THE USE OF BACTERIAL SULFATE REDUCTION IN THE TREATMENT OF DRAINAGE FROM COAL MINES[1]

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Abstract. Bacterial sulfate reduction is a naturally-occurring process that proceeds only in the absence of oxygen and in the presence of sufficient organic carbon and sulfate. The net by-products of sulfate reduction, hydrogen sulfide and bicarbonate, can precipitate metal sulfides and neutralize acidic water, respectively. An experimental wetland was designed and built by the Bureau of Mines to maximize contact between mine drainage and the anaerobic zone of the organic substrate, where sulfate reduction takes place. Preliminary results from the wetland were encouraging. Iron concentrations decreased from 237 to 27 ppm, pH increased from 2.9 to 6.5, and alkalinity increased from 0 to 1077 ppm when the mine drainage was forced through the anaerobic zone. The observed improvement in water quality was due to both the inherent chemical characteristics of the organic substrate as well as bacterial activity. Bacterial sulfate reduction rates in the organic substrate ranged from 2 to 600 nmol cm^{-3} day^{-1}. Sulfate reduction rates are high enough to significantly affect the water quality of acid mine drainage. The sulfate-reducing bacteria effectively precipitate many heavy metals as insoluble sulfides and may also be useful in treatment processes designed to improve the water quality of metal mine drainage.

Introduction

At over 300 mine sites in the bituminous coal region of the eastern United States, acid mine drainage (AMD) is being treated biologically in constructed wetlands (Hedin 1989). In general, the processes at work in these systems are aerobic. The oxidation of ferrous iron to ferric iron and the subsequent precipitation of iron oxyhydroxide floc, for example, are dominant processes:

$$Fe^{2+} + 0.25O_2 + 1.5H_2O \dashrightarrow FeOOH\ (solid) + 2H^+ \qquad (1)$$

Ferrous iron tends to autoxidize in aerated solutions at pH values greater than 6, while in more acidic water naturally-occurring bacteria catalyze the reaction (Singer and Stumm 1970). Although iron oxidation and hydrolysis processes are effective at removing much of the iron from AMD, these processes do nothing to help raise the pH of the water or decrease the acidity. In fact, the pH of water can be lowered by these reactions (Equation 1). Many constructed wetlands with circumneutral pH and iron-contaminated inflow water actually produce water with a lower pH (Brodie et al. 1988; Kepler 1990).

Ironically, bacterial processes capable of increasing the pH and alkalinity of AMD entering constructed wetlands are already found there, but current wetland designs do not take advantage of them. Probably the most useful of these processes for treating AMD is bacterial sulfate reduction, a naturally-occurring process that proceeds in many environments in the absence of oxygen and in the presence of suitable organic substrates and sulfate. Sulfate-reducing bacteria use organic carbon and sulfate in the process of anaerobic respiration:

$$2CH_2O + SO_4^{2-} \dashrightarrow H_2S + 2HCO_3^- \qquad (2)$$

The reaction has promise in the treatment of acid and metal-contaminated mine waters because the by-products of the reaction, hydrogen sulfide and bicarbonate, can be used to precipitate many metals and raise the pH of the water, respectively. When AMD percolates into stream bottoms or constructed wetlands containing abundant organic matter and debris, bacterial sulfate reduction can proceed. Wetlands constructed to treat AMD are generally built to include a large amount of organic matter, and anaerobic conditions develop rapidly within

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[1] Paper presented at the 1990 Mining and Reclamation Conference and Exhibition, Charleston, West Virginia, April 23-26, 1990.

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Proceedings America Society of Mining and Reclamation, 1990 pp 409-416

DOI: 10.21000/JASMR90020409

https://doi.org/10.21000/JASMR90010409

that material. Calculations using data from natural wetlands and constructed wetlands have suggested that the use of a sulfate reduction treatment system is theoretically feasible (Hedin, Hammack, and Hyman 1989). In this study, we present data collected from a constructed wetland designed to incorporate bacterial sulfate reduction as a treatment process.

Materials and Methods

An experimental wetland was constructed at the Friendship Hill National Historic Site, located in Fayette County, PA. The inflow to the wetland comes from a stream severely polluted with water draining from an abandoned drift mine 1 km upstream of the wetland. The inflow water has a pH of about 2.5, total iron concentration of 50 to 250 ppm, and a sulfate concentration of 1000 to 2500 ppm.

