Bioremediation by Sulfate Reducing Bacteria of Acid Mine Drainage

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Abstract

Mining activity produces metal sulfide wastes - particularly pyrite - which remain in the mine long after operations cease. When water percolates through a mine where oxygen is present, a series of chemical reactions occur which produce extremely low pH and high concentrations of toxic sulfate and metal ions. This toxic leachate can cause severe aquatic habitat degradation downstream of the mine. This study addresses this environmental problem by harnessing the metabolism of sulfate reducing bacteria, whose ability to reduce sulfate produces carbonate which neutralizes acids and sulfide, which chemically stabilizes toxic metal ions as solid metal sulfides. Batch reactors were set up with mine leachate, bacterial culture, a growth medium, and various sources of organic carbon. Results have shown that bacterial reactions caused copper and zinc reductions of 100%, pH increases of up to 2, and decreases in toxicity of 100%.

Introduction

Acid mine drainage (AMD), a major environmental hazard that affects aquatic ecosystems around the world, results from the oxidation of metal sulfides, particularly pyrite (FeS$_2$). Discharges from abandoned sulfide ore mines (the most commonly mined sulfides being sulfur, copper, zinc, lead, gold, silver, and uranium) often contain high concentrations of metal sulfides. In lotic environments such as streams, these metal sulfides become rapidly oxidized though chemical and microbial processes (Gray 1998, Bonnissel-Gissinger *et al.* 1998, Evangelou and Zhang 1995). The resulting sulfate and hydrogen ions lower pH significantly as well as add toxic metal ions to the environment (Gray 1998). Acid mine drainage causes the degradation of aquatic systems through acidification, high concentrations of iron and sulfate, and elevated levels of soluble toxic metals. Under the acidic conditions resulting from AMD, the oxidation of pyrite proceeds by the following reaction (Bonnissel-Gissinger *et al.* 1998):

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$
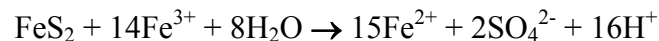
This reaction demonstrates the polluting capability of the oxidation of pyrite – every mole of pyrite becomes converted to 16 moles of hydrogen and 2 moles of sulfate. This reaction serves as a template for the similar oxidation reactions of most metal sulfides, which also contribute acidity, sulfate, and toxic metal ions to the aquatic environment.

Once oxidation has occurred, its products damage the ecosystem in a number of ways. First, the ferric precipitate common to AMD destroys vegetation by blanketing the soil layer and clogging the substrate interstices (Gray 1998). Evidence has also shown that AMD has been responsible for marked declines in aquatic species and ecosystem diversity, as well as productivity. And the toxicity of extreme concentrations of heavy metals and acidity has been linked to total elimination of certain fish species in some aquatic ecosystems (Gray 1998).

This study investigated bioremediation as a solution to AMD. In past experimentation, the most common method of combating AMD has been the construction of a treatment wetland downstream from the mine (Machemer *et al.* 1993; Mitsch and Wise 1997). Such wetlands take up the polluted water, and through microbial processes similar to those used in this experiment purify the water. The wetlands further remove metals through uptake into plant tissues. The drawback of this method is that the problem is not dealt with at the source,

but rather after the polluted water has already had an environmental effect. The goal of this project was to determine if sulfate reducing bacteria can be used *inside* the mine itself such that the leachate is not toxic.

Prior research has shown that bacterial metabolism can be of significant use in the removal of metals from wastewater (Dvorak *et al.* 1992). Sulfate reducing bacteria oxidize simple organic molecules using the sulfate ion as an electron acceptor. This process produces hydrogen sulfide(H_2S) and the bicarbonate ion(HCO_3^-). Hydrogen sulfide readily reacts with heavy metal ions to immobilize the metals as insoluble metal sulfides, while the bicarbonate ions buffer the pH to significantly higher levels (Dvorak *et al.* 1992). Thus, sulfate is removed as hydrogen sulfide gas and immobile metal sulfides, metals are removed as metal sulfides, and pH is raised, improving water quality. In order to maintain bacterial metabolism, the bacteria must be given both an organic carbon source (as food) and some growth substrate for attachment (the bacteria cannot survive in open water).

AMD from an abandoned mine site was treated in batch reactors with an organic carbon source, a growth substrate, and a culture of sulfate reducing bacteria. The mine site lies in the hills east of Oakland, California. Streams originating in the watershed currently show strongly visible signs of habitat degradation such as vegetation loss, low pH, and coating of riparian substrate with oxidized ferric precipitate. It is hypothesized that the bacterial reactions described above will improve this severely toxic water to quality levels similar to unpolluted streams.

