Acid Mine Drainage: Flow-Through Experiments

Examining Pyrite Dissolution Rates

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ABSTRACT

Pyrite is an iron sulfide mineral which forms with coal deposits and readily weathers in the presence of water and air (Madigan et al., 2000). At many mine sites the presence of pyrite causes a major environmental problem known as acid mine drainage. Bacteria contribute to the problem by utilizing an electron from ferrous iron retrieved from pyrite dissolution. The bacteria oxidize the ferrous iron to ferric iron, creating their energy source. Pyrite dissolution increases the concentration of hydrogen ions in the water, increasing acidity at the mine site and down stream. One strain of bacteria, *Acidithiobacillus ferrooxidans* are prevalent at many mine sites. Duplicate experiments involving column studies compared the rates of pyrite dissolution by measuring total dissolved iron content in output solutions. The control experiment included pyrite with water at a pH value of 2 (pH controlled by concentrated HCl). One variation of the experiment was done with water at a pH value of 2 and ~5.0g/L ferric iron. And another variation was done with pH 2 water and *A. ferrooxidans*. Results showed that the addition of *A. ferrooxidans* to the reaction vessel increased the dissolution rates of pyrite. The addition of ~5.0 g/L of ferric iron caused the largest increase in pyrite dissolution rates. Results showed that ferric iron increased pyrite dissolution by three orders of magnitude greater than water and oxygen. Data from these experiments and future experiments will help researchers gain a better understanding of the acid mine drainage systems and will help to devise more effective plans for remediation.

BACKGROUND

Bacteria can be classified by their metabolism; these classifications include chemolithotroph, autotroph, and heterotroph (Madigan et al., 2000). Chemolithotrophs obtain their energy by oxidizing inorganic compounds such as minerals. Autoptrophs use carbon dioxide as their sole carbon source. Heterotrophs obtain their energy by oxidizing organic compounds such as glucose (Madigan et al., 2000). These terms can be combined to classify bacteria more specifically. For example, chemolithoautotrophs oxidize inorganic compounds and use carbon dioxide as their sole carbon source. The bacterial source of concern at acid mine drainage sites are chemolithoautotrophs. *Acidithiobacillus ferrooxidans* oxidize the sulfide in pyrite (FeS_2) to sulfate (SO_4^{2-}) as their inorganic energy source and utilize carbon dioxide as their sole carbon source. In bacteria and other biological systems, energy conservation involves redox reactions where electron transport is coupled to the formation of adenosine triphosphate (ATP), an energy storage molecule necessary for life, growth and the reproduction of living organisms. Electrons are transported via the electron transport chain which includes an electron donor and acceptor. Figure 1 shows the electron transport chain for *A. ferrooxidans* where ferrous iron acts as the electron donor and oxygen is the terminal electron acceptor. Once again, this reaction is coupled with the formation of ATP. When *A. ferrooxidans* oxidize the pyrite (FeS_2), they release the ferrous iron (Fe^{2+}) which is soluble in low pH waters (Madigan et al., 2000). This allows *A. ferrooxidans* to take an electron from the ferrous iron (Fe^{2+}). The bacteria use a protein to capture the electron and then use another series of proteins to shuttle the electron across their membrane and into the inside of the cell (See Figure 1).

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INTRODUCTION

Acid mine drainage occurs across the country at many mine sites. The US currently has 2,062 operating coal mines; ten years ago that number was double. (US Department of Labor, Mine Safety and Health Administration, 2002). This number is small compared to other courtiers such as China, which has 23,000 operating coal mines (Xinhua News Agency, 2002). The number of operating mines in China has been reduced by thirty percent in the last year (Xinhua News Agency, 2002). The rising costs of mine operations along with safety and environmental concerns has caused this reduction. (US Department of Labor, Mine Safety and Health Administration, 2002).

Worldwide 3 trillion tons of dirt and rock are moved every year to extract minerals (Economic Geology, 2000). The clean-up costs for individual mines vary from a few hundred thousand dollars to more than one billion dollars. At the Iron Mountain mine, in California, the clean up cost for the government is expected to be upwards of one billion dollars (US Department of Justice, 2000). The burden of these costs falls mainly on the taxpayers' shoulders, both on the federal and state level. The cost of the clean up at many of these mines exceeds the mining companies' resources and so federal and state governments are called upon to help with the costs to prevent the companies from going bankrupt and potentially leaving an even higher environmental hazard. Understanding the science behind the problem will help create more cost efficient remediation solutions.

When pyrite is exposed to air, oxidation of the iron sulfide to sulfate occurs along with the production of hydrogen which creates an acidic environment (See Equation 1)(Madigan et al., 2000). The removal of overburden rock and soil above the coal

through mining exposes the coal and pyrite to air, initiating the oxidation reaction. These reactions mainly occur in two places: the exposed coal seams and in waste piles. The pyrite in the coal is not useful and so the pyrite is discarded into waste piles. Pyrite, discarded coal, and overburden rock and soil sit in waste piles and oxidation occurs. Movement of water through the exposed coal and waste piles mobilizes the hydrogen created in the oxidation reaction. As a result, the drainage areas from the coal mines become very acidic. In these wet, acidic, environments acidophiles, acid loving bacteria, thrive. They survive by oxidizing ferrous iron from the pyrite; and in doing so a ferric hydroxide precipitate is formed. (Madigan et al., 2000). In the acid mine environments, many bacteria have been discovered, some working in tandem. *A. ferrooxidans* are believed to be of most concern.

There are three important reactions that occur in the acid mine environment that are of interest. The first is the oxidation of pyrite which occurs in the presence of oxygen and water resulting in the release of ferric iron (Fe^{3+}), sulfate (SO_4^{2-}), and hydrogen (H^+) according to the reaction:

$$FeS_2 + 7/2O_2 + H_2O \rightarrow Fe^{3+} + 2SO_4^{2-} + 2H^+ \qquad \text{(Equation 1)}$$
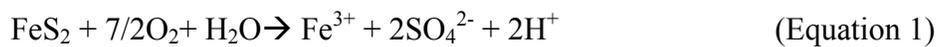
The buildup of hydrogen ions produced in this reaction decreases the pH of the water, causing the water to become acidic. The ferric iron (Fe^{3+}) produced by this reaction (Eq 2) oxidizes pyrite:

$$FeS_2 + 14Fe^{3+} + 8 H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad \text{(Equation 2)}$$

