

EFFECTS OF A SPHAGNUM PEAT ON THE QUALITY OF A SYNTHETIC ACIDIC MINE DRAINAGE

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Artificial (constructed) wetland systems have attracted considerable attention in connection with the treatment of acidic mine waters. A study was undertaken to examine the effects of Sphagnum spp. and decomposed peat on pH, and iron and sulfate concentrations, in laboratory-scale reactors receiving synthetic mine water. Effluent was monitored for pH, iron, and sulfate. Depth profiles were examined for iron, sulfate, sulfide, pH, Eh, and sulfate-reducing bacteria. Addition of iron in the ferric state to the experimental units resulted in fluxing of highly visible oxidized iron which, according to profile and effluent data, underwent transformation to ferrous iron under reducing conditions. The addition of sulfate to the system correlated well with parameters of sulfate reduction, i.e., level of sulfate-reducing bacteria enriched in the peat, reducing Eh, sulfate loss, and concentrations of sulfide obtained. Under conditions of sulfate reduction significant decreases occurred in the effluent concentrations of total iron, soluble iron, ferrous and ferric iron, and sulfate following addition of sulfate to the synthetic mine water. Decreases in these chemical constituents were associated with the formation of iron sulfides important to the long-term retention of iron in the wetland environments.

INTRODUCTION

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Acidic mine drainage, a waste product of both deep and surface coal mining, is formed when water and air contact sulfide-bearing minerals associated with the coal. Such disturbance of coal seams stimulates a series of chemical, and biochemical oxidation-reduction reactions mediated by iron- and sulfur-oxidizing bacteria, e.g., Thiobacillus ferrooxidans and I. thiooxidans. Acidic mine drainage characteristically contains high concentrations of metal ions, particularly iron species, and sulfate often at levels greater than 1000 mg/L. The mineral acidity of the waters vary, with pH values in the ranging from pH 2-3 up to pH 6.

Treatment of acidic mine drainage is required in order to meet water quality standards set by Federal and State regulations. Although costly and labor intensive, physical-chemical processes have been the usual methods of treatment. Lime, limestone, or sodium hydroxide are employed for neutralization of the acid waters followed by oxidation and precipitation of the metals. More recently, biologically based systems, including freshwater wetlands, have been investigated for application to the partial or full treatment of acidic mine waters (Unz and Dietz, 1986). Interest in wetlands for acidic mine water renovation has developed in response to the coal mining industry's need for a more economical method of treating acidic mine drainage. cursory research on volunteer wetlands receiving acidic mine drainage has led to the belief that such environments may effect a decrease in the mineral constituents of the mine waters. These observations prompted laboratory and field investigations aimed at understanding the advantages and limitations of wetlands for acidic mine drainage treatment and the underlying mechanisms responsible. Gerber et al. (1984) studied *Sphagnum* moss placed in laboratory troughs and found the biomass capable of uptake and absorption of iron and manganese from synthetic mine water. Snyder and Aharrah (1984) examined a *Typha* community existing in a mine water region and observed considerable amounts of accumulated iron and manganese associated with the plants. Tarleton et al. (1984), based on results from constructed microcosms containing *Sphagnum* moss, proposed mechanisms of iron removal by wetland systems which included plant uptake, microbial oxidation and precipitation, absorption by organic deposits, and sulfide precipitation.

Rapid accumulation of incompletely decayed plant material in wetland communities results in extensive peat deposits and the formation of anaerobic conditions. Decomposition by obligate and facultative anaerobic bacteria may account for a large percentage of the reduction of organic material in a wetland system. In addition to pathways of intramolecular fermentation, organic carbon compounds may be anaerobically respired utilizing ferric iron and the sulfur of sulfate as final electron acceptors.

Relative to free ferrous iron, ferric iron is fairly insoluble in water becoming more so at higher pH values. In natural aqueous environments, ferric iron is precipitated as an insoluble oxide, oxyhydroxide, or jarosite. Tarleton et al. (1984) noted that iron oxides accumulated within their experimental units owing to the low solubility of oxidized iron. The stability of oxidized iron deposits in wetlands is not well understood. Oxides of iron are subject to microbial reduction when exposed to anaerobic conditions (Nealson, 1983). In addition, ferric iron may be reduced to ferrous iron by radical groups associated

with humic materials and sulfides (Szilagy, 1971 and Temple, 1964). The possibility exists that appreciable solubilization of the precipitated mineral deposits may occur in wetlands used to treat acid mine drainage.