The constructed wetland was built in the summer of 1988 and was divided into three cells (Fig. 1a). The first cell is a holding pond that does not treat the AMD. The remaining two cells are each divided into three different treatment lanes separated by Fiberglas sheeting. In cell two, the AMD flows across the surface of the organic substrate, but in cell three, subsurface infusion pipes can be used to carry AMD down to the bottom of the organic substrate layer (Fig. 1b). Individual ball valve controls are used to adjust AMD water flow into each lane.

The six treatment lanes were each constructed with a 15 cm (6 in) layer of gravel on the bottom, covered by a 46 cm (18 in) layer of spent mushroom compost obtained from a mushroom farm near Worthington, PA. The basal gravel layers in the cells consisted of crushed limestone in lanes B2, B3, C2, and C3, and noncalcareous river gravel in lanes A2 and A3. Whole cattail plants were transplanted to all cells and the compost was flooded with 8 to 20 cm (3-8 in) of AMD water.

Surface water samples were collected from nine locations in the wetland biweekly for eight months. Measurements of pH in the field were made using an Orion SA230 portable pH meter. Alkalinity (as $CaCO_3$ equivalents) was determined by titrating a 50-ml sample with 0.2 N H_2SO_4 to pH 4.8. Acidity was measured on a 50-ml sample that was first boiled with 1 ml of 30% H_2O_2 and then titrated with 0.1 N NaOH to pH 8.3. Total iron concentrations were determined for acidified water samples using an ICP atomic emission spectrophotometer. Sulfate concentrations were determined by barium chloride titration, using thorin as the end point indicator.

Platinum electrode potentials in organic substrate cores taken from the wetland were measured within 20 minutes of core removal. Measurements were made at 4 cm intervals throughout each core. A platinum wire was inserted through a silicone plug located at each sampling interval and a reference electrode was suspended in the surface water overlying the substrate. The wire and electrode were attached to a voltage meter which was standardized using Zobell's solution (Eh = +430 mV) prior to each measurement series.

Sediment sulfate reduction rates and total reduced sulfur concentrations were determined using the method of Herlihy and Mills (1985), which is a modification of the method of Jorgensen (1978). Vertical cores of organic substrate from the wetland were transported to the laboratory within eight hours of their removal. Five to 8 ml subcores were taken from two depths in each core. The subcores were immediately injected with 50 uL of carrier-free ^{35}S-labeled Na_2SO_4 (5 mCi/mL; New England Nuclear). Subcores were incubated at in-situ temperature for 2 to 14 days. Incubation times were chosen to allow 5 to 50% of the labeled sulfate to be converted to sulfide. After incubation, the subcores were frozen and stored at -4° C prior to analysis. The amount of labeled sulfide formed during incubation was determined by acid distillation of the subcores and the collection of the released hydrogen sulfide in traps containing a solution of 10% NaOH. Total reduced sulfur concentrations reported in this paper consist of the sum of an acid volatile sulfide (AVS) fraction (hydrogen sulfide, bisulfide ion, and iron monosulfide) and a chromium reducible sulfur (CRS) fraction (elemental sulfur and pyrite). Sulfate reduction rates were determined as described by Jorgensen (1978).

Interstitial porewater samples were collected using equilibrators similar to those designed by Hesslein (1976). These equilibrators consist of thick Plexiglas stakes containing wells of approximately 30 ml volume spaced 2 cm apart along

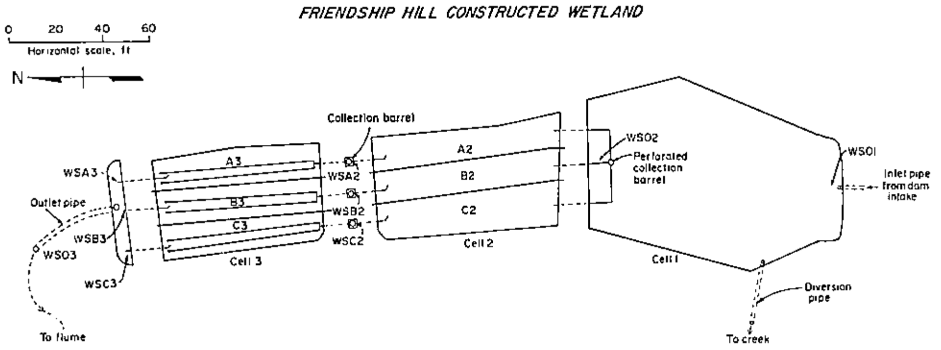

Figure 1a. Plan view of Friendship Hill constructed wetland.