As stated previously, these two reactions oxidize pyrite and begin as soon as the pyrite is exposed to air and moisture. Bacteria catalyze a reaction where they use an electron from ferrous iron and create ferric iron. By creating ferric iron, the bacteria

drive the oxidation of pyrite, creating 16 moles of hydrogen for every one mole of ferric

iron produced (See equation 2). *A.ferrooxidans* utilize an electron from ferrous iron

(Fe^{2+}) producing ferric iron (Fe^{3+}) by the reaction:

$$2Fe^{2+} + \tfrac{1}{2} O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \hspace{2cm} \text{(Equation 3)}$$

This production of ferric iron (Fe^{3+}) coupled with the oxidation of the pyrite

(FeS_2) drives the pyrite weathering cycle and produces large amounts of hydrogen atoms,

perpetuating the acidity of the environment. In these acidic environments potentially

toxic trace elements, such as arsenic and mercury, become mobilized in the environment

which can cause damage to biota when the elements occur in quantities larger than trace

amounts. Pyrite is often found with trace amounts of arsenic which can become a source

of groundwater contamination as arsenic is released during the pyrite weathering reaction

(Kolker and Nordstrom, 2002). In one specific incidence in Alabama, pyrite containing 4

to 180 mg/kg of arsenic was found to be the source of arsenic polluting surrounding

streams (Goldhaber et al., 2001). These oxidation reactions and the role that bacteria and

pyrite play have significant impacts on the environment.

In the mine sites studied in the literature where *A. ferrooxidans* were found, pH

values ranged from 0.7-3.0 (Edwards et al., 2000) and concentration of ferric iron from

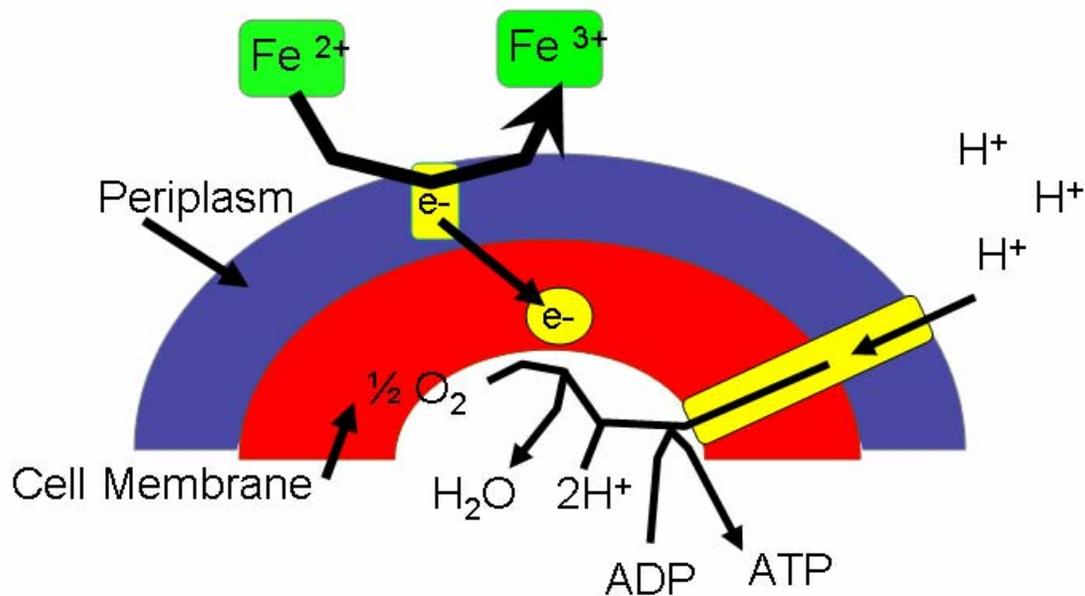1.0 g/L to 20.0 g/L (Fowler et al., 1999; Edwards et al., 1998).

Figure 1. The cell membrane of *A.ferrooxidans* and the electron transport chain. The bacteria utilize an electron from ferrous iron, oxidizing the ferrous iron to ferric iron. The bacteria use a protein to take the electron from the ferrous iron. At each step where the electron is transferred from one protein to another, a small amount of energy is released. At the final step, the electron is used to synthesizes ATP, the energy required for life. Oxygen acts as the electron acceptor in this reaction. This final step is coupled with a hydrolysis reaction.

A number of bacteria have been isolated from acid mine drainage environments; however the predominant bacteria seen at these sites are the acidophile, *A. ferrooxidans* (William et al., 1956). *A.ferrooxidans* was one of the first bacterial species isolated from an acid mine environment (Colmer and Hinkle, 1947; Temple and Kohler, 1954; Leathern et al., 1956) and can be found in almost all bituminous coal environments (Lazaroff, 1963). In addition to creating acidity, they have also been found to adhere to pyrite surfaces (Bennett and Tributsch, 1977). They do this by means of a sulfur binding protein that enables them to closely adhere to the sulfide in the pyrite which in turn allows the bacteria to be closer to the pyrite surfaces and may help them oxidize the pyrite as well (Ohmura et al., 1996).

Other studies have focused on what happens inside of the mine shafts and tunnels rather than in the waters draining from the mine sites. Recent studies found a new Archaea, *Feroplasma acidarmanus*, in high abundances inside the Iron Mountain mine in California. Researchers were surprised to find a very small population of A.*ferrooxidans,* instead, the new Archaea dominated the population (Edwards et al., 2000). These Archaea have only been found at the Iron Mountain mine site in California so the extent of their environmental range is unknown.

Four different experiments were conducted to simulate and study the acid mine environment. A continuous flow system was created with removable components that could be sterilized and/or changed as necessary. The initial experiment used water with a pH value of 7. The pH values of output water were monitored throughout. This was done to perfect the experiment design. For subsequent experiments, water was supplied to the system with a pH value of 2, similar to the low pH waters found in acidic mines (Edwards et al., 2002). Using a peristaltic pump, the water was pumped towards a column of pyrite which was packed with sterile cotton to keep the pyrite from leaving the system. To create an environment similar to a mine, the pyrite had to be exposed to oxygen. An aquarium pump was used to add filtered air to the column so that the pyrite was exposed to pulses of water followed by air, followed by water. This mimicked the continually wetting and drying of pyrite found at mine sites (See figure 2).

Batch reactions are another design frequently used in acid mine drainage experiments. They typically consist of a glass flask with pyrite on the bottom and low pH water which will sit or shake for a period of days. Other variables are added such as bacteria and ferric iron. Small aliquots of solution will be removed from the experiment

for analyses, however, no fresh reactants are introduced. Because there is no removal of

output solution and no introduction of fresh input solution, these reactions are not able to

efficiently mimic the drainage area of acid mine environments.