Methods

Study Site All AMD water samples were taken from the Leona Heights Mine, an abandoned sulfur mine in the hills east of Oakland, California. The small creek running out of the mine showed strong visible signs of metal pollution including the characteristic brown-orange coloration of iron precipitate. In addition, toxic levels of several metals and sulfate were found in the creek. Both this creek and the terminal conduit of the watershed, located at the inlet to Lake Aliso several miles downstream in Oakland have been found to be toxic to *Ceriodaphnia dubia*, a zooplankton species commonly used in aquatic metal toxicity testing.

Bacterial Remediation Experiment This study cultured sulfate reducing bacteria in the presence of AMD, an organic carbon source, a substrate on which to grow, and conditions

otherwise similar to those inside a mine shaft. The goal was a determination of whether SRBs can have a significant effect of reducing concentrations and toxicity of metal and sulfate ions while raising pH in water sampled from abandoned mine sites.

The bacterial remediation experiment consisted of filling three one-liter sample bottles for each treatment with AMD water from a polluted mine site, a carbon source, a bacterial culture, and dried cattails as a growth substrate. The three experimental treatments contained varied organic carbon sources for the bacterial reaction as listed in Table 1. The control reactors contained only AMD water and cattails.

Name of Treatment	Bacteria	Carbon Source	Growth Medium
Control	NO	NONE	Dead Cattail
PCI	YES	Pine Needles	Dead Cattail
OCI	YES	Oak Leaves	Dead Cattail
ECI	YES	Eucalyptus Leaves	Dead Cattail

Table 1. Bacterial Remediation Experiment, Site 1, Reactor Treatments. Only the three treatments PCI, MCI, and SCI include all ingredients necessary for bacterial metabolism – growth substrate and carbon source.

The dead cattail clippings were added as an intended substrate for the bacteria to grow on, while the pine needles, oak leaves, and eucalyptus leaves were intended as organic carbon sources for the bacteria. Before use in the reactors, the dead cattails were soaked in water for two weeks in order to leech out as much carbon as possible, thereby making their contribution as a carbon source negligible.

The reactors were stored in covered, insulated boxes to keep temperature fluctuation to a minimum and to keep out light. This helped to approximate actual mine shaft conditions. Every week, 5mLs were removed from the sample bottles for analysis. The sample bottles were analyzed for sulfate, iron, zinc, and copper concentrations on a weekly basis. In addition, pH was measured on a biweekly basis. Sulfate measurements were taken with an Ion Chromatograph (Dionex DX 100) with a pulse electrochemical detector using a Dionex Ionpac AS4A-SC column (detection limit~0.1ppm). Metal analysis was performed by Flame Atomic Absorption Spectroscopy (detection limit~1ppm), and pH measurements were taken

with a digital pH meter. After every sampling, reactors were purged of any oxygen with nitrogen gas in order that anoxic conditions typical of an actual mine shaft be maintained.

Two months into the experiment, approximately four grams of carbon source were added to two of the three batch reactor replicates for each treatment.

Toxicity Testing Toxicity tests were performed on *Ceriodaphnia dubia*, a species of zooplankton classically used for its intolerance to many aqueous heavy metals (Nimmo *et al.* 1990, Ribeiro, *et al.* 2000). Tests were performed according to standard EPA protocol (USEPA 1993). Ten replicates were used for each water sample. Table 2 summarizes each treatment.

Sample 1	*C. dubia* culture water
Sample 2	Control Batch Reactor water (diluted 1:10 w/Horseshoe Creek)
Sample 3	Eucalyptus-remediated water (diluted 1:10 w/Horseshoe Creek)
Sample 4	Pine-remediated water (diluted 1:10 w/Horseshoe Creek)
Sample 5	Oak-remediated water (diluted 1:10 w/Horseshoe Creek)
Sample 6	Horseshoe Creek water

Table 2. Toxicity Testing Experiment Water Samples

Each sample replicate contained one individual *C. dubia* and was investigated after forty-eight hours for mortality and after every 24 hours thereafter for five days for number of new live births of *C. dubia* young. In treatments including water from the batch reactors, a 1:10 dilution of sample water to Horseshoe Creek water was used for the toxicity test. Horseshoe Creek is an unpolluted stream in the same watershed as the Leona Heights mine. From flowrate measurements throughout the watershed, it was determined that the impacted creek directly below the mine is diluted 1:10 when it reaches the watershed terminus at Lake Aliso. This realistic dilution level was used for the toxicity tests.