Sulfate reduction occurs in nearly any environment where strong reducing conditions prevail. A well known sulfate-reducing environment is the salt marsh wetlands of coastal regions. The richly organic, anaerobic sediments and high concentrations of sulfate present in this environment provide for high rates of sulfide production mediated by sulfate-reducing bacteria. Although organically rich sediments exist in freshwater systems, production of sulfide is usually limited by the low concentrations of sulfate present. Smith and Klug (1981) demonstrated increased potential for sulfate reduction with increased supplements of sulfate in experiments with freshwater sediments. Tuttle et al. (1968) obtained evidence of sulfate-reducing bacteria in wood chip dams intercepting acidic mine drainage and Herlihy and Mills (1985) observed sulfate reduction in the sediments of a lake receiving acidic mine drainage. Results obtained by these workers indicate that sulfate reduction may ameliorate the characteristics of acidic mine drainage through increased pH and removal of dissolved iron in the form of iron sulfides. Tarleton et al. (1984) identified iron sulfides in their experimental microcosms, although, in small amounts compared to other iron fractions. The importance of sulfate reduction to the binding of metal ions in wetlands has not been resolved.

The present study was conducted to examine the impact of synthetic mine waters on laboratory microcosms, fashioned from transplanted wetland *Sphagnum* spp. and peat, with respect to (1) metal oxide transformations within the reducing zone of the peat and (2) potential for sulfate reduction in the peat with respect to increasing the pH and decreasing iron concentrations through formation of insoluble sulfide deposits.

MATERIALS AND METHODS

Experimental Units

Three rectangular chambers (30 cm x 40 cm x 20 cm) were constructed of plexiglass and fitted with inlet and outlet ports to provide a water depth of 12 cm. A variable speed, multichannel, model 1203 Harvard peristaltic pump (Harvard Apparatus Co., Millis, MA) was used to deliver synthetic acidic mine water from a 55-L storage vessel to the units at a continuous flow rate of 2.8 mL/min. Tygon tubing was used in pumping connections between the feed reservoir and the units and silicon tubing (0.125 inch ID) was inserted in the peristaltic pump. The entire apparatus was located in an environmental room maintained at 20°C and

Table 1. Composition of synthetic water applied to the microcosms during experimentation

Component	Influent Composition(mg/L)			
	Water	Stage1	Stage2	Stage3
Fe(III)	0	30.0	0	0
Fe(II)	0	0	30.0	30.0
SO ₄	0	0	0	100.0
Cl ¹	26.5	81.5	62.5	1.0
Ca	2.0	2.0	2.0	9.0
Mg	2.0	2.0	2.0	7.5
Na	5.0	5.0	5.0	5.0
K	6.0	6.0	6.0	6.0
NH ₄ -N	1.5	1.5	1.5	1.5
NO ₃ -N	0.5	0.5	0.5	0.5
PO ₄ -P	1.1	1.1	1.1	1.1
pH	3.5	3.5	3.5	3.5

¹ Chloride concentrations > reported value due to pH adjustment with HCl

60 percent RH. A cool white fluorescent light source providing approximately 120 ft-candles to the surface of microcosms was placed on a 12-hour on-off cycle.

Microcosms

Sphagnum spp. and underlying peat were obtained from the Bear Meadows area, a natural wetland in Central Pennsylvania. The Sphagnum biomass and the peat were removed successively and placed in plastic bags. The collected materials were transported to the laboratory and immediately placed in the experimental units which, subsequently, were filled with water from the collection area and allowed to stabilize under controlled conditions. During the stabilization period, which lasted approximately 4 weeks, water loss due to evaporation was replenished with distilled water. After the initial period of stabilization, a synthetic water, adjusted to a pH of 3.5 with HCl and containing mineral salts without iron and sulfate (table 1), was pumped into the microcosms. The microcosms were maintained on synthetic water for a 10-week period. Experimentation with the three microcosms was conducted in three successive stages (treatments) within the same experimental vessels. The chemical composition of the synthetic water used in each stage is given in table 1. Stage one units received synthetic water with oxidized iron (Fe III) in order to examine the effects of the peat on the stability of ferric iron and iron oxide deposits. Stage two and stage three units received synthetic water containing reduced iron (Fe II) and, in addition, stage three units received synthetic water which contained sulfate to determine the existence of sulfate reduction in the microcosms and its effects on the quality of the synthetic mine drainage. Effluent

