chalcopyrite (Goyne et al., 2006), but this is unlikely under anaerobic conditions.

The anaerobic experiments did not yield as much total sulfate as the aerobic chalcopyrite oxidation experiments. Contrary to some studies, it has been discovered that the dissolution of chalcopyrite is actually inhibited by $Fe(III)_{(aq)}$, and $Fe(II)_{(aq)}$ is 2.7 times more effective in the leaching of copper from chalcopyrite than $Fe(III)_{(aq)}$ (Third et al, 2000). These authors suggested that the reduction potential (E_h) is an important control during oxidation of chalcopyrite, and that an E_h value of ~650 mV is optimal for leaching, whereas a higher concentration of $Fe(III)_{(aq)}$ causes the E_h to increase, thus slowing the process down. The lack of an initial $Fe(III)_{(aq)}$ concentration may account for why the aerobic experiments resulted in more complete oxidation than the anaerobic experiments. This is at least partially shown in the faster rate of [4.0×10^{-9} (mol/(m^2*sec))] for biological oxidation of chalcopyrite by O_2. The slowest rate of oxidation of [2.6×10^{-10} (mol/(m^2*sec))] during this study was for the abiotic aerobic experiments. This would be expected since bacteria are required for both the oxidation of sulfur species at the conditions of this study and recycling of $Fe(III)_{(aq)}$, which is very slow at pH < 3 (Schwertmann and Fitzpatrick, 1992; Morgan and Stumm, 1998) and was initially absent in the aerobic experiments. Similar results were observed during the abiotic oxidation of pyrite by O_2 (Balci et al, 2007). The initial absence of $Fe(III)_{(aq)}$ could also explain why the biological aerobic oxidation of chalcopyrite was much faster than the anaerobic oxidation of chalcopyrite because the bacteria recycle $Fe(III)_{(aq)}$ through the action of Equation 1-11, the regenerated ferric iron could then help to further abiotically oxidize the

chalcopyrite. This would also allow a slower increase in iron concentrations, which wouldn't slow the leaching of chalcopyrite as suggested by Third et al (2000) and therefore permit faster oxidation by O_2. The calculated rates for oxidation of chalcopyrite by $Fe(III)_{(aq)}$ and O_2 are slightly slower but similar to an abiotic rate of [9.6×10^{-9} (mol/(m^2*sec))] previously reported (Rimstidt and Chermak, 1994). Rimstidt and Chermak (1994) conducted their experiments in the presence of $Fe(III)_{(aq)}$ and oxygen and therefore their experiments were not truly anaerobic as in this study. Therefore, it is difficult to compare their results with the results of this study. However, it is interesting that Rimstidt and Chermak (1994) used a starting concentration of 1 mM $Fe(III)_{(aq)}$, which is an order of magnitude lower than the concentration of 10 mM used in this study. Based on the above discussion the lower concentration of $Fe(III)_{(aq)}$ could explain why the rate reported by Rimstidt and Chermak (1994) is faster.

4.2 Kinetic Oxygen Isotope Fractionation Between SO_4, H_2O, and O_2

Anaerobic oxidation of chalcopyrite by $Fe(III)_{(aq)}$, as expected incorporated 100% water-derived oxygen into sulfate formed. This result has also been obtained for the biological and abiotic anaerobic oxidation of pyrite (Taylor et al, 1984b; Balci et al, 2007) and sphalerite (Balci, 2005). Because H_2O is the only source of oxygen in sulfate during the anaerobic oxidation of chalcopyrite, the $\varepsilon^{18}O_{SO4\text{-}H2O}$ value can be closely approximated from the y-intercept value of Figure 3-3.