Acute toxicity was determined by mortality rates of *C. dubia* after the first 48 hours of incubation. Chronic toxicity was determined through the number of births of *C. dubia* juveniles per day over the five day period following the acute test.

Results

Bacterial Remediation Experiment   The data from the Oakland mine site shows a general downward trend in copper concentrations for averages over the three replicates for the three experimental reactors (pine needles, eucalyptus, and oak). In all three cases, copper concentrations were brought to below detectable limits within a four week period (Figure 1). The control reactors showed a slight rise over the course of the experiment.
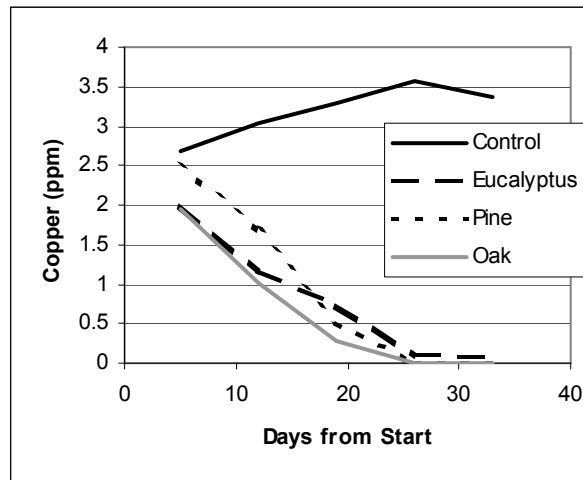

Figure 1. Copper concentration with time in batch reactors. Detection limit for analysis is ~0.1ppm. Values below equipment detection limit are plotted as zero.

Zinc measurements over the course of the experiment did not fall in all cases as occurred with copper concentrations. The experimental reactors containing oak leaves as the carbon source show a sharp decline in zinc concentration in the fourth week after the start of the experiment (Figure 2). From that point on, zinc levels were below detection limits. The reactors with pine needles as a carbon source showed no sign of zinc sulfide precipitation until the addition of carbon to the reactors 73 days into the experiment. After that carbon addition, the pine needle reactors showed a decrease in zinc concentration all the way to below detectable limits. The eucalyptus reactors showed no decline in zinc concentration throughout the experiment, even after the second carbon addition.
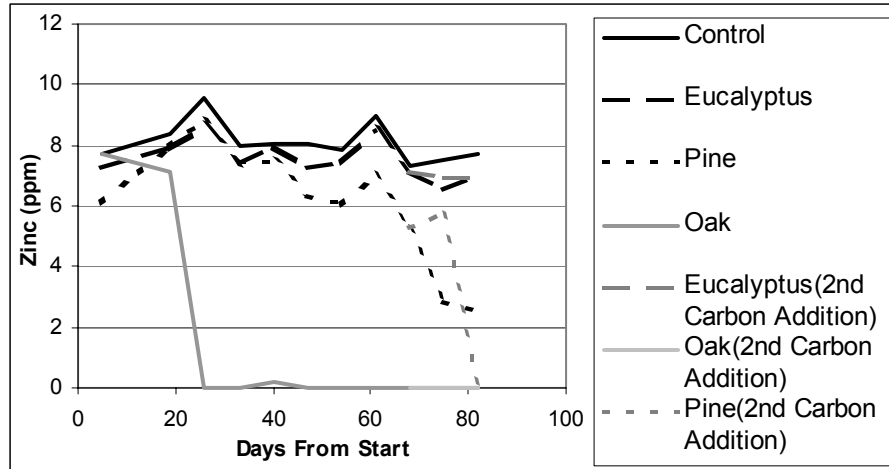
Figure 2. Zinc concentration with time in batch reactors. Detection limit for analysis is ~0.1ppm. Values below equipment detection limit are plotted as zero. Note that data in reactors after 2nd carbon addition are designated by additional lines in grey.