total, soluble (ferric plus ferrous), and ferrous iron, sulfate, and pH were measured every other day during treatment. Each stage was operated until the effluent parameters monitored remained stable for a period of 2 weeks. Depth profile analysis of the microcosms was made at the conclusion of an experimental run and included a determination of ferrous and total iron, sulfates, sulfide, pH, eH, and sulfate-reducing bacteria counts. Profiles of Eh and pH were measured in the microcosms at a distance of 1 cm from influent and effluent walls and at 10, 20, and 30 cm of the width and the values obtained from each depth averaged. Remaining profiles were determined for the effluent end of the tanks only with samples collected for each location from individual depths combined into one average sample. Between experimental stages, tanks were allowed to stabilize for a period of 3 weeks while receiving the initial synthetic feed water(table 1).

Analytical Procedures

Total and ferrous iron was determined using the phenanthroline method (APHA, 1975). Ferrous iron was determined directly. Total iron was determined after acidification of samples and reduction of ferric iron with hydroxylamine. Ferric iron was determined indirectly by subtracting ferrous iron from total iron. Sulfate analysis was performed turbidimetrically (APHA, 1975). Sulfide was analyzed by the methylene blue method (APHA, 1975). The pH and E_h values were measured with an Orion 399A pH/mV Analog Meter (Orion Research, Cambridge, MA) equipped with a Fisher Universal Glass pH electrode and a Fisher Platinum Indicating E_h Electrode (Fisher Scientific Co., Pittsburgh, PA). Sulfate-reducing bacteria were enumerated using a modification of the 5-tube MPN method described by Postgate (1984). Serially diluted samples were dispensed to 24-well Corning tissue culture plates (Fisher Scientific Co., Pittsburgh, PA) and incubated at 23°C in an anaerobic chamber under an atmosphere of N₂.

Data Analysis and Statistical Procedures

Effluent results of stage 2 and stage 3 units were statistically compared to evaluate the effects of sulfate reduction on iron and pH. Data collected during the last 2 weeks of stage two and stage three were averaged and statistically compared using a one-tailed ANOVA of comparison of means (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Ferric Iron Reduction

In the first stage of experimental work, microcosms, which had been purged of

Table 2. Depth profile counts of sulfate reducing bacteria of stage two and stage three.

Tank	Sulfate-Reducing Bacteria(counts/mL)			Sulfate-Reducing Bacteria(counts/mL)		
	1	2	3	1	2	3
Depth (cm)						
2	5.6	36	360	68000	3600	6800
4	4.4	68	68	6800	4400	1100
7	880	3200	680	1800	28000	8800
10	56	5200	104	36000	25000	36

deposits may be unstable towards solubilization and transformation within a major segment of the peat column.

Sulfate Reduction

The potential for sulfate reduction in a low pH, freshwater environment was examined in stages two and three of the study. Microbial sulfate reduction in wetland environments at decidedly acid pH values is not considered to be a major activity and when such an event occurs there is usually the suspicion that the pH values represent bulk water measurements not reflective of the sediment microzonal pH (Tuttle et al., 1968). The results presented appear to support the premise of microbial sulfate-reducing activity at depressed pH values in the experimental microcosms. High numbers of sulfate-reducing bacteria (table 2) were found at all depths of microcosms studied, however, negligible influent sulfate concentrations (<0.5mg/L) in stage two of the study precluded significant sulfide formation. Nevertheless, high levels of sulfate-reducing bacteria existed in stage two units in spite of the absence of exogenous sulfate. The profile of the stage two microcosm (fig. 2B) reveals that reducing conditions were eventually established throughout the sediment even though sulfate was limited. In addition, significant reductions of effluent sulfate (as much as 10 mg SO₄⁻²/L by comparison of influent and effluent [fig. 3A] values) were achieved by stage three microcosms. Depth profiles on stage three microcosms depicting measurements of sulfate and sulfide (fig. 4) and E_h (fig. 3) point to dissimilatory sulfate reduction as the principal process of sulfate removal. Decreasing sulfate concentrations with depth were observed in all three microcosms during stage three experiments. The possibility that the gradually diminished sulfate concentrations may have been due to diffusion limitations in the sediments rather than sulfate transformations was dispelled in consideration of the constant effluent sulfate concentrations, depth profiles of conservative ions, and the balance of sulfide produced against