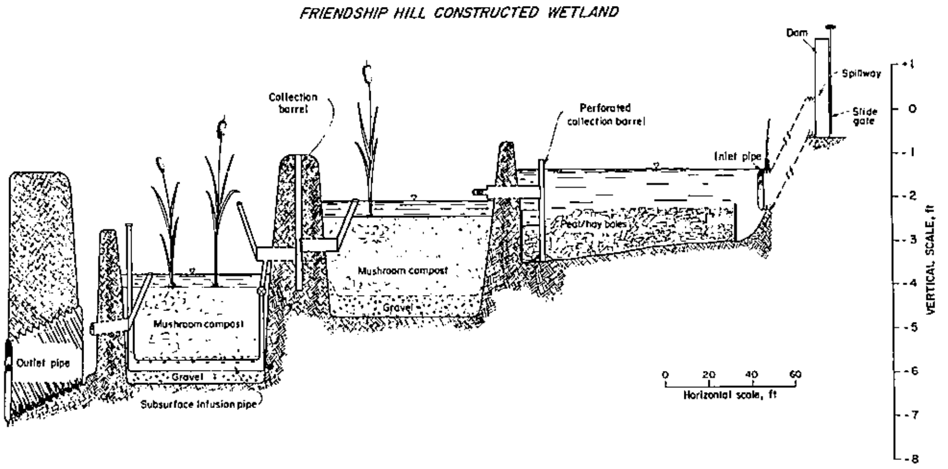

Figure 1b. Typical longitudinal cross-section of the Friendship Hill constructed wetland.

the length of the stake. The wells were filled with deionized and deoxygenated water and covered with a 0.2 um membrane filter sheet. The apparatus was pushed down into the organic substrate of the wetland and allowed to equilibrate with the interstitial water for three to four weeks. Ten ml samples were then extracted from each well and placed in vials containing 1 ml of 2 N zinc acetate. Sulfate concentrations in these samples were determined by liquid chromatography and were used in the calculation of sulfate reduction rates.

The percent volume of water in subcores used to measure sulfate reduction rates was determined by measuring the difference in weight of similar subcores before and after drying, and assuming the density of water to be equal to 1 g cm^3.

Laboratory experiments were also done to evaluate potential metal toxicity to sulfate-reducing bacteria. Metals were added as chloride salt solutions to Postgate's medium B for sulfate-reducing bacteria (Postgate 1984). Final metal concentrations ranged from 5 to 380 ppm. The media were inoculated with a mixed culture of sulfate-reducing bacteria isolated from mushroom compost. The bacteria were incubated at 30^o C for four weeks and the growth or lack of growth was noted. After four weeks, the media were filtered and metal concentrations in the supernatant were determined.

Results and Discussion

Experimental Wetland

Study of the Friendship Hill constructed wetland showed that increasing contact between AMD and the anaerobic zone of the organic substrate improved the quality of the water leaving the wetland. When subsurface infusion pipes were turned on and the AMD was forced down into the anaerobic substrate, an immediate increase in pH and alkalinity and a net removal of total iron were observed (Fig. 2, 3, and 4). During this time, the pH of the outflow water increased to as high as 6.5, the alkalinity increased up to 500 ppm, and the total iron concentration decreased from 200 to 20 ppm. In contrast, little improvement in water pH or alkalinity was observed when the AMD flowed predominantly across the surface of the organic substrate. When the subsurface infusion pipes were turned off, the observed improvements in water quality disappeared.

It is probable that much of the initially observed improvement in water quality was due to the chemical characteristics of the spent mushroom compost used to construct the wetland. The neutralization potential capacity of fresh mushroom compost is about 3.5% $CaCO_3$ equivalents (Hammack and Hedin 1989). If we consider that approximately 35 tons of compost were placed in each lane containing a subsurface drainage system, and we know that approximately 3.3 x 10^5 liters of AMD of 1000 mg L^{-1} acidity passed through the compost during 31 days of operation, approximately 30% of the total initial alkalinity of the compost would have been exhausted. If all of this alkalinity could be used to treat AMD before it left the wetland, chemical neutralization might be expected to continue for three months. However, this assumes that the water flows through the wetland in such a way that all compost is contacted equally and no portion of the compost is exhausted of its neutralization potential before any other. In reality, flow patterns are likely to occur that use up the neutralization potential of the compost along major flow paths and result in less effective neutralization of the AMD. In addition, bacterial sulfate reduction can be expected to contribute neutralization potential to the compost with time, adding to the total pool of alkalinity. The potential significance of this biological process is addressed later in this section.