Iron concentrations in the batch reactors behaved markedly different than zinc or copper concentrations. Throughout the experiment, the control reactors exhibited a slight decline in iron concentration while concentrations in all experimental reactors remained relatively constant (Figure 3).
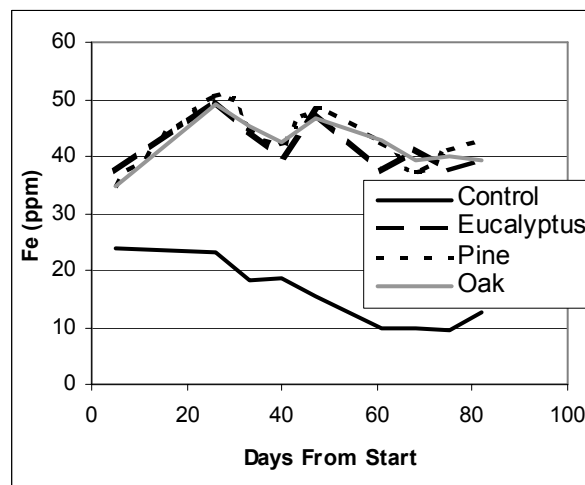

Figure 3. Iron concentration with time in batch reactors. Detection limit for analysis is ~0.1ppm. Values below equipment detection limit are plotted as zero.

Sulfate concentrations in the batch reactors varied little over the course of the experiment. Figure 4 displays sulfate concentration in all treatments for each week of batch reactor sampling. Very little deviation from the control reactors was observed.
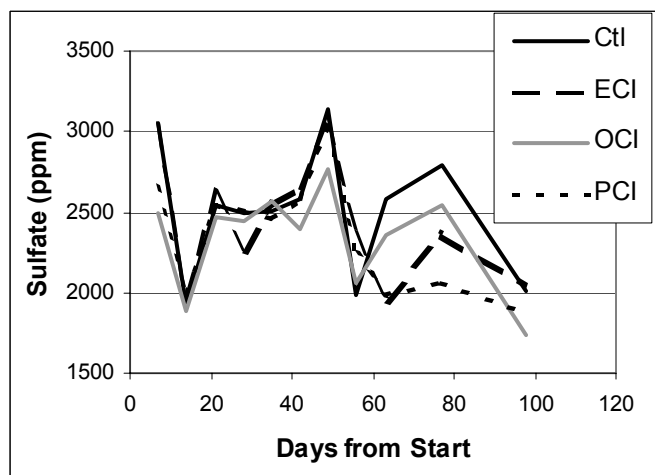
Figure 4. Sulfate concentration in batch reactors. Instrument variation from sampling to sampling is believed to have caused the significant variation common to all treatments.

Finally, pH levels in all experimental reactors rose from an initial value of 2.8 over the course of the experiment while the control reactors remained constant. In the case of the oak reactors, which showed the highest increase, pH levels of up to 4.6 were observed at the end of experimentation.
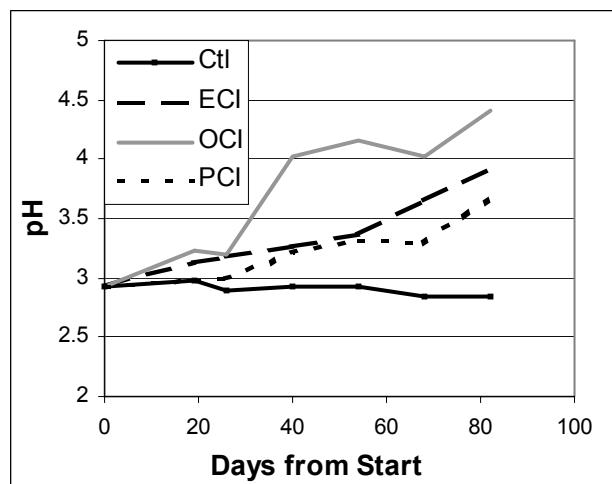

Figure 5. pH in batch reactors. Carbonate produced by bacterial metabolism acts as a buffer which neutralizes acid generated by chemical processes within the mine.

Toxicity Tests In acute toxicity tests, the unremediated control water (diluted 1:10 with unpolluted Horsehoe Creek water) was toxic in all replicates. A 100% mortality rate

was observed in all cases. In the case of all remediated water samples (diluted 1:10 with unpolluted Horsehoe Creek water), the zooplankton culture water, and Horseshoe Creek water, no *C. dubia* deaths were recorded during the acute time period. During the chronic testing, the oak water samples were not found to be toxic to *C. dubia* reproduction and showed a statistically similar number of births per day to the unpolluted Horseshoe Creek water and zooplankton culture water. The pine-remediated water samples were not wholly toxic to *C. dubia* reproduction, however these showed statistically fewer live births than the unpolluted samples. The eucalyptus water samples showed very few live births and appeared to be toxic to *C. dubia* reproduction.

