DETERMINATION OF THE RATE OF SULFIDE PRODUCTION IN A CONSTRUCTED WETLAND RECEIVING ACID MINE DRAINAGE[1]

by
Julia S. Reynolds, Steven D. Machemer, Thomas R. Wildeman,
David M. Updegraff, and Ronald R. Cohen[2]

Abstract. The Big Five wetland in Idaho Springs, Colorado was built to treat mine drainage with low pH and high concentrations of heavy metals. Field studies indicated that sulfate reduction and sulfide precipitation are important processes in removing metals from the mine drainage. The approximate summer in situ rate of sulfide production by sulfate-reducing bacteria at the wetland was determined to be 1.2 micromoles of sulfide per gram of dry substrate per day by a laboratory serum bottle experiment. Preliminary results indicated that the addition of sodium lactate or an extract of hay significantly increased the rate of sulfide production in the bottles. Sulfate and dissolved metal concentrations decreased, whereas acid volatile sulfide concentration and pH increased with time in the serum bottles. The conditions in the serum bottles were similar to conditions in the wetland output, indicating that the laboratory scale systems could be used to estimate the remediation potential of a constructed wetland for the treatment of mine drainage.

Additional key words: Sulfate-reducing bacteria, heavy metals remediation.

Introduction

Acid mine drainage from abandoned mining operations contributes significantly to water pollution in Colorado streams. Artificial wetlands are currently being considered as possible passive treatment systems for drainage from these sources, utilizing natural biological and geochemical processes to treat the water. The Big Five wetland in Idaho Springs, Colorado was constructed to determine the effectiveness of such a system in treating mine drainage with low pH and high concentrations of heavy metals and sulfate. The design, construction, and initial operating results of the Big Five wetland treatment system are given in Howard, et al. (1989a and 1989b).

Research projects over the past few years have studied several metal removal processes that could be occurring in the Big Five wetland, including adsorption of metals onto organic materials in the substrate, uptake of metals by the vegetation, and precipitation of metals as sulfides (Machemer et al., 1990, Laudon, 1989). Results of laboratory and field studies indicate that organic adsorption processes are important during the start-up phase of the constructed wetland. However, after several months of operation, organic adsorption sites are filled by the metals and sulfide precipitation becomes the dominant process of metal removal from the incoming mine drainage (Machemer and Wildeman, 1991).

Production of hydrogen sulfide from sulfate by dissimilatory sulfate-reducing bacteria has been shown to be an important process in the sulfur cycle of marine and fresh water environments (Smith and Klug, 1981). Sulfate-reducing bacteria mineralize organic matter in anoxic waters and sediments using sulfate as an electron acceptor according to the

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[1] Paper presented at the 1991 National Meeting of the American Society for Surface Mining and Reclamation, Durango, Colorado, May 14-17, 1991. Publication in this proceedings does not preclude authors from publishing their manuscripts, whole or in part, in other publication outlets.

[2] Julia S. Reynolds is a Graduate Student and Ronald R. Cohen is an Associate Professor in the Department of Environmental Sciences and Engineering Ecology; Steven D. Machemer is a Graduate Student and Thomas R. Wildeman and David M. Updegraff are Professors in the Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO, 80401.

Proceedings America Society of Mining and Reclamation, 1991 pp 175-182
DOI: 10.21000/JASMR91010175
https://doi.org/10.21000/JASMR91010175

following reactions:

$$2CH_2O + SO_4^{-2} \rightarrow S^{-2} + 2CO_2 + 2H_2O \qquad (1)$$

$$S^{-2} + 2CO_2 + 2H_2O \rightarrow H_2S + 2HCO_3^- \qquad (2)$$

The products of sulfate reduction (i.e., hydrogen sulfide and bicarbonate ions) are desirable for the treatment of acid mine drainage as the increase in alkalinity will help raise the pH of the water and the hydrogen sulfide can react with the dissolved metals to form sulfide precipitates (Herlihy et al., 1987).

Results of microbiological studies of the Big Five wetland substrate indicate that sulfate-reducing bacteria are present in large numbers, with viable counts ranging from 1900 to 28,000,000 bacteria per gram of dry substrate (Batal, 1989). Analysis of outflow from the wetland shows a decrease in sulfate concentration of approximately 10-20%, significant decreases in concentrations of iron, zinc, and copper (i.e., consistently complete removal of copper and up to 100% removal of iron and zinc depending on flow rate), and an increase in pH as compared to the incoming mine drainage (Machemer and Wildeman, 1991). Significant increases in the sum of acid volatile sulfides (i.e., metal monosulfide precipitates such as FeS and ZnS) plus elemental sulfur over levels measured in the original substrate material have been demonstrated in areas of the Big Five wetland containing mushroom compost. Acid volatile sulfides (AVS) plus elemental sulfur represented 0.15% of the total sulfur in the original compost material. After ten months of operation of the wetland, the compost contained 1.1% of the total sulfur as AVS plus elemental sulfur. However, no significant increases in organic or pyritic forms of sulfur were detected in these areas after the same period of time (Laudon, 1989). All of these results indicate that bacterial sulfate reduction is an important process in the treatment of mine drainage at the Big Five wetland.