The first time that underflow drains were turned on in the Friendship Hill wetland, there were large differences in the chemical composition of water flowing from the lanes containing either calcareous or noncalcareous gravel. The pH, alkalinity, and total iron concentration in the treated water were all improved in the subsurface flow lanes, but especially in those containing calcareous gravel (Fig. 2, 3, and 4). This suggests that dissolution of the limestone initially played a major role in improving the water quality. However, the second time the subsurface infusion

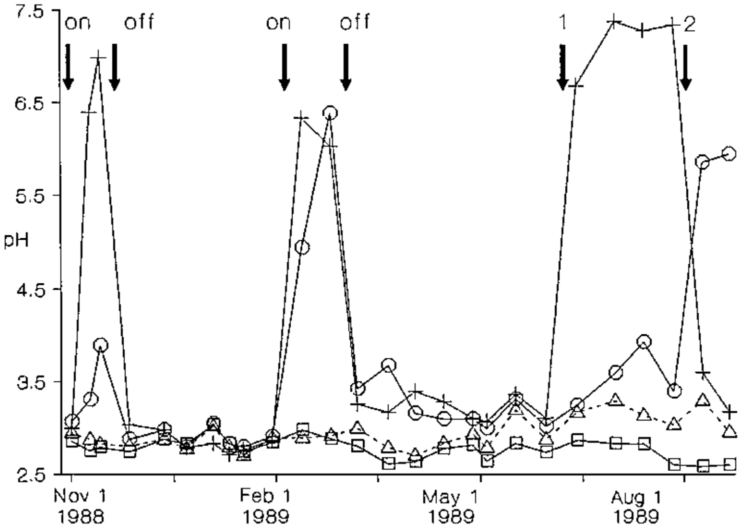

Figure 2. The pH of water in the Friendship Hill constructed wetland system during the study period. Symbols indicate: untreated water flowing into the wetland (open squares); water leaving subsurface flow only cell in wetland (open triangles); water leaving subsurface flow wetland cell containing noncalcareous gravel (open circles); water leaving subsurface flow wetland cell containing limestone gravel (plus signs). Arrows mark times when the subsurface system was turned on and off. Symbols "1" and "2" denote when underflow dams were installed in the calcareous and noncalcareous gravel lanes, respectively.

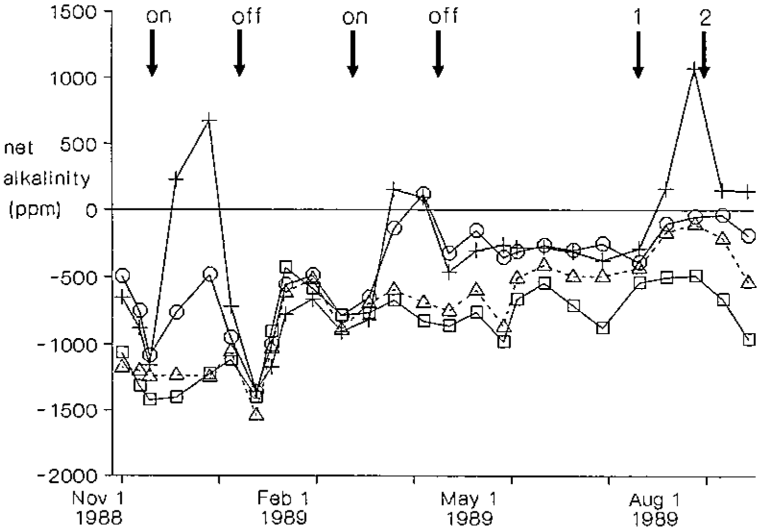

Figure 3. Alkalinity (as ppm CaCO3) of water in the Friendship Hill wetland system during the study period. Symbols are the same as described for Fig. 2. Acidity is considered negative alkalinity.