Figure 2 A close-up view of a pyrite column reaction vessel. The length of the vessel is 2.25 inches.
Each end is packed with sterile cotton to prevent the pyrite from escaping the vessel. The vessel is a
modified, sterile syringe. Various sizes of tubing and a pipette tips were used to modify the system and
create a continuous flow.

The kinetics of pyrite dissolution were examined as well with respect to the rate

laws governing flow-through experiments. The equation that best models the dissolution

rates is:

$$\text{Rate} = k_0 \cdot S_a \cdot e^{-Ea/RT} \cdot X_{H+}^{n_{H+}} \cdot \prod_i X_i^{n_i} \cdot g(I) \cdot f(\Delta G) \qquad \text{(Equation 4)}$$

Where k_0 is a constant (which varies with the mineral in dissolution), S_a is the reactive

surface area of the mineral, Ea/RT is the activation energy divided by temperature in

Kelvin (T) and the gas constant (R). In this equation (Eq. 4) X_i and X_{H+} are the

concentrations of ions adsorbed on the mineral surface and n_{H+} and n_i are the reaction

orders given the surface species. And finally, $g(I)$ is a function of the ionic strength, and f

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(ΔG) is the free energy exchange for the reaction in question (Ganor et al., 1999). Although this equation can be used to calculate the rate of a reaction from first principles, rate equations can be derived from kinetic flow-through experiments by using the equation:

$$\text{Rate} = (q*(C_{out} - C_{in}))/(k_0 * Sa) \qquad \text{(Equation 5)}$$

Where q is the rate of flow in $m^3 s^{-1}$, C_{out} is the concentration of the output solution, C_{in} is the concentration of the input solution in $moles \cdot m^{-3}$, k_0 is a constant, and Sa is the surface area in m^2 (Cama et al., 2000). This rate equation is modeled for a mass balanced equation in which the composition of the output solution reaches a constant value. Using this equation (5) to model dissolution will yield a pyrite dissolution rate in $moles \cdot m^{-2} \cdot s^{-1}$. Measuring the pH and total dissolved iron of the inputs and outputs will provide a way to monitor the change from the input solution to the output solution.

Previous work done in this field has included batch and column experiments. Batch experiments conducted by Fowler, Holmes, and Crundwell, 1999 used electrical current to change the ferric iron to ferrous iron which provided the bacteria in the experiment with a continuous flow of energy. This experiment, however, used an unclassified strain of *A. ferrooxidans*. Ohmura, Kitamuria, and Saiki, 1993 have studied the adhesion of *A. ferrooxidans* to pyrite and other minerals however, the experiments were batch reactions and the researchers did not monitor ferric or ferrous iron rates with respect to time. They did find a correlation though between an increase in ferrous iron and a decrease in *A. ferrooxidans* attachment to pyrite. Marchand and Silverstein, 2002 investigated the influence on pyrite oxidation with mixed bacterial cultures using batch experiments which did not provide fresh reactants to the system. Using a mixed bacterial

culture makes it difficult to determine individual species contributions to the reactions. Other researchers have focused on acid mine drainage using batch reactions (Konishi et al., 1994) and/ or an unclassified strain of *A. ferrooxidans* (Espejo and Romero, 1987; Mesa et al., 2002). Research focusing on oxidation rates and adhesion has been conducted, however it is limited in its application to real life systems because of the batch reactors used to conduct the experiments. By creating a flow-through reactor the natural environment can be mimicked. Using bacteria that have been classified is important as well. An unidentified strain does not help other scientist build upon the research that has been conducted. Using unidentified strains causes problems because it is difficult to discover contamination and very difficult for future researchers to duplicate or expand upon previous research. Previous work in this area has not included modeling of the dissolution rates of pyrite or use of flow rate equations.

Flow-through reactions using a classified strain of *A.ferrooxidans* have not been conducted. Using the genome sequencing strain of *A. ferrooxidans* from American Type Culture Collection in the described experiment will allow others to build upon this research. This project obtained data concerning flow-through dissolution rates by creating a simple flow-through reactor and examining the effects of pyrite dissolution through iron concentration. Using both low pH water and neutral pH water allowed a baseline to be established, and acted as a control group. Varying subsequent experiments by simple parameters allowed researchers to see an increase in dissolution rates when ferric iron was added to the reaction and an increase in dissolution rates when bacteria were added.

METHODS

Four experiments were conducting using a flow-through apparatus shown and described in Figure 3. Research grade pyrite (Wards Scientific) was ground with a mortar and pestle and sieved to have an average diameter of 350 μm. The pyrite powder was washed in an ultrasonic bath containing 1.0 M (moles/liter) HCl for 20 minutes, and rinsed with ultra pure filter sterilized water (18.2MΩ-cm H_2O) until fine particles were removed and the pH of the rinse water reached a value of 7. Pyrite was then rinsed in absolute methanol and dried with nitrogen gas. It was stored in nitrogen gas to prevent oxidation from occurring prior to use in experiments.

A flow-through reactor was made using Pyrex bottles, silicone tubing, and a peristaltic pump. For each experiment, 5.0 grams of clean pyrite was placed in a sterile syringe with sterile cotton at each end to prevent the pyrite from leaving the reactor. In the first experiment, ultra pure water at a pH value of 7 was used. The pH of the water coming out of the reactor was measured every twenty minutes for six hours. In the second experiment, ultra pure water was used at a pH value of 2. The pH was controlled by adding concentrated HCl (11.6 moles/L). This experiment was run for one week and water from the reactor was collected once every twenty-four hours. The decision of the change in collection time was made based on previous work done in the field which allowed the reactions to take place over days and weeks rather than hours. Although pH values were measured in the first experiment, they could not be used in subsequent experiments. Because the rest of the experiments were conducted with pH 2 water, the concentration of hydrogen in the input solution was high, and changes in pH in the output solution were no longer detectable. Instead, total dissolved iron was measured from the