The objectives of this study included designing a laboratory scale system to determine an approximate in situ rate of sulfide production by sulfate-reducing bacteria by measuring the change in AVS in the substrate over time and to determine the effects of nutrient amendments on the rate of sulfide production. Another purpose of constructing these laboratory scale wetland substrate systems was to compare values of various parameters such as pH, concentrations of dissolved metals and sulfate, and temperature in the serum bottles to output waters at the Big Five wetland. From these comparisons, the usefulness of serum bottle systems in initial treatability studies could be evaluated.

Materials and Methods

Collection of Samples and Preparation of Serum Bottles

In June 1990, a 2-liter sample of substrate was collected from an area of the wetland containing mushroom compost. Twelve liters of mine drainage were collected from the adit of the Big Five Tunnel and deoxygenated in the field with nitrogen.

The substrate sample was homogenized on a paint shaker for five minutes. Subsamples of approximately 20 grams were then transferred to 120 ml serum bottles in a glove bag under a nitrogen atmosphere. The bottles were sealed in the glove bag. The following day, 70 ml of deoxygenated mine drainage was added by syringe to each bottle. The bottles were then incubated at 18° C. This temperature was chosen to simulate summer conditions at the wetland.

Determination of Dissolved Metals, Sulfate, and pH in Serum Bottles

Several parameters were determined to be important for comparing conditions in the serum bottles to output waters from the wetland, including pH and concentrations of dissolved metals and sulfate. At approximately five day intervals, an aliquot of the aqueous phase of the serum bottles was removed by syringe. Concentrations of dissolved metals (i.e., zinc, copper, iron, and manganese), sulfate concentration, and the pH of this subsample were determined. Concentrations of dissolved metals were measured by atomic absorption. The sulfate concentration was determined gravimetrically as $BaSO_4$.

Rate of Sulfide Production

Four bottles were analyzed on the first day after the mine drainage was added for acid volatile sulfides to provide a baseline level of AVS in the substrate sample. AVS was determined with a trapping train similar to the one described by Jorgensen and Fenchel (1974). The bottles were connected to the trapping train and approximately 25 ml of 6M HCl was added by syringe. The H_2S that was produced was flushed from the serum bottles with O_2-free nitrogen and trapped in the first of two test tubes containing 1N NaOH connected in series. No sulfide was ever detected in the second test tube. Approximately 2 ml of 7.5 M NH_4OH was added to an aliquot of the first trap, and this solution was titrated with 0.01M $AgNO_3$ to determine the amount of sulfide trapped. The efficiency of the trapping train was determined to be 93% by acidifying a known quantity of ZnS in place of the substrate sample. AVS values determined by titration were corrected accordingly.

Subsequent determinations of AVS were made on triplicate samples at approximately 5-day intervals. The rate of sulfide production was determined by taking the difference in AVS per gram of dry substrate in the subsequent samples from the average AVS per gram of dry substrate in the baseline samples and dividing by the time interval.

Poisoned Controls

Poisoned controls were prepared by adding 11 ml of 5% sodium azide to each of three serum bottles for a final concentration of 0.5%. AVS in these bottles was determined at 15, 35, and 70 day intervals by the method described above.

Nutrient Amendments

Nutrient amendments of either sodium lactate (final concentration of 100 mg/l) or 30 ml of an extract of hay were added to a set of serum bottles. The sodium lactate was chosen because lactate has been found to be a substrate utilized by sulfate-reducing bacteria (Dicker and Smith, 1985). Hay has been used at the Big Five wetland as insulation for the substrate and has appeared to enhance the activity of the sulfate-reducing bacteria. The hay extract was prepared by soaking a sample of hay in distilled water for approximately one week and then removing the supernatant. The AVS produced in these bottles was measured by the method described above approximately two weeks after the addition of the amendments.

Results

Rate of Sulfide Production

Table 1 presents the AVS per gram of dry substrate produced in the serum bottles over 76 days at approximately 5-day intervals. The average rate of sulfide production in the bottles at each time interval is also presented. The initial rate was 1.7 umoles of sulfide per gram of dry substrate per day for the first 5-day interval. From 5 to 35 days the rate was relatively constant at approximately 1.2 umol/g/day. Beginning with the 40-day time period, the rate of sulfide production began to decrease and this trend continued for the rest of the experiment.