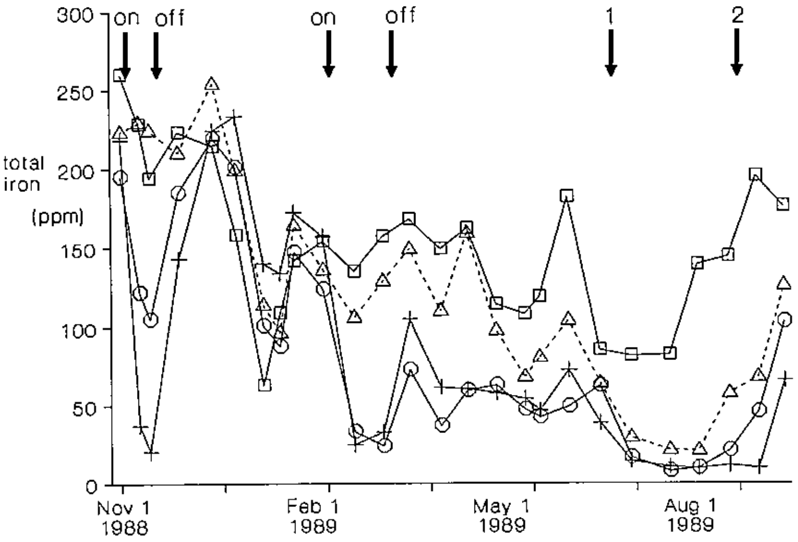

Figure 4. Total iron concentrations in water in the Friendship Hill wetland system during the study period. Symbols are the same as described for Fig. 2.

pipes were turned on, the noncalcareous gravel lanes were just as effective as those containing limestone gravel. Although it was hoped that the anaerobic environment established in the organic substrate of the wetland would prevent the armoring of the gravel with iron oxyhydroxides and/or other deposits, it appeared that this probably occurred anyway. This explanation seems reasonable considering that the surface water introduced to the gravel layers contained both oxygen and ferric iron. These data imply that there is no significant acid neutralization advantage in using limestone gravel in the construction of wetlands employing anaerobic systems. This is in agreement with the laboratory results of Hammack and Hedin (1989).

Soon after the subsurface infusion pipes were turned on for the second time at Friendship Hill, they became clogged with iron oxyhydroxide floc and organic debris. An alternative method of introducing AMD to the anaerobic regions of the wetland without the use of pipes was then devised. This method involved the installation of a sheet of Plexiglas in a test lane of the wetland, placed so that water in the lane was dammed behind the sheet and was forced to flow downward and beneath the dam. This is somewhat analogous to the hay bale dams found in some wetlands. Water downstream of these dams often has a higher pH (Hedin et al. 1988; Stark et al. 1988), although this is in part due to sulfate reduction activity occurring within the hay bales themselves. In the Plexiglas dam method used in the Friendship Hill wetland, the dammed water was forced to make contact with the basal gravel and anaerobic compost layers. Initially, water quality improvements were observed again (Fig. 2, 3, and 4); however, after a few weeks of operation the water quality was no longer affected positively by the dam system, presumably due to the exhaustion of alkalinity in the substrate and the disruption of the anaerobic microbial community.

Measurements of pH in the porewater of the organic substrate at the Friendship Hill wetland showed that the pH generally remained between 6.0 and 7.0, even though the surface water usually had a pH of 2.5 to 3.5. This high porewater pH most likely reflects the influence of alkaline substances present in the compost as well as those produced by the natural bacterial population. When the Plexiglas dam was used, the increased flow of AMD through the relatively small volume of compost located on either side of the dam exhausted the neutralization potential of the compost. Porewater pH values deep in the compost above the dam after its operation were all low (3.2 to 4.5), supporting this hypothesis.

Sulfate Reduction Rates

During the course of this study, the redox potentials measured below 15 cm deep in the organic substrate of the constructed wetland decreased steadily (Table 1). This is an indication of the gradual exhaustion of oxidized chemical species and the accumulation of reduced species.

Table 1. Redox potentials determined with a platinum electrode at depths below 15 cm in the substrate of the Friendship Hill wetland (n = 6 to 20).

	Pt Electrode Potential (mV)	
Date	Min	Max
11/01/88	32	449
11/15/88	-18	238
12/16/88	-84	33
01/31/89	-46	-10
05/31/89	-108	-9

Sulfate reduction rates measured in the organic substrate of the Friendship Hill wetland were highly variable, ranging from 2 to 600 nmol

cm^{-3} day^{-1} (Fig. 5). This reflects, in part, the heterogenous nature of the organic compost, which contains materials such as straw, manure, and corncobs. Maximum detectable rates of sulfate reduction activity during the study period are comparable to rates found in coastal marine sediments (0.4 to 3000 nmol cm^{-3} day^{-1}; Edenborn et al., 1987). Sulfate reduction rates remained consistently low throughout the winter but increased as the summer progressed, consistent with the predicted overall increase in biological activity with seasonally higher temperatures (Fig. 5). The concentration of total reduced sulfur compounds also increased in the wetland substrate with time (Fig. 6). This confirms that the by-products of bacterial sulfate reduction do accumulate in the wetland substrate and can probably be used to affect the quality of AMD under properly engineered conditions.