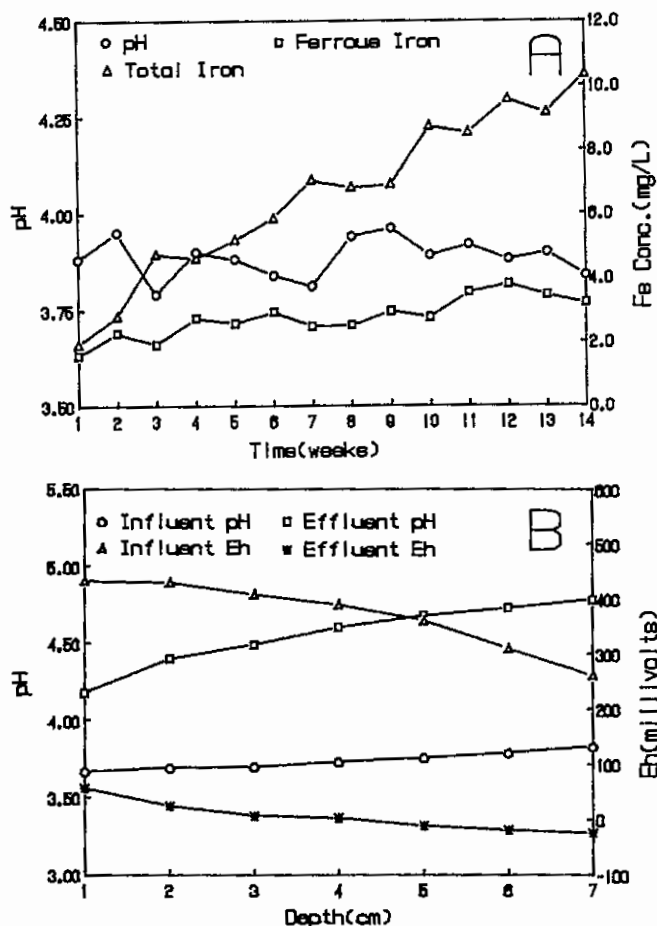


Fig.1. A.Effluent quality of stage one microcosms. B.Depth profile of influent and effluent pH and Eh parameters of stage one microcosms. Values are the mean of individual measurements on three microcosms.

all traces of ferrous iron, received synthetic water containing iron in the oxidized form. The charging of ferric iron(Fe(III)) to the experimental units resulted in the formation of highly visible iron oxide deposits in the peat. Subsequent appearance of ferrous iron in the effluent of the microcosms was considered evidence of internal reduction of ferric iron and the iron oxide deposits. The increased effluent iron levels, both total and Fe(II), over time (fig. 1A) may reflect the transformation of a greater proportion of the iron oxides deposited in the reducing zone. Depth profile data obtained at the end of the run are presented in fig. 1B. Both influent and effluent redox potential were markedly depressed with depth and were lower at the effluent region of the units. Stage one ferrous iron concentrations (fig. 5) increased slightly as E_h values diminished with depth. Interestingly, ferric iron reduction occurred at relatively high E_h values within the microcosms, suggesting that iron oxide