Figure 1 shows the rate of sulfide production in the serum bottles compared to the rate in the poisoned controls. The rate in the controls was minimal compared to the serum bottles, indicating that the production of sulfide in the wetland substrate is predominantly the result of microbial activity.

Effect of Nutrient Amendments

The rates of sulfide production measured in amended bottles were compared to rates measured in unamended serum bottles for corresponding time intervals. These rates in umoles of sulfide per gram of dry substrate per day were as follows:

Sodium lactate	2.4
Hay extract	4.3
No amendment	0.91

The addition of sodium lactate resulted in a rate approximately 2.5 times greater than the rate measured in the unamended bottles. The addition of hay extract resulted in a rate approximately five times greater than in the unamended bottles.

Comparison of Serum Bottles to Mine Drainage and Wetland Output Waters

Table 2 presents a comparison of pH, temperature, concentrations of dissolved metals, and sulfate concentration for serum bottles over a 35-day time period. Mine drainage and output waters from the Big Five wetland treatment system were measured at approximately the same time as the substrate sample was collected.

Table 2 shows that concentrations of dissolved metals in the baseline sample bottles (i.e., measured the day after adding the mine drainage) were lower than the concentrations in the mine drainage. Several processes could have contributed to this immediate removal of some of the metals, including precipitation due to the increase in pH, adsorption onto organic materials enhanced by the agitation of the substrate, and precipitation with residual sulfides in the substrate sample.

Over the course of the experiment, the pH of the serum bottle systems increased, the concentration of

TABLE 1. AVS Production and Rate of Sulfide Production in Serum Bottles.

TIME (days)	B*	5	10	15	20	25	30	35	40	48	55	66	76
AVS (umol/g)	43	52	55	59	68	73	77	84	81	85	89	99	100
RATE (umol/g/d)	**	1.7	1.2	1.1	1.2	1.2	1.1	1.2	0.95	0.86	0.84	0.84	0.74

* Baseline samples

** Not calculated

Note: Values for AVS and rate are the average of three replicates except for values on day 0 (Baseline), which are the average of four replicates and days 10 and 25, which are the average of duplicate samples.