The value of 3.8‰ for $\varepsilon^{18}O_{SO4\text{-}H2O}$ measured from the anaerobic experiments (Figure 3-3) compares well with similar values measured from the oxidation of other

aqueous sulfide and sulfide minerals, which typically fall in a range of 0 to 4.1‰ (Lloyd, 1968; Taylor et al., 1984b; Van Everdingen and Krouse, 1985; Balci, 2005; Balci et al., 2007). Sulfate produced from biological and abiotic anaerobic oxidation of pyrite has been shown to display a value of 3.6‰ and 2.9‰ for $\varepsilon^{18}O_{SO4\text{-}H2O}$, respectively (Balci et al., 2007). During biological oxidation of pyrite by O_2 for 44 days and 20 days, $\Delta^{18}O_{SO4\text{-}H2O}$ values of 4.1 and 4.7‰, respectively, were measured (Balci et al, 2007). However, these slightly higher values may reflect the incorporation of small amounts of more ^{18}O enriched O_2.

Values of 16.3 and 16.0‰ for $\Delta^{18}O_{SO4\text{-}H2O}$ were recently calculated for pyrite oxidation by O_2 for two biological pyrite oxidation experiments involving two different grain sizes, ~100μm and ~200μm, respectively (Pisapia et al, 2007). These values were calculated using both a bulk equation model (BEM) (Taylor et al, 1984b: Van Everdingen and Krouse, 1985) and an electron transferred model (ETM) (Toran and Harris, 1989). Both models assumed that the dissolution of pyrite was non-stoichiometric (NS), meaning that S/Fe ratio of the iron and sulfur released from pyrite is not equal to two and both models produced the same values for the two grain sizes. Pisapia et al (2007) also calculated $\Delta^{18}O_{SO4\text{-}H2O}$ values based on both models with stoichiometric (S) release of sulfur and iron, and the calculated values were 2.0 and 5.6‰, respectively, for the two different grain sixes, and were once again identical for both models. However, as in this study, Pisapia et al (2007) had a large initial sulfate concentration that they claimed to have taken into account in their calculations. However, unlike this study they did not vigorously explain how this

was done, nor did they take into account the extra error involved in these calculations, making their data suspect.

The estimated $\varepsilon^{18}O_{SO4\text{-}H2O}$ values for the aerobic chalcopyrite oxidation experiments were 8.5, 6.3, and 6.5‰ for the abiotic, biological short and long term experiments, respectively (Figure 3-6). All of these values are conspicuously different than the $\varepsilon^{18}O_{SO4\text{-}H2O}$ value of 3.8‰ measured in the anaerobic experiments (Figure 3-6). However, each of the aerobic experiments may have incorporated a small amount of molecular O_2 as suggested by the slopes in Figure 3-6 that are significantly different from one. For the aerobic experiments, plots of $\delta^{18}O_{SO4}$ vs. $\delta^{18}O_{H2O}$ suggest that 51, 92, and 94% of the oxygen in sulfate is derived from water for the abiotic, biological short and long term experiments, respectively (Figure 3-6). However, when considering the error propagation for the abiotic, biological short and long term experiments, there could be as much as 100% water-derived oxygen incorporation, or as little as 0, 78, and 82% water oxygen, respectively, incorporated into the sulfate. However, even if the water-derived oxygen values were lower, this results in little change with the estimated $\Delta^{18}O_{SO4\text{-}H2O}$ values of 7.2, 6.3, and 6.3‰ for the abiotic, biological short term and biological long term experiments respectively.

Under abiotic conditions with O_2 as the oxidant, Taylor et al (1984b) found that 94 and 72% of the oxygen in sulfate formed during pyrite oxidation was water-derived under submerged and wet/dry conditions, respectively. They further found that 65 and 23% of the oxygen in sulfate was water-derived under biological aerobic oxidation of pyrite by O_2 under submerged and wet/dry conditions, respectively. However, Balci et al (2007) found that 85, 92, and 87% of the oxygen in sulfate was

water-derived for the oxidation of pyrite by O_2 under biological short (20 days) and long term (44 days), and abiotic conditions, respectively. In contrast, biological oxidation of elemental sulfur and sphalerite by O_2 showed no O_2 incorporation into sulfate and $\varepsilon^{18}O_{SO4\text{-}H2O}$ values ranged from 7.0 to 9.6‰ (Balci, 2005). Similar $\varepsilon^{18}O_{SO4\text{-}H2O}$ values were also measured during biological and abiotic oxidation of elemental sulfur and sphalerite by Fe(III)(aq) under anaerobic conditions (Balci, 2005). These results suggest that the oxidation of chalcopyrite is more similar to pyrite oxidation than sphalerite oxidation.