samples after collection. The third reaction was with ultra pure water at a pH value of 2 and ~5.0g/L $FeSO_4$. The amount of ferric iron to add was determined from measured amounts in mines found in the literature (Fowler et al., 1999). This reaction ran for one week and water was collected from the reactor once every twenty-four hours. The fourth experiment incorporated *A. ferrooxidans* into the experiment and water with a pH value of 2. The experiment ran for one week and output solution was collected every twenty-four hours.
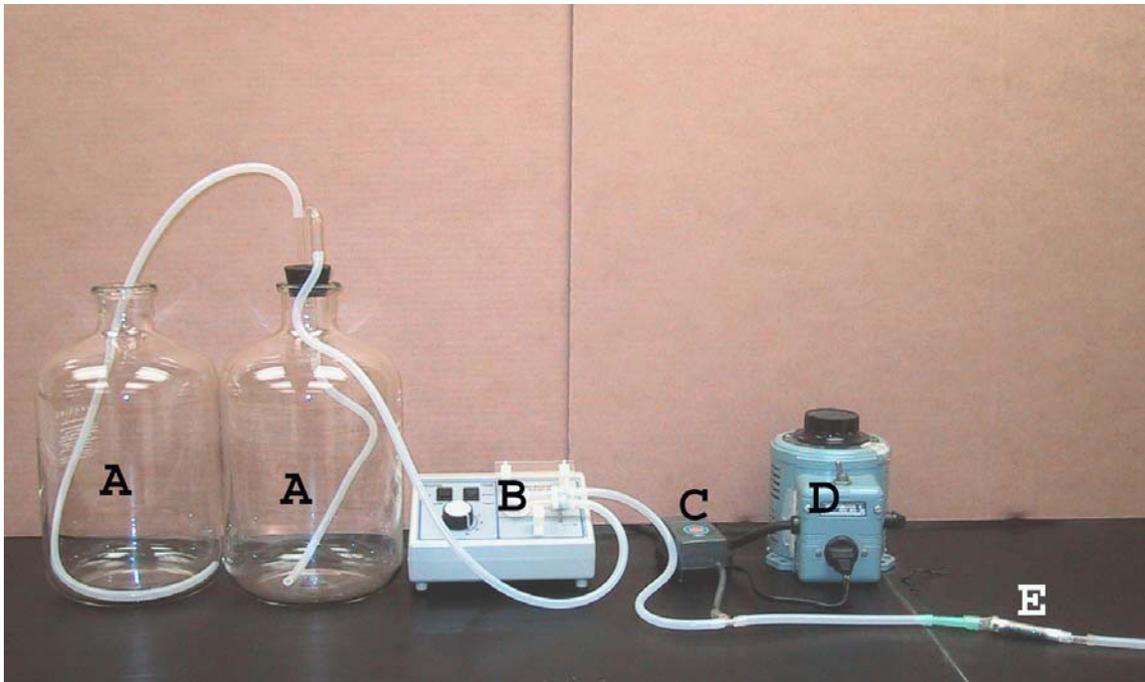
Figure 3. Experiment Design. Pyrex vessels labeled A, held input solution. A variable flow rate peristaltic pump, labeled B, (VWR brand, medium flow) controlled flow rate. The aquarium pump (C) was connected to a Powerstat variable autotransformer (D) which allowed control over the AC current flowing to the pump. This allowed the rate of air going into the experiment to change. The pyrite column, packed with cotton, is labeled E.

A pure culture of *A. ferrooxidans* (#23270) was obtained from American Type Culture Collection (ATCC). They were grown on ATCC 2039 media as instructed by ATCC, 2003. The media was prepared by aseptically combining two solutions. The first

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solution, A, was prepared by mixing together 0.8g $(NH_4)_2SO_4$, 2.0g $MgSO_4 \cdot 7H_2O$, 0.4g K_2PO_4, 5.0mL Wolfe's Mineral Solution, and 800.0mL ultra pure water. The pH value was adjusted to 2.3 with H_2SO_4 and solution A was filter sterilized. The second solution, B, was prepared by dissolving 20.0g $FeSO_4 \cdot 7H_2O$ into 200.0mL ultra pure water and rapid filter sterilizing. Wolfe's Mineral Solution was purchased from ATCC, it consists of 1.5g Nitrilotriacetic acid, 3.0g $MgSO_4 \cdot 7H_2O$, 0.5g $MnSO_4 \cdot H_2O$, 1.0g NaCl, 0.1g $FeSO_4 \cdot 7H_2O$, 0.1g $CoCl_2 \cdot 6H_2O$, 0.1g $CaCl_2$, 0.1g $ZnSO_4 \cdot 7H_2O$, 0.01g$CuSO_4 \cdot 5H_2O$, 0.01g $AlK(SO_4)_2 \cdot 12H_2O$,0.01g H_3BO_3, 0.01g $Na_2MoO_4 \cdot 2H_2O$, 1.0 L ultra pure water. This mineral solution was prepared by adding the nitrilotriacetic acid to approximately 500mL of water. The pH value was adjusted to 6.5 with KOH until the nitrilotriacetic acid was dissolved. The remaining water was added and the remaining compounds were added. All media preparation was performed with heat sterilized, autoclaved instruments and filtered with single use, sterile 0.2μm filter membranes with sterile, single use vessels attached. *A. ferrooxidans* were cultured every two weeks to maintain bacteria in the active growth stage. The ferrous sulfate ($FeSO_4 \cdot 7H_2O$) provided the electron (energy) source for the bacteria. The bacteria were cultured aerobically, using oxygen as the terminal electron acceptor and carbon dioxide as the carbon source, and incubated in glass test tubes at 26 °C and shaken at 200 rpm.

Because of the flow-through nature of the experiment, a system had to be devised to keep the bacteria inside the reaction vessel. Filters provided too much resistance to the pump and shut down the experiment. Vessel adaptation was also required due to the bacteria preference for glass over plastic. A new vessel was made using a 15mL glass beaker (See figure 4). Pyrite and bacteria were deposited at the bottom of the vessel

where air and water could enter and exit with minimal chance of dislodging the bacteria.

By allowing the bacteria to grow for a few days on the pyrite, they were allowed time to

adhere to the pyrite to further prevent them from exiting the vessel during the experiment.

Figure 4. Bacteria reaction vessel. 5.0 grams of pyrite are in the bottom. A glass tube was suspended above the liquid line to bring fresh input solution into the vessel. Another glass tube was suspended just below the air/ water interface to remove output solution. Cotton was not used because it would have introduced organics into the system that might have interfered with bacterial growth.