The results of both chronic and acute testing are summarized in Figure 6.
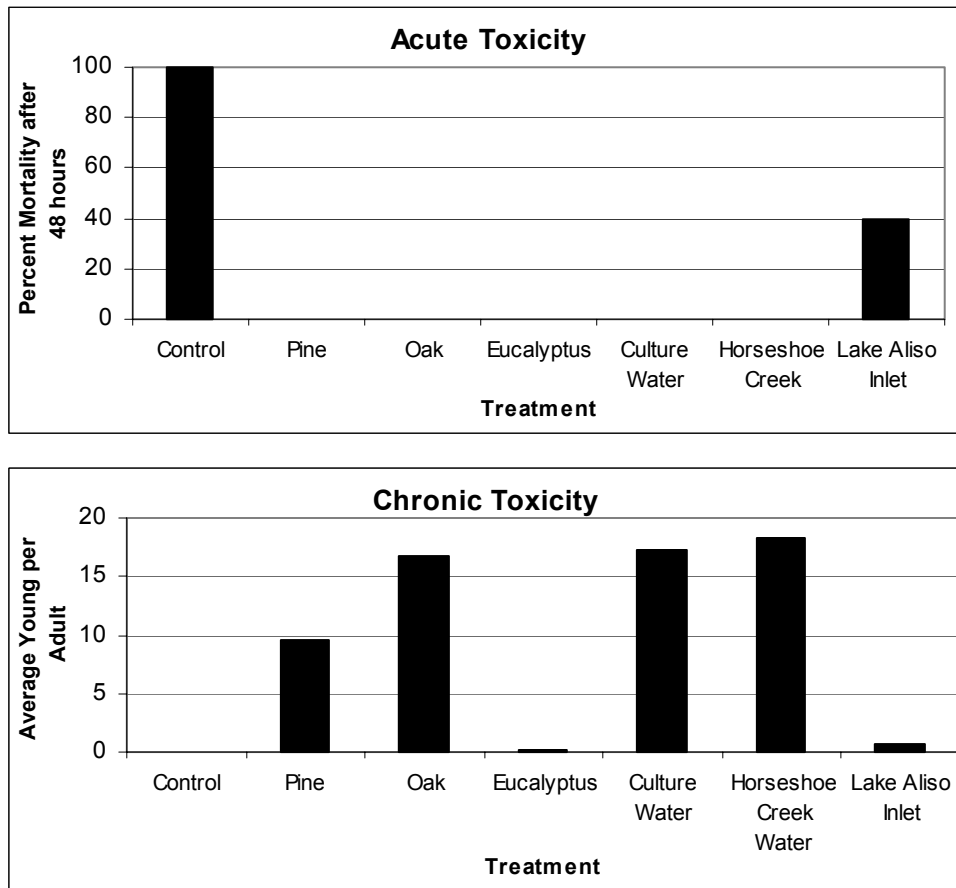

Figure 6. Toxicity testing results. Zooplankton culture water, Horseshoe Creek water, and the oak batch reactor water are statistically similar while the pine batch reactor water is statistically more toxic than oak.

Discussion

Bacterial Remediation Experiment   The results of this experiment show that SRB, when given proper nutrients and growth substrate, can help remediate Acid Mine Drainage. SRB have the ability to simultaneously reduce toxic metal concentrations and raise acidic pH.   The removal of copper to below detection limits in all reactors (Figure 1) can be attributed to the precipitation of copper sulfide (CuS) out of solution.   Sulfide produced by bacterial metabolism spontaneously bonds with available metal ions in solution; however, which metals bond first is determined by the solubility product (K_{sp}) of the metal sulfide precipitate.   Of the three metals most commonly found in AMD – iron, zinc, and copper – copper has by far the lowest sulfide solubility product.   Copper levels in the reactors thus dropped first, and iron, whose sulfide has by far the largest solubility product, showed no significant reduction in the time frame dictated by the experiment.

Zinc sulfide, which has a slightly lower solubility product than copper sulfide precipitated out of solution in the pine and oak batch reactors after the precipitation of copper sulfide.   The zinc dropout in the pine reactors after the second carbon addition indicates that the reactors may have reached a stagnation point due to carbon limitation.   Therefore, the addition of more carbon seems to have spurred more sulfide production which led to ZnS precipitation and subsequent removal of soluble zinc.