The $\delta^{18}O_{SO4}$ values shown in Table 3-6 and Figure 3-6 show good agreement between the biological short and long term experiments. Whereas the $\delta^{18}O_{SO4}$ values from the abiotic experiment are conspicuously different. The relative amount of water oxygen incorporated into the sulfate formed during the aerobic chalcopyrite oxidation experiments is not in agreement with the net stoichiometry of Equations 1-4 or 1-5. The copper and iron released could be catalyzing the oxidation of chalcopyrite by O_2, which may be especially important for the abiotic experiments that displayed a slower oxidation rate (Chen and Morris, 1971). This could account for the apparently greater incorporation of molecular oxygen in the abiotically formed sulfate relative to the biological experiments. However, since the sulfate production was limited, and the associated error propagation was high in the abiotic experiments, the amount of water-derived oxygen in the sulfate could vary anywhere from 0-100%. Therefore, it is not possible to accurately discern a difference between the biological and abiotic experiments.

Using the $\varepsilon^{18}O_{SO4\text{-}H2O}$, value of 3.8‰ from the anerobic chalcopyrite oxidation experiment and Equation 2-5, the $\varepsilon^{18}O_{SO4\text{-}O2}$ values were calculated for the aerobic experiments. These $\varepsilon^{18}O_{SO4\text{-}O2}$ values were -10.7, 11.7, and 24.4‰ for the abiotic and biological short and long term experiments, respectively. These results are confusing as the three were expected to be similar based on a previous oxygen isotope study of pyrite oxidation that showed similar values between abiotic and biological experiments (Balci et al., 2007). This may once again have to do with the high initial sulfate concentration in the abiotic experiments and the inherent error in Figure 3-3. Furthermore, the error propagation of the aerobic experiments suggests that the biological short and long term experiments could have actually incorporated more or less water-derived oxygen than the linear regression suggests. Using values of 78 and 82% water-derived oxygen for the biological short and long term experiments, respectively, changes the $\varepsilon^{18}O_{SO4\text{-}O2}$ values to -8.6 and -5.1‰ for the biological short and long term experiments. If the abiotic experiment incorporated 60-68% water oxygen then the $\varepsilon^{18}O_{SO4\text{-}O2}$ value would change from -10.7‰ to a range between -8.5 to -5.6‰, and consequently the three values from the aerobic experiments would generally agree. These results illustrate the difficulty associated with accurately measuring $\varepsilon^{18}O_{SO4\text{-}O2}$ when sulfate production rates are not adequately high and/or the % of O_2 incorporation is very small.

Assuming the $\varepsilon^{18}O_{SO4\text{-}O2}$ value from the oxidation of chalcopyrite is between -5 to -11‰, this value would then agree with similar values from the literature. Taylor et al. (1984b) reported $\varepsilon^{18}O_{SO4\text{-}O2}$ values of -4.3‰ and -11.4‰ for abiotic and biological oxidation of pyrite, repectively. Balci et al. (2007) calculated $\varepsilon^{18}O_{SO4\text{-}O2}$

values of -10.0, -10.8, and -9.8 for their biological short (20 days) and long term (44 days) and abiotic experiments, respectively, for pyrite oxidation by O_2. Pisapia et al (2007) calculated $\varepsilon^{18}O_{SO4\text{-}O2}$ values ranging from -24.3 to -28.5‰ and -8.9 to -14.6‰ for non-stoichiometric (NS) and stoichiometric (S) reactions, respectively. As previously mentioned these values are potentially suspect, although their estimates based on stoichiometric reactions appear to more closely agree with other studies (Taylor et al, 1984b; Balci et al, 2007).

4.3 Kinetic Sulfur Isotope Fractionation Between SO_4 and Chalcopyrite

Fractionation effects between sulfate and sulfide minerals are typically small to non-existent (Nakai and Jensen, 1964; McCready and Krouse, 1982; Taylor et al., 1984b; Fry et al., 1986; Balci et al., 2007). This means that the sulfate produced has $\delta^{34}S$ values very similar to the starting material (Gavelin et al., 1960; Nakai and Jensen, 1964; Field, 1966; Rye et al., 1992). However, some researchers have reported values of $\varepsilon^{34}S_{SO4\text{-}Sulfide}$ that were different from the starting material (Kaplan and Rittenberg, 1964; Taylor et al., 1984b; Toran and Harris, 1989).