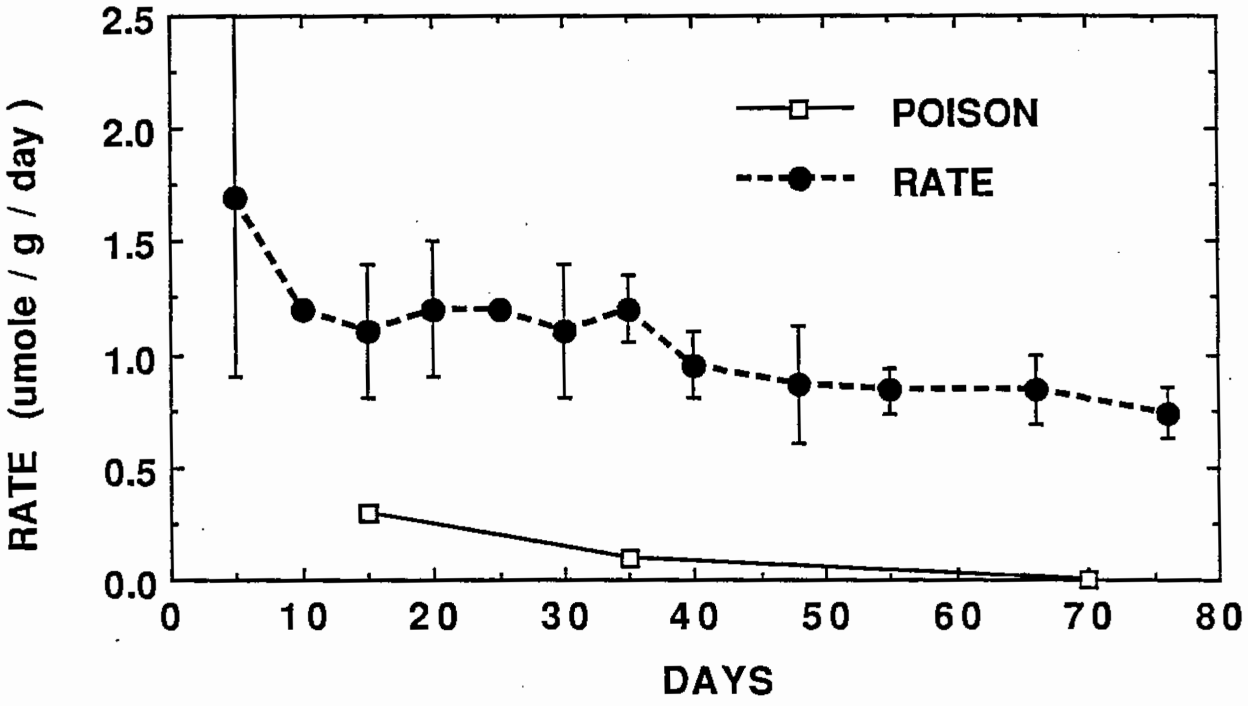

FIGURE 1. Rate of Sulfide Production in Serum Bottles and Poisoned Controls. Error bars represent one standard deviation. Data points without error bars are the average of two samples.

sulfate in the aqueous phase decreased, and the concentrations of dissolved metals decreased. These are the same trends observed when samples of output waters from the Big Five wetland are compared to samples of the incoming mine drainage.

Discussion

Results of these studies indicate the laboratory scale method developed for determining the rate of sulfide production in a wetland substrate could also be used as an inexpensive, initial treatability study of acid mine drainages. A relatively constant rate of sulfide production was measured over a 30-day period. For a treatability study, the serum bottles could be run for just two weeks to obtain a reasonable estimate of the rate of sulfide production in a given system. In addition, the serum bottles could be used to test the effectiveness of different substrates and combinations of nutrient amendments on treating a particular source of mine drainage.

The sulfide production rate measured in the laboratory could be used to estimate loading rates of metals that could be effectively removed by a constructed wetland. Several methods have been used to estimate loading rates. One method is based on the concept that the wetland operates most efficiently if a suitable microenvironment for the sulfate-reducing bacteria is consistently maintained (Reynolds, et al., 1991). The most important environmental factors are reducing conditions and a pH around 7. These conditions should exist when sulfide is being produced in excess of the amount required to precipitate all of the metals in the incoming mine drainage. In this case, metals are the limiting reagent in the precipitation of metal sulfides. The sulfide production rate measured in serum bottles could be used to estimate a loading rate for metals that would result in metals rather than sulfide being the limiting reagent.

Finally, measurements of other parameters in the serum bottles such as sulfate concentration, concentrations of dissolved metals, and pH could be used to qualitatively predict conditions in the output waters expected after the wetland treatment system is established.

Summary

The summer <u>in situ</u> rate of sulfide production by bacteria in the Big Five wetland substrate was estimated to be 1.2 umol/g/day. The rate of sulfide production measured in poisoned control bottles was minimal compared to the serum bottles, indicating that the process is predominantly microbiological. Preliminary results indicated that the addition of sodium lactate or an extract of hay significantly increased the rate of sulfide production in the serum bottles. Trends in sulfate concentrations, pH, and concentrations of dissolved metals were similar to conditions measured in output waters from the Big Five wetland, indicating that the serum bottle systems could be used in initial treatability studies for mine drainage.

Acknowledgments

The authors thank Holly Fliniau of the U.S. EPA, Region VIII, and Edward Bates of the U.S. EPA Risk Reduction Engineering Laboratory, Cincinnati, Ohio, for their assistance on the project.

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TABLE 2. Comparison of pH, Temperature, Sulfate, and Metal Concentrations in Mine Drainage, Wetland Output, and Serum Bottles.

SAMPLE	pH	TEMP. (°C)	SO_4^{-2} (mg/L)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)	Zn (mg/L)
Mine Drainage	3.0	15.9	1720	0.573	38.6	30.6	8.58
Wetland Output	6.7	18.1	1460	<0.050	0.640	15.8	0.069
Serum Bottles Baseline*	6.1	18.0	1680	<0.050	10.5	15.5	0.036
Serum Bottles (5 days)*	6.2	18.0	1660	<0.050	7.28	10.8	0.534
Serum Bottles (10 days)**	6.3	18.0	1610	<0.050	4.70	9.78	0.272
Serum Bottles (15 days)**	6.3	18.0	1530	<0.050	8.80	15.3	0.129
Serum Bottles (20 days)**	6.4	18.0	1410	<0.050	8.06	12.0	0.368
Serum Bottles (25 days)**	6.4	18.0	1470	<0.050	6.90	11.3	0.221
Serum Bottles (30 days)**	6.4	18.0	1350	<0.050	4.93	9.72	0.213
Serum Bottles (35 days)**	6.7	18.0	1240	<0.050	4.51	10.6	0.162

* Values for these samples are the average of four replicates.

** Values for these samples are the average of three replicates.

Note: Mine drainage and wetland output values are the average of two samples collected in June, 1990.