Iron analyses were made using a Hack kit and FerroVer reagent. This method

allowed measurement of iron in the ranges of 0.02 to 3.30 mg/L. FerroVer reagent is a

combination of two chemicals: 1, 10-phenanthroline and hydroxylamine hydrochloride

(Steehler, 2003). The hydroxylamine hydrochloride reduces the ferric iron to ferrous iron

through the reaction (Steehler, 2003):

$$2Fe^{3+} + 2NH_2OH + 2OH^- \rightarrow 2Fe^{2+} + N_2 + 4H_2O \qquad \text{(Equation 6)}$$
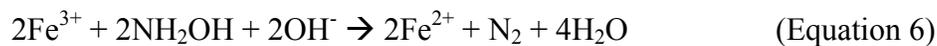
The ferrous iron then forms a colored complex with the 1, 10- phenanthroline through the

reaction (Steehler, 2003):

$$Fe^{2+} + 3C_{12}H_8N_2 \rightarrow FeC_{36}H_{24}N_6^{2+} \qquad \text{(Equation 7)}$$

This reaction can be shown visually (Steehler, 2003):

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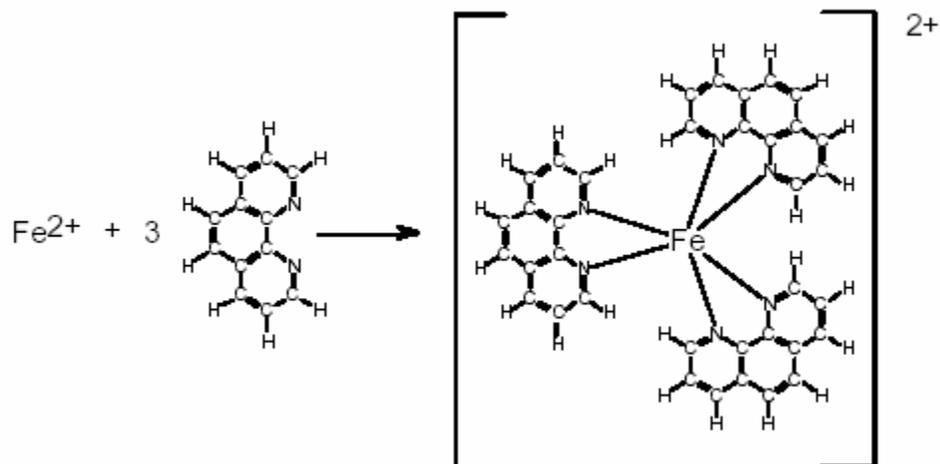
The result of the reaction is an orange-colored complex (Hach, 1980). The intensity of

the color is proportional to the amount of iron in the sample and this can be measured

with a spectrophotometer (Hach, 1980). For these experiments a Hach Direct Reading

(D/R) 2000 spectrophotometer was used at a wavelength of 510 nm. At the beginning of

each sample set the first sample was measured three to five times to obtain a mean and

standard deviation for the sample set.

RESULTS/DISCUSSION

Analyses were conducted for the total iron content of all samples collected from

the experiments which utilized water with a pH value of 2. Results showed that the time

of analysis (whether two minutes after collection or two days) was insignificant.

In the first experiment involving water with a pH value of 7, the changes

measured in pH were used to calculate the dissolution rates (Using equation 5). The

calculated rates matched the literature rates for the dissolution of pyrite. Samples were

measured every twenty minutes over six hours. The rates observed in the experiments

ranged between 2.01E-9 to 2.18E-11 moles•m^{-2}•s^{-1} compared to rates on the order of

1.0E9 moles•m^{-2}•s^{-1} in the review literature (Ehlrich, 2002). The agreements in dissolution rate proved a successful experimental design.

In the second experiment with ultra pure water with a pH value of 2, the rate of pyrite dissolution over one week reached equilibrium rapidly. After the third day, the rates of iron coming out of the system were very low. (See Figure 4) This was unexpected however several variables could have caused this to happen. Because a column flow-through reactor was used, there was nothing inside the column to keep the pyrite stirred and so equilibrium with the exposed faces could have been reached very quickly. This would support the initial high pulse of iron as the pyrite surfaces reacted with the first pulses of water and air. As the week went on, less and less fresh pyrite faces were available, decreasing the rate of iron coming out of the experiment.

Dissolution rates calculated for this experiment were lower than the rates found in the literature. The rates obtained for this experiment, using equation 5, ranged from 1.99E-13 to 9.62E-13 moles•m^{-2}s^{-1}, +/- σ=3.26E-1313 moles•m^{-2}s^{-1}. This suggests that pH may have an effect on the rate of pyrite dissolution.

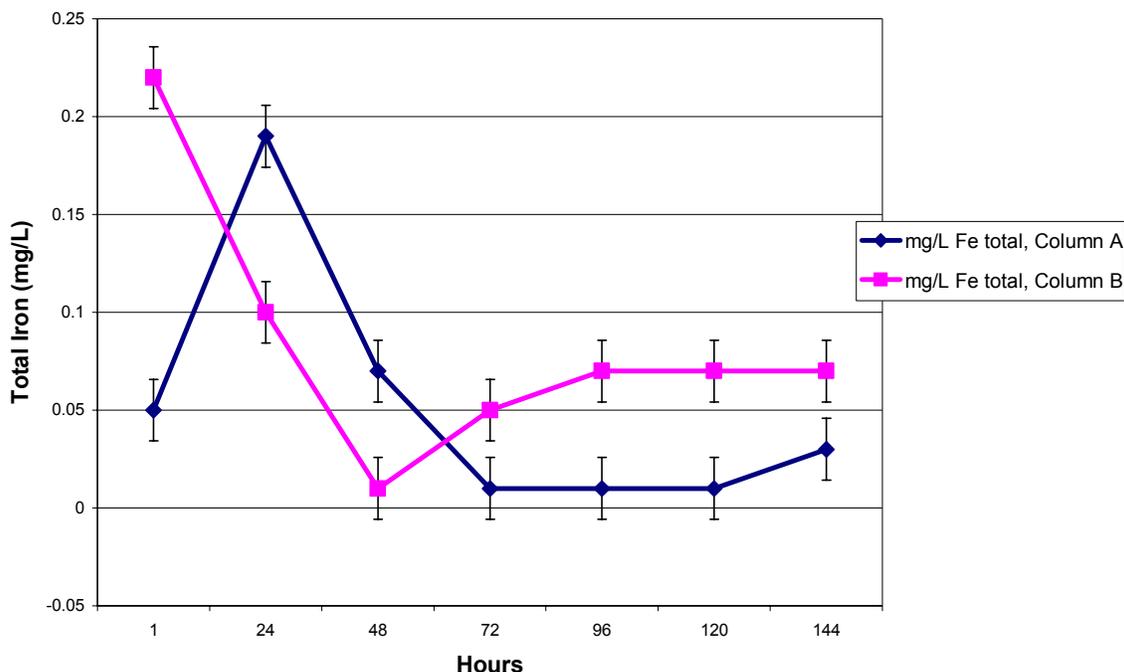## Total Iron (mg/L) vs. Time Air and pH 2 Water

Figure 5. Total iron (mg/L) measurements vs. time for the experiment with pyrite and water with a pH value of 2. A large flux of iron at the beginning of each experiment was followed by a decrease in total iron as time increases. σ for both columns is +/- 0.0158 mg/L total iron.