An $\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ value of ~-3.8‰ was measured in this study for all the anaerobic experiments. The results presented here for the anaerobic chalcopyrite experiments are reasonably close to an $\varepsilon^{34}S_{SO4\text{-}FeS}$ value of -2.6‰ for abiotic oxidation of H_2S under anaerobic conditions (Lewis and Krouse, 1969). Anaerobic oxidation of pyrite by $Fe(III)_{(aq)}$ showed an ($\varepsilon S_{SO4\text{-}FeS2}$) value of -0.75‰, whereas oxidation of pyrite with O_2 in the absence of $Fe(III)_{(aq)}$ did not show significant fractionation (Balci et al., 2007). Balci et al (2007) attributed these sulfur isotope

fractionation effects to the incomplete oxidation of intermediate sulfur species formed during pyrite oxidation by $Fe(III)_{(aq)}$ under low pH conditions as reported elsewhere in the literature (Schippers and Sand, 1999; McGuirre et al, 2001). Therefore, previous results and those presented here suggest that $Fe(III)_{(aq)}$ is important in the non-biological fractionation of sulfur isotopes during sulfide mineral oxidation, probably due to incomplete oxidation and the formation of sulfur intermediates.

One study found that $\varepsilon^{34}S_{SO4\text{-}Sulfide}$ value varied between -2 to -5.5‰ for the aerobic biological oxidation of FeS_2 and ZnS (Toran and Harris, 1989). Pisapia et al (2007) reported $\Delta S_{SO4\text{-}FeS2}$ values of -1.3 and 0.4‰ for their S and NS processes, respectively, during aerobic oxidation of pyrite, which compare with a value of 0.1‰ reported by Balci et al (2007). Pisapia et al's (2007) results for sulfur fractionation are probably fairly accurate given that their reported $\delta^{34}S$ value from the initial sulfate was 4.2‰ and very similar to the $\delta^{34}S$ value of 4.1‰ for the pyrite. Aerobic oxidation of elemental sulfur has shown an $\varepsilon^{34}S_{SO4\text{-}S0}$ value of -1 and 1.2‰ (McCready and Crouse, 1982; Balci, 2005). Aerobic thiosulfate oxidation produced a $\varepsilon^{34}S_{SO4\text{-}S2O3}$ value of -4.7‰ (Chambers and Trudinger, 1978), which is very similar to the results of the anaerobic experiments from this study. Biological and abiotic aerobic oxidation of sodium sulfide showed $\varepsilon^{34}S_{SO4\text{-}Na2S}$ values of -15 and 5.2‰, respectively (Kaplan and Rittenberg, 1964; Fry et al, 1988). Values of -1.5 and -0.5‰ were measured for $\varepsilon^{34}S_{SO4\text{-}CuFeS2}$ in this study for the aerobic biological and abiotic experiments, respectively. These values are not as depleted in ^{34}S as in the anaerobic oxidation of chalcopyrite. These values seem to be closer to $\varepsilon^{34}S_{SO4\text{-}S0}$ value of -1‰ for oxidation of elemental sulfur (McCready and Crouse, 1982),

although Balci (2005) recently reported a fractionation of +1.3‰ for the oxidation of elemental sulfur by *A. ferrooxidans*. Since $Fe(III)_{(aq)}$ was initially absent from the aerobic experiments of this study, the role of $Fe(III)_{(aq)}$ as an oxidant and attendant formation of sulfur intermediates, may account for the larger depletion in ^{34}S in sulfate for the anaerobic experiments relative to the aerobic experiments.