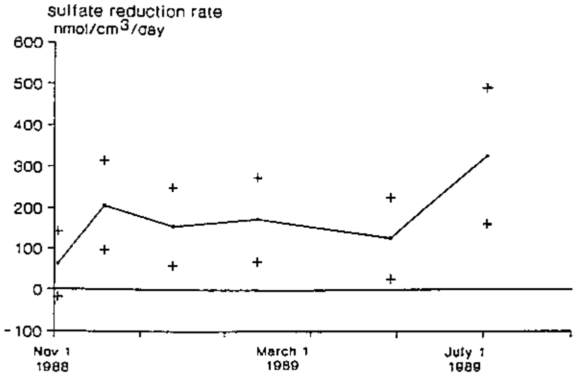

Figure 5. Sulfate reduction rates measured in the organic substrate of the Friendship Hill wetland. x ± SD is shown for each sampling date (n = 10 to 40).

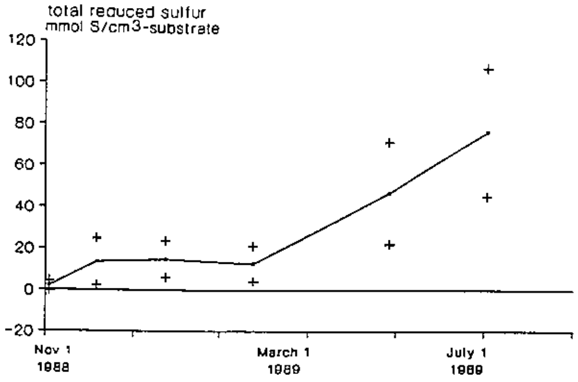

Figure 6. Total reduced inorganic sulfur concentrations in the organic substrate of the Friendship Hill wetland. x ± SD is shown for each sampling date (n = 10 to 40).

It is of interest to consider the potential role of bacterial sulfate reduction in the neutralization of AMD flowing through a wetland constructed to incorporate this process as a treatment method. If the maximum sulfate reduction rates measured in the Friendship Hill wetland (600 nmol cm^{-3} day^{-1}) were maintained throughout the entire volume of organic substrate (4.16×10^4 L) for six months, and AMD chemical composition and flow rates were as described previously (acidity = 1000 mg L^{-1}, flow rate = 7.5 L min^{-1}), 23% of the AMD flowing through the wetland during that time could be neutralized by bacterial activity alone. This percentage could be increased significantly by enhancing the sulfate reduction rate or by lowering the flow rate of AMD through the system. Even low bacterial sulfate reduction rates will lengthen the period of time that AMD can be fully neutralized, until the alkaline components in the substrate are exhausted. It will require additional study to determine if higher rates of sulfate reduction can be stimulated and maintained by improving wetland design and construction practices.

If practical sulfate reduction treatment systems can be developed, they may also be useful in the treatment of wastewater containing heavy metals. Laboratory experiments demonstrated that sulfate-reducing bacteria were active in solutions containing high concentrations of metals (Table 2). The bacteria effectively decreased the concentrations of many of the metals that were added to their growth media, forming insoluble precipitates with Ni, Cu, Zn, Cd, and Pb. These preliminary data indicate that biological systems may have practical application to the treatment of mine effluents containing selected heavy metals that precipitate with hydrogen sulfide.

Table 2. Metal toxicity tests on a mixed culture of sulfate-reducing bacteria.

	Metal Concentration (ppm)			
Metal	Toxicity	After Inoculation[1]	After Growth[2]	Percent Removal
Cd	104	35	0.1	> 99
Cu	> 14	14	< 0.1	> 99
Mn	> 223	223	60	73
Ni	> 211	211	0.4	> 99
Pb	> 5	5	0.5	89
Zn	310	194	2	99

[1] Metal concentration immediately after inoculation.

[2] Metal concentration at the end of the experiment.

Conclusions

Although it has been known for several years that bacterial sulfate reduction takes place in wetlands constructed for the treatment of acid mine drainage, the rate measurements reported here represent the first to be published in the literature to our knowledge. The study has found that sulfate reduction occurs at relatively high rates, but that these rates are highly variable in the heterogeneous environment of the wetland organic substrate, and they vary seasonally. It is apparent from laboratory and field studies that this process may prove to be useful in the treatment of acid and metal mine drainage if properly engineered systems can be designed. However, the problem of designing and constructing an effective system incorporating bacterial sulfate reduction for the long-term treatment of mine drainage remains to be solved.

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