Iron levels in the control reactors show a decrease over the course of the experiment, while remaining level in the experimental reactors.   This can most likely be attributed to dissolved oxygen levels in the different reactors.   In the controls, where the reactors were not kept anoxic by bacterial activity, iron oxide formed which precipitated out of solution, causing a decrease in soluble iron.   Iron oxide readily forms in the presence oxygen, while copper oxide and zinc oxide do not, resulting in a decrease in iron without a decrease in either of the other metals.   In the experimental reactors, no oxygen was present - because the reducing conditions caused by the bacterial metabolism use up any available oxygen - thereby preventing iron oxide precipitation and possibly causing the dissolution of any iron oxide which might have been present in the water samples.

Sulfate data for the bioremediation experiment shows no real variation from the controls throughout the experiment.   Equipment variation from week to week might have caused minor changes in sulfate concentration throughout the experiment, but because the

concentration of sulfate in the reactors was several orders of magnitude higher than that of the metals, a large reduction in sulfate concentrations would not be expected through precipitation alone. And although some sulfate was removed by reduction to H_2S, carbon was the limiting reactant in the SRB metabolism and therefore sulfate could never be completely removed.

Finally, the pH levels recorded during the study indicate that SRB metabolism can cause a rise in pH. Acidity was reduced in all experimental reactors due to the production of the buffering bicarbonate ion from bacterial metabolism. In the oak reactors, which performed better than any of the other carbon sources, pH was raised close to 2 points.

Toxicity Testing The results of the toxicity tests prove the remediating effect of SRB metabolism. Even when diluted ten times in order to approximate conditions well downstream of the mine, 100% zooplankton mortality was observed in the unremediated control water, while in the case of the oak batch reactors, no acute or chronic toxicity was observed at all. These results are the best indication that SRB can drastically improve the quality of AMD polluted water

Conclusions

The results of this study have shown that SRB have the potential for remediation of AMD when the proper nutrients and growth substrate are provided. Copper levels were reduced to below detection limits in all cases. Zinc levels were completely reduced in two of the three treatments. While iron was not reduced, the toxicity testing results indicate that even without reduced iron levels, the remediated water far less toxic than the unremediated water.

Further research, which might include a more large scale model of a potential remediation system, is needed to determine whether this technique of AMD remediation is feasible. While there are many positive results from SRB metabolism, there are negative consequences as well. The reduction of sulfate produces hydrogen sulfide (H_2S) which results in a very pungent odor. Any system used for AMD cleanup would have to take into account the public nuisance that this odor might cause. In addition, as shown by the fact that carbon limitation may have occurred in the experimental batch reactors, the bacteria need a constant supply of organic carbon in order to keep the metabolism going. Therefore, constant carbon additions would have to be a part of any large scale system. Nevertheless,

this approach indicates a possible solution to AMD which is far cheaper to operate than a conventional wastewater treatment plant.

Acknowledgments

References

Bonnissel-Gissinger, Pascale, Marc Alnot, Jean-Jacques Ehrhardt, and Philippe Behra. 1998. Surface Oxidation of Pyrite as a function of pH. *Environmental Science and Technology* **32**:2839-2845.

Dvorak, D. H., R. S. Hedin, H. M. Edenborn, and P. E. McIntire. 1992. Treatment of metal-conaminated water using bacterial sulfate reduction: Results from pilot scale reactors. *Biotechnol. Bioeng.* **40**:609-616.

Evangelou, V. P. and Y. L. Zhang. 1995. A Review: Pyrite Oxidation Mechanisms and Acid Mine Drainage Prevention. *Critical Reviews in Environmental Science and Technology* **25**(2):141-199.

Gray, N. F. 1998. Acid mine drainage composition and the implications for its impact on lotic systems. *Water Research* **32**(7):2122-2134.

Machemer, Steven D., Julia S. Reynolds, Leslie S. Laudon, and Thomas R. Wildeman. 1993. Balance of S in a constructed wetland built to treat acid min drainage, Idaho Springs, Colorado, U.S.A. *Applied Geochemistry* **8**:587-603.

Mitsch, William J., and Karen M. Wise. 1997. Water quality, fate of metals, and predictive model validation of a constructed wetland treating acid mine drainage. *Water Research* **32**(6):1888-1900.

Nimmo, D. R., M. H. Dodson, P. H. Davies, J. C. Greene, M. A. Kerr. 1990. Three studies using *Ceriodaphina* to detect nonpoint sources of metals from mine drainage. *Research Journal WPCF* **62**(1).

Ribeiro, R, I. Lopes, A. M. M. Pereira, F. Goncalves, A. M. V. M. Socies. 2000. *Bull. Environ. Contam. Toxicol* **64:**130-136.

United States Environmental Protection Agency. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. USEPA, Environmental Monitoring Systems Laboratory, Cincinatti, Ohio.