Even though sulfur intermediate species such as sulfite were not identified in this study, the possibility that intermediates were present would help to explain the results of this study for the oxidation of chalcopyrite by $Fe(III)_{(aq)}$. If an intermediate sulfur species were forming during this reaction, they could preferentially incorporate the heavier isotopes of sulfur (Seal, 2003). This in turn would leave the sulfate in solution depleted in ^{34}S compared to the chalcopyrite mineral as determined for my anaerobic experiments. Because the aerobic chalcopyrite experiments did not have a high initial concentration of $Fe(III)_{(aq)}$ this process may not have contributed to the formation of these same intermediates during that experiment and thus explain the apparent lack of sulfur isotope fractionation.

4.4 Oxidation Mechanisms of Metal Sulfides

Metal sulfide minerals generally fall into two different categories, acid insoluble and acid soluble (Schippers and Sand, 1999; Schippers, 2004). There are only three acid insoluble sulfide minerals- pyrite (FeS_2), molybdenite (MoS_2), and tungstenite (WS_2), with all other metal sulfide minerals being designated as acid soluble. Because of this distinction, it is thought that there are two different mechanisms to account for the oxidation of each type of metal sulfide. The acid

insoluble metal sulfides are thought to be resistant to proton attack because their valence bands are solely composed of metal orbitals with pairs of sulfur atoms forming non-bonding orbitals (Schippers, 2004). Therefore, in the absence of bacteria, these minerals generally require a stronger oxidant such as $Fe(III)_{(aq)}$ to be oxidized.

The acid soluble sulfide minerals are thought to undergo a mechanism known as the polysulfite mechanism (Schippers, 2004). In this mechanism protons or a stronger oxidant such as $Fe(III)_{(aq)}$ activate the surface of the mineral by releasing the metal moiety from the mineral surface while simultaneously adsorbing protons. The protons are later released as they or other oxidants further attack the surface, and the sulfide moiety of the mineral is oxidized to elemental sulfur. Since elemental sulfur is insoluble under low pH conditions and has been detected under various experimental conditions during the oxidation of acid soluble sulfides (Garcia et al, 1995; Curutchet et al, 1996), its exact role during the oxidation process needs to be evaluated. Furthermore, the role of sulfur oxidizing bacteria becomes important since the bacterial oxidation of S^0 by O_2 incorporated only water-derived oxygen into sulfate (Balci, 2005). Although the $\varepsilon^{18}O_{SO4\text{-}H2O}$ values observed for biological anaerobic oxidation of pyrite (3.6‰) (Balci et al, 2007) and chalcopyrite (3.8‰) were similar, these two minerals had greatly different $\varepsilon^{34}S_{SO4\text{-}sulfide}$ values (-0.7‰) and (-3.8‰), respectively. This could be a result of the difference between acid soluble and acid insoluble sulfide minerals.

The oxidation of sulfide to sulfate undergoes a valence change from -2 to +6. However, typically only one or two electrons are transferred between reactants in

most reactions (Luther, 1987), which means that intermediate sulfur species are expected. Elemental sulfur and other intermediate sulfur species such as thiosulfate and sulfite have been detected in previous studies of sulfide oxidation (Yin et al., 1995; Yuehua et al., 2002; Rodríguez et al., 2003). The role of these intermediates during the oxidation of metal sulfide minerals and their impact on the mechanism must also be considered.

In terms of the biological experiments, the bacteria are obviously catalyzing mineral oxidation, yet the oxygen isotope results are not in agreement with the net stoichiometry for any of the given equations for chalcopyrite oxidation (Equations 1-4 and 1-5). This is especially true because the long and short term aerobic biological experiments are both trending toward 100% water oxygen incorporation. Four possibilities exist to explain the near complete incorporation of water oxygen into the resulting sulfate and thus, deviations from expectations based on simple stoichiometry. One possibility is that sulfite and water are exchanging oxygen atoms prior to the sulfite becoming oxidized to sulfate, which is known to occur rapidly at low pH, as discussed previously (Krouse et al., 1991; Reedy et al., 1991). Another possibility is that the bacteria, in the process of extracting energy during the enzymatic oxidation reaction through their electron transport system (ETS), preclude O_2 from making direct contact with the sulfur atoms as proposed previously (Balci, 2005). A third possibility is that the bacteria may be oxidizing intermediate sulfur species such as elemental sulfur, which has been shown to form sulfate with oxygen derived exclusively from water (McCready and Krouse, 1982; Balci, 2005). Additionally, bacteria are able to oxidize elemental sulfur at low pH's, during which