In the third experiment, ~5 g/L of ferric iron was added to the input water with a pH value of 2. This was done to simulate the high concentrations of ferric iron in acid mine environments caused by the activity of bacteria (Equation 2). The initial measurement of iron in the water was very high and as time increased, the amount of ferric iron in the system decreased. This was most likely because of a decrease in fresh pyrite faces available for reaction in the column.

Dissolution rates for this experiment were calculated using equation 5 and values ranged from 2.32E-9 to 9.95E-10 moles•$m^{-2}s^{-1}$, +/- σ=1.91E-10 moles•$m^{-2}s^{-1}$. These rates are much higher than the rates obtained without ferric iron in solution (the second experiment). This correlates well with equation 2 which showed how ferric iron weathers

and oxidizes pyrite. These rates are lower than those previously calculated which suggest pH may factor into dissolution rates.

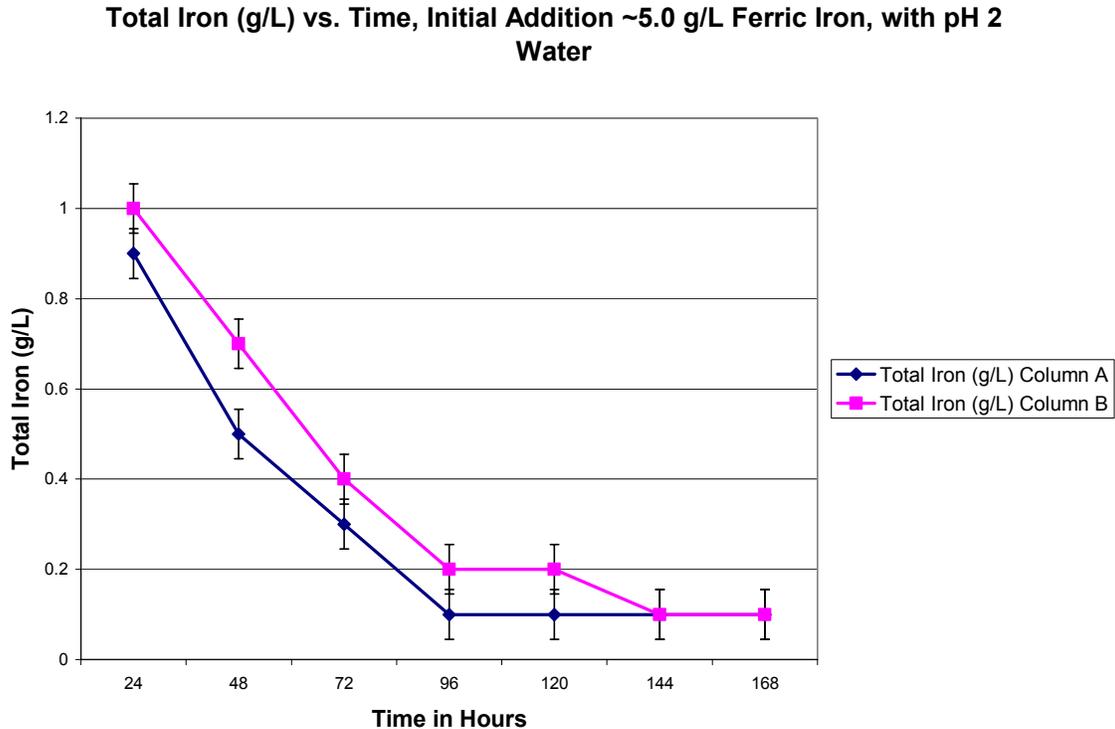**Total Iron (g/L) vs. Time, Initial Addition ~5.0 g/L Ferric Iron, with pH 2 Water**

Figure 6. Total iron (g/L) vs. time for the experiment with an initial input of ~5.0g/L of ferric iron and water with a pH value of 2. The initial pulse of output solution had a high amount of iron and then as time increases, total iron decreased. The values of total iron are much higher than the previous experiment. σ for the columns +/-0.055g/L total iron.

In the fourth experiment, ultra pure water was used with a pH value of 2 and 5.0mL of *A.ferrooxidans* culture. The initial rates of iron in the output solution were high. After the first twenty four hours, the rates of dissolved iron coming out of the experiment were extremely low (See Figure 5). This indicates that the bacteria probably came out of the experiment, either with some pyrite or in the output solution. Rates of pyrite oxidation are 3 to 100 times faster with *A. ferrooxidans* than they are with oxygen (Edwards et al., 1998). It's possible that the bacteria exited the vessel after the first twenty four hours because the pH of the water was controlled with HCl and the bacteria

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prefer SO_4^{2-} to Cl^-. Hydrochloric acid was used to control pH over sulfuric acid because

of an initial plan to measure sulfate concentrations along with iron. The introduction of

hydrochloric acid could have caused the bacteria that were adhered to the pyrite to

release, making them more likely to escape from the vessel during the experiment.

Dissolution rates were calculated for this experiment using equation 5 and the rates

ranged from 7.73E-12 to 9.95E-14 moles•$m^{-2}s^{-1}$, +/- σ=2.77E-13 moles•$m^{-2}s^{-1}$.