abiotic oxidation of elemental sulfur did not occur (Balci, 2005). Therefore, any elemental sulfur formed initially that is later oxidized by bacteria would produce sulfate that has its oxygen derived from water. The fourth explanation for increased incorporation of water oxygen could result from additional oxidation of chalcopyrite by the $Fe(III)_{(aq)}$ formed by the bacteria during oxidation of chalcopyrite as shown in Equation 1-11 and reported elsewhere (Balci et al, 2007).

As to the second possibility, because bacteria may be directly involved in the oxidation of chalcopyrite, during oxidation the electrons can flow through the ETS, which involves several reactions with the final one involving the reduction of oxygen to form water and not sulfate. Since the reduction of oxygen to form water is biochemically separate from the oxidation of the sulfur, this could explain why more water oxygen is becoming incorporated into sulfate as proposed in the following mechanism (Kelly, 1982; Balci, 2005). During the biological oxidation of elemental sulfur, oxidation proceeds through sulfite with two of the oxygen atoms coming from water and the third from molecular oxygen as seen in Equation 4-1. However, sulfite can rapidly undergo oxygen isotopic exchange with water (Lloyd, 1968; Holt et al, 1981). The sulfate subsequently incorporates more water oxygen as shown in Equation 4-2 below. The net stoichiometry is displayed in Equation 4-3.

$$S^0 + O^*_2 + 2\ H_2O \longrightarrow SO_2(O^*)^{2-} + H_2O^* + 2\ H^+ \quad (4\text{-}1)$$

$$SO_2(O^*)^{2-} + H_2O + 0.5\ O^*_2 \longrightarrow SO_3(O^*)^{2-} + H_2O^* \quad (4\text{-}2)$$

$$S^0 + 1.5\ O^*_2 + 3\ H_2O \longrightarrow SO_3(O^*)^{2-} + 2\ H_2O^* + 2\ H^+ \quad (4\text{-}3)$$

The relatively high incorporation of water oxygen into sulfate during the oxidation of chalcopyrite by O_2 could be explained through one or more of the above possibilities. The formation of sulfite was not verified in this study, although if it did form this would suggest that oxidation of chalcopyrite proceeds through the acid insoluble mechanism where formation of sulfite is more likely to occur. This is supported by the $\varepsilon^{18}O_{SO4\text{-}H2O}$ values, which are in good agreement with pyrite, an acid insoluble sulfide, and not sphalerite, an acid soluble sulfide (Balci et al, 2007). The data suggest that the aerobic abiotic experiments did not incorporate as much water derived oxygen and this may be due to catalytic oxidation of the chalcopyrite by iron or copper, which would cause a greater incorporation of molecular O_2. However, the tremendous amount of error for the aerobic abiotic oxidation of chalcopyrite experiment makes interpreting the data difficult.

4.5 Covellite Oxidation by O_2

There was no distinction between the aerobic biological and abiotic covellite oxidation experiments in terms of changes in pH and sulfate concentrations with time (Figure 3-7). As suggested for chalcopyrite, the copper as it is released from the mineral may be catalyzing the oxidation of the sulfide to sulfate as previously suggested (Chen and Morris, 1971). This catalytic effect has been observed to be greater at neutral and alkaline pH's. However, this catalytic effect has also been observed to occur quite rapidly, within several days, under low pH conditions. However, the pH increased dramatically at the beginning of my experiment to near neutral conditions and significant appearance of sulfate didn't occur until after ~40

days. Therefore, because of this theorized catalytic effect by Cu(II) it is feasible that Cu(II) may have catalyzed covellite oxidation by O_2 during my experiments as it was immediately released from the mineral near the beginning of the experiment. Also, previous studies showed that the products of sulfide oxidation by a catalyst like copper are pH dependent, with thiosulfate dominating at alkaline pH's, and other species such as sulfate occurring in higher concentrations at neutral pH (Cline and Richards, 1969; Chen and Morris, 1971). Although two previous studies were conducted under similar neutral pH conditions, Cline and Richards (1969) showed a higher concentration of sulfate relative to thiosulfate in their experiments. This difference was attributed to metals in the seawater samples not found in the experiments conducted by Chen and Morris (1971). Additionally, the catalytic effect by Cu(II) could explain why both the abiotic and biological samples had a fairly large inferred incorporation of oxygen atoms into the sulfate, as this catalytic effect utilizes molecular oxygen. However, no studies have been performed to investigate the relative incorporation of water and molecular oxygen atoms during catalytic oxidation of a sulfide mineral.