Total Iron (mg/L) vs. Time, Initial Addition 5.0 mL *A. ferrooxidans* , with pH 2 Water
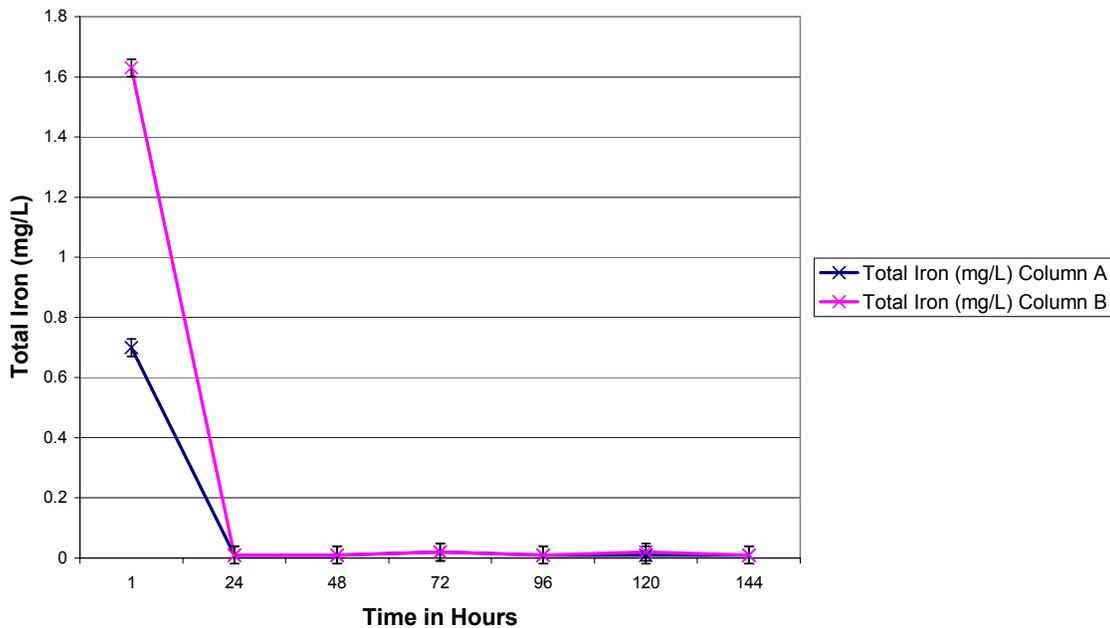

Figure 7. Total Iron (mg/L) vs. time for the experiment with 5.0mL *A. ferrooxidans* and water with a pH value of 2. The initial measurement of total iron is high and subsequent measurements are low. σ for the columns is +/- 0.029 mg/L total iron.

A comparison of dissolution rates shows that the addition of ferric iron increased

the pyrite dissolution rates the most (See figure 8). This fits with the literature

information which states that ferric iron drives pyrite oxidation reactions (equation 2).

As stated before, the problem with chemolithoautotrophs such as *A. ferrooxidans* is the

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production of ferric iron which in turn drives the weathering of pyrite increasing the acidity of the environment.

Dissolution Rates of Pyrite, Comparison of Ultra Pure Water, Addition of ~5.0 g/L Ferric Iron, and Addition of 5.0mL *A. ferrooxidans*
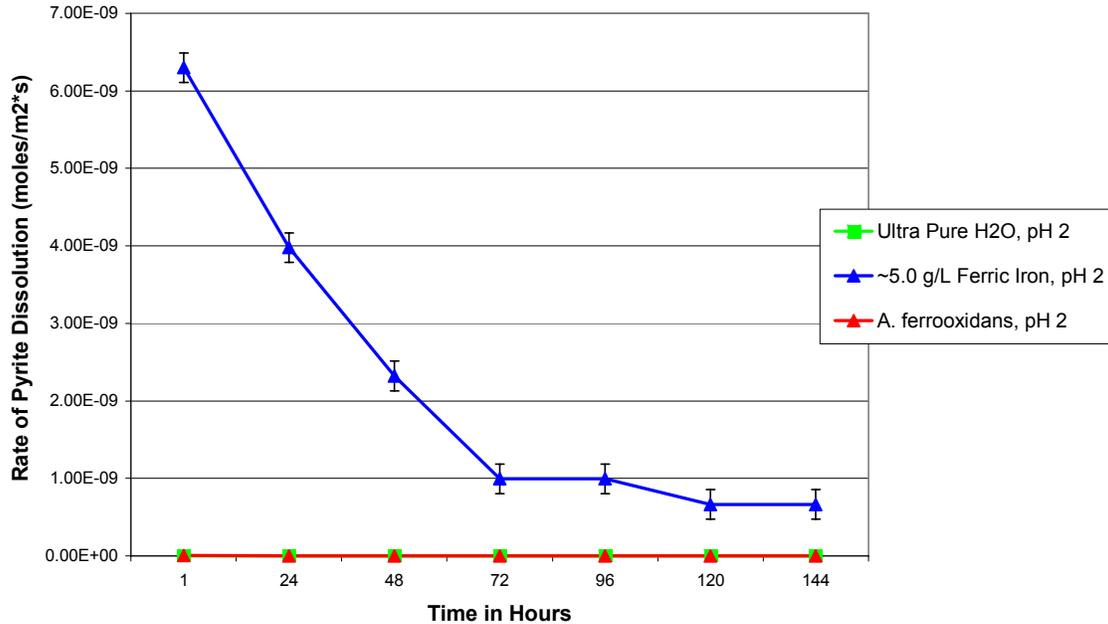

Figure 8. A comparison of the dissolution rates of pyrite and water at a pH value of 2 (green); ~5.0g'L ferric iron , pyrite, and water at a pH value of 2 (blue); and *A.ferrooxidans,* pyrite, and water at a pH value of 2. The ferric iron had the highest rates of dissolution.

By removing the ferric iron experiment from the graph, a closer look can be taken at the differences in dissolution rates between the bacteria experiment and the pyrite and oxygen experiment (see figure 9). The dissolution rate for the experiment with *A. ferrooxidans* begins seven times higher than the rate of the experiment with water and air. This matches the literature rates which cite dissolution of pyrite with bacteria 3 to 100 times higher (Edwards et al., 1998). The marked drop in the dissolution rate after the first twenty four hours supports the hypothesis that the bacteria unintentionally exited the experiment vessel.

Overall, the experiments showed that an addition of ferric iron or of *A. ferrooxidans* both increase the rate of pyrite dissolution. With the experiments conducted, the pyrite was most reactive in the first twenty four hours. Because of the fresh input solution, these reactions happen more quickly than those in batch reactors.