Since the bacteria did not increase the rate of covellite oxidation the difference of 10% for water-derived oxygen between the long term biological and abiotic samples may be a result of insufficient sulfate production and not statistically meaningful. Because the net production of sulfate only exceeded the starting sulfate concentration by no more than 90%, the original sulfate in the system may be interfering with accurate measurements of the $\delta^{18}O_{SO4}$ produced in each experiment. This combined with the large error associated with the oxygen isotopic analysis

makes it impossible to interpret any perceived differences between the aerobic biological and abiotic covellite oxidation experiments and thus precludes further discussion regarding the $\delta^{18}O_{SO4}$ results.

CHAPTER 5

CONCLUSIONS

In the anaerobic oxidation of chalcopyrite by $Fe(III)_{(aq)}$ experiments there was a large drop in pH, which is predicted by both Equations 1-1 and 1-2. However, Equation 1-1 predicts a much larger increase in [H+] than Equation 1-2 does. This suggests that Equation 1-1 is probably more important during the anaerobic oxidation of chalcopyrite. Since the data suggests that sulfur intermediate species are produced because of the non-stoichiometric fraction of sulfur and the slow formation of sulfate, these results suggest that indirect or the indirect contact mechanism is important during this process.

Oxidation of chalcopyrite is slower under anaerobic conditions, possibly due to a passivating layer of some precipitating species, perhaps jarosite, elemental sulfur, or some other intermediate sulfur species. Formation of these intermediates may explain the significant fractionation of S isotopes ($\varepsilon^{34}S_{SO4\text{-}CuFeS2}$) of ~ -3.8‰ observed during the anaerobic chalcopyrite oxidation experiments, which showed no measurable difference between the biological and abiotic experiments. The oxygen isotope fractionation effect between water and sulfate ($\varepsilon^{18}O_{SO4\text{-}H2O}$) during anaerobic oxidation of chalcopyrite by $Fe(III)_{(aq)}$ was estimated to be ~3.8‰.

Results from the aerobic experiments suggest that bacteria significantly increase the rates of leaching and oxidation of chalcopyrite by O_2. Under biological conditions, the pH dropped almost immediately. Only Equation 1-5 predicts this behavior for aerobic chalcopyrite oxidation. Equation 1-5 also predicts that almost all

of the oxygen in sulfate was derived from molecular O_2. However, aerobic oxidation of chalcopyrite showed 92, 94, and 51% incorporation of water-derived oxygen into the resulting sulfate formed during the biological short and long term, and abiotic experiments, respectively, which is not in agreement with the net stoichiometry for any of the published reactions for the oxidation of chalcopyrite by O_2. If Equation 1-5 is relevant to the biological oxidation of chalcopyrite by O_2, then the lack of O_2 incorporation might result from the ETS system of the bacteria, which effectively separates the molecular oxygen from the oxidation of sulfide to sulfate. In contrast to the oxidation of chalcopyrite by $Fe(III)_{(aq)}$, this would also suggest that direct contact is required between the bacteria and the mineral. Additional possibilities to explain the predominance of water-derived oxygen in sulfate during the biological oxidation of chalcopyrite by O_2 include the following: 1) Exchange between water and sulfite, 2) additional oxidation of elemental sulfur or sulfide by $Fe(III)_{(aq)}$, or 3) subsequent oxidation of an elemental sulfur intermediate could all contribute to a higher than expected amount of water-derived oxygen in the sulfate formed during the biological experiments.