Pyrite Dissolution Rates vs. Time, *A. ferrooxidans* Compared to Ultra Pure Water
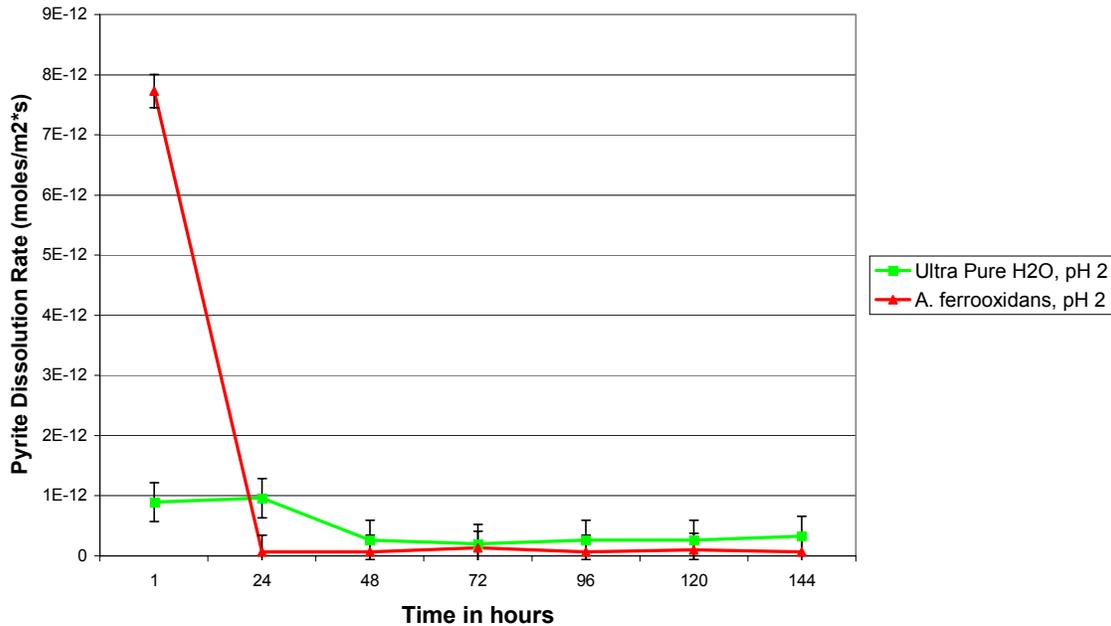

Figure 9. Dissolution rates of pyrite and air in water with a pH value of 2 (green) compared to dissolution rates of pyrite with bacteria in water at a pH value of 2 (red). The dissolution rates of the bacteria experiment drop after 24 hours. The rates then drop below those of pyrite in air, supporting the hypothesis that the bacteria must have escaped from the reaction vessel shortly after the experiment was started. The rates of dissolution when the bacteria are present are seven times greater than the rates of pyrite dissolution in water and air alone.

Multiple, unforeseen problems were encountered with the growth of the *A. ferrooxidans*. The media for the bacteria, ATCC 2039 broth, had a tendency to precipitate minerals out of solution. Multiple batches of media incorporating different brands of chemicals were prepared to remediate the precipitation problem. The *A.*

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ferrooxidans have a very slow growth period making it difficult to detect growth until approximately two weeks after culturing. They also precipitate an insoluble iron hydroxide as they grow which is difficult to distinguish from the ATCC 2039 media precipitate. Due to the complexities listed above, the initial culture sent from ATCC did not grow. The replacement culture, which arrived two weeks later, leaked en route to College Park and may have been contaminated. Successful growth of the bacteria was achieved from the second replacement which arrived five weeks after the first culture.

FUTURE EXPERIMENTS

Based on my experience, several adjustments to the procedures would dramatically improve the success of future experiments. A filter needs to be added into the experiment design which would prevent the bacteria from escaping from the pyrite column. A variation on this could incorporate the addition of bacteria to dialysis tubing. Dialysis tubing is a porous membrane that would allow proteins across however the bacteria would not be able to cross the membrane. The bacteria in the dialysis tubing would be wrapped around the pyrite and all of this would be placed into a reaction vessel. This could have very interesting results including being able to detect the presence of proteins as the bacteria adhere to the pyrite through the dialysis membrane. Also, future work might include finding the total iron content of the output solution with input water at a pH value of 7 to see how well total iron can be an indicator of dissolution rates. More parameters can be also added to the experiment design such as varying the air input, and/ or using a larger column to allow for more mixing of pyrite and water. Increasing the ferric iron source with bacteria in the column would allow one to see an

additional effect on the total iron rate not seen in this research. Furthermore, this type of experiment would more closely mimic the acid mine environment. Coal can also be added to the pyrite to see an additional affect on dissolution rates. Later experiments might be conducted using actual source material from a mine site.

Another complement to these experiments would be to use larger cubes of pyrite and classify the pyrite faces by making schematic drawings of the cubes. These cubes would be placed into a similar experiment and subjected to the same set of flow-through experimental parameters. The pyrite cubes could then be looked at in an Environmental Scanning Electron Microscope using gold or another soft metal to mount the cubes of pyrite. This technology would allow us to see the size and shape of the dissolution pits in the pyrite and provide a very nice complement to the column experimental data.   Using a soft metal like gold over epoxy thick sections of pyrite would allow one to look at the pyrite before and after the experiment without worrying about the effects of the acidic waters on the epoxy and the chemicals that might be released from the degradation of the epoxy in acidic waters.

SUMMARY & CONCLUSIONS

The four experiments conducted by the researcher mimicked the acid mine drainage experiment by incorporating low pH water, additional ferric iron and an acidophile, *A. ferrooxidans*. The initial experiment using water with a pH value of 7 provided an opportunity to perfect the experiment design. Dissolution rates for this experiment agreed within an order of magnitude to rates in the literature (Ehlrich, 2000). The first variation on this experiment involved water with a pH value of 2 and yielded

slower dissolution rates. These rates were calculated by measuring the total iron in the

output solution using equation 5. This experiment acted as a control, as pyrite dissolution

rates at low pHs have not been previously measured. In the second variation of the

experiment, ferric iron was added in concentrations of ~5.0 g/L. This simulated the high

concentration of ferric iron produced by bacteria in acid mine environments. Pyrite

dissolution rates increased by three orders of magnitude compared to the control

experiment. In the final experiment, *A. ferrooxidans*, a bacteria common to acid mine

environments, was added. Dissolution rates of pyrite with the bacteria in the reaction

vessel were seven times higher than the control experiment. However, these dissolution

rates dropped below the rates of the control after twenty four hours, indicating the

bacteria probably exited the reaction vessel. These rates and rate comparisons between

the three experiments provide new data to the scientific community. The experiment

design closely mimics the environment in acid mine drainage areas. Use of a classified

bacterium, *A. ferrooxidans*, allows future research to build upon the experiments

discussed above.

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APPENDIX

"I pledge on my honor that I have not given or received any unauthorized assistance on this assignment/examination."

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