By using the estimated $\varepsilon^{18}O_{SO4\text{-}H2O}$ value of 3.8‰ from the anaerobic experiments, the $\varepsilon^{18}O_{SO4\text{-}O2}$ value is estimated to be 11.7, 24.4, and -10.7 ‰ for the biological short and long term, and abiotic experiments, respectively. However, the error analysis of this data causes uncertainty in the reliability of these values. Sulfur isotopes were only slightly fractionated during the aerobic oxidation of chalcopyrite.

For covellite, *A. ferrooxidans* had no effect on the oxidation of this mineral under aerobic conditions. This is possibly due to the immediate increase in pH to

near neutral conditions, thus changing the system from acidic to neutral, which is not favorable to *A. ferrooxidans*. The $\delta^{18}O_{SO4}$ values for aerobic covellite oxidation showed 71, 49, and 62 % incorporation of water oxygen atoms into the resulting sulfate formed for the biological short and long term, and abiotic experiments, respectively. These results are not in areement with the net stoichiometry of Equations 1-6 and 1-7. However, as for the abiotic aerobic oxidation of chalcopyrite, the error propagation associated with these experiments makes it impossible to accurately interpret the $\delta^{18}O_{SO4}$ results. The aerobic oxidation of covellite may also have been catalyzed by leached metal cations such as $Cu(II)_{(aq)}$ and $Fe(III)_{(aq)}$.

CHAPTER 6

IMPROVEMENTS FOR FUTURE STUDIES OF COPPER SULFIDE OXIDATION

The most obvious improvement that could have been made with my experimental design was to have ensured that minimal sulfate was added to the media at the beginning of the experiment. This would have permitted a clearer and more straight forward interpretation of the measured $\delta^{18}O_{SO4}$ from the covellite oxidation experiments and the aerobic abiotic chalcopyrite oxidation experiments. This in turn would have permitted more accurate estimates of the % oxygen incorporated into sulfate from O_2 and water, as well as $\varepsilon^{18}O_{SO4\text{-}H2O}$ and $\varepsilon^{18}O_{SO4\text{-}O2}$ values.

Measurement of sulfur intermediate species, such as dissolved sulfoxy anions or those formed on the mineral surface, such as elemental sulfur, would also have helped with interpreting the measured $\varepsilon^{18}O_{SO4\text{-}sulfide}$ values. Dissolved sulfur intermediates could have been measured either by IC or precipitation methods, and mineral surface products could be analyzed by X-Ray diffraction or Raman spectroscopy. These procedures would be especially important for experiments such as the anaerobic chalcopyrite experiments where sulfate may have precipitated, intermediates may have formed, and where non-stoichiometric amounts of the mineral elements are released. It would be prudent to measure both dissolved and surface intermediates in future experiments.

The aerobic experimental results did not always agree between monitoring flasks, which had samples periodically removed for chemistry, and separate flasks

that were set aside for isotopic analysis, which were only chemically monitored at the beginning and end of the experiments. A larger initial volume of media in the monitoring flasks would help to minimize differences, which could be associated with a greater disturbance of the settled material in the monitoring flasks, or partial removal of mineral during a sampling event. Also, not enough media sample was available for analysis of copper and iron measurements, more initial media would have allowed for more of these metal determinations. This would also have been helpful as then determining the exact valence state of the copper in solution, and whether or not the valence state changes could have been done as well. Potentially $Cu(II)_{(aq)}$ could have the same role of oxidizing a mineral as $Fe(III)_{(aq)}$.

For the bacterial studies, controlling the pH during an experiment would be important, as in the covellite oxidation experiment. Ensuring that the pH remained acidic may have allowed the acidiphillic bacteria to grow and oxidize the mineral. This would also have to be done in parallel with the abiotic control experiment.

Improvements might also be made for the anaerobic studies utilizing $Fe(III)_{(aq)}$ as the oxidant. Because precipitation of sulfate or a sulfur containing species was inferred by my results, and the high $Fe(III)_{(aq)}$ concentration used was implicated as potentially causing this effect, controlling the $Fe(III)_{(aq)}$ concentration might be important. This could be done by either starting with a lower $Fe(III)_{(aq)}$ concentration or by adding small amounts incrementally during the experiment, perhaps after each sampling event.

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