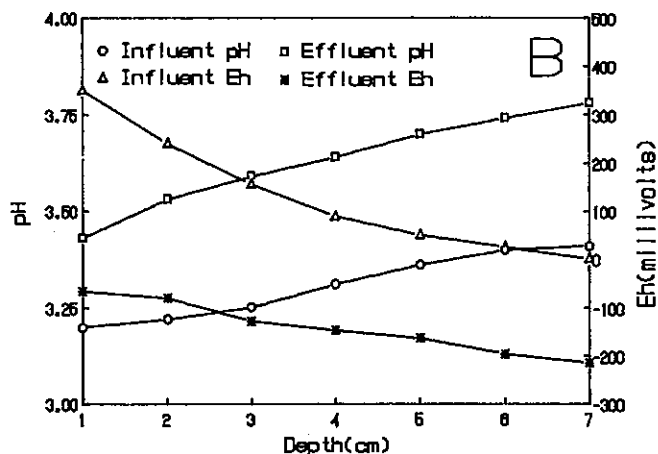
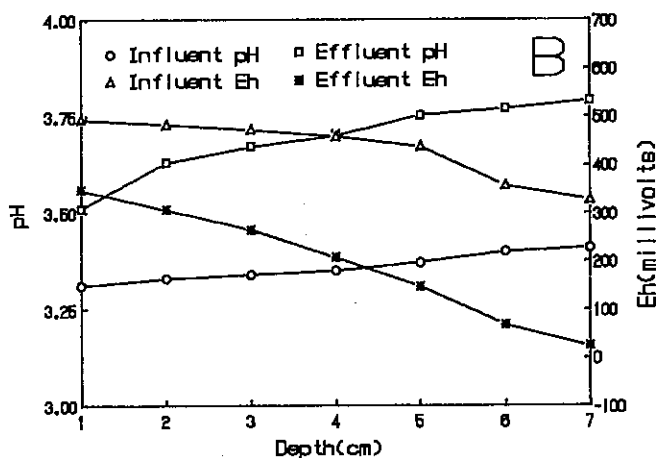
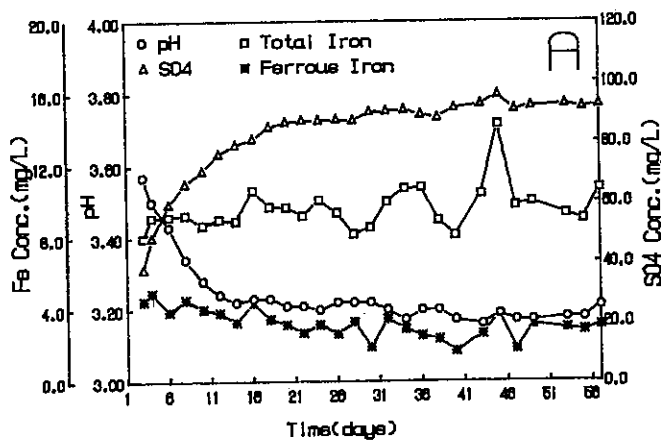
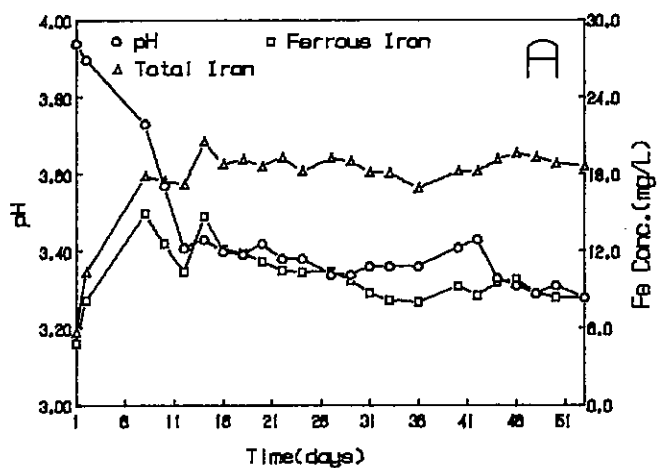


Fig. 2. A. Effluent quality of stage two microcosms. B. Depth profile of influent and effluent pH and Eh parameters of stage two microcosms. Values are the mean of individual measurements on three microcosms.

Fig. 3. A. Effluent quality of stage three microcosms. B. Depth profile of influent and effluent pH and Eh parameters of stage three microcosms. Values are the mean of individual measurements on three microcosms.

sulfate lost. The large amounts of sulfide produced by stage three microcosms must have been derived from the sulfate charged to these units since no detectable sulfide was produced in sulfate-limited stage two units. Comparisons of E_h values (figs. 2B and 3B) in the microcosms during stages two and three revealed stage three E_h values were more characteristic of the reducing conditions expected where sulfate reduction would be occurring and in which sulfide would be present. The lower E_h values found in stage three units are likely to be as a result of the reduction of oxidized form of sulfate to sulfide.

Sediments displaying active sulfate reduction might be expected to effect an increase in pH owing to consumption of hydrogen ions by sulfide ions and to precipitate ferrous iron as FeS . The effect of sulfate reduction on effluent iron concentrations and pH was analyzed by statistically comparing the effluent results (figs. 2A and 3A) obtained with stage two (minor sulfate reduction) and stage three (major sulfate reduction)

systems; both of which received a ferrous iron influent. The investigation found there to be significant decreases in all effluent iron concentrations from stage 2 to stage 3, but no significant increases in pH could be established.

Effluent iron concentrations (all forms) were lower by at least 50 percent in stage three units as compared to stage two units. High concentrations of effluent ferrous iron would be expected, as a result of increased reduction, in stage 3 experimental units as a result of low E_h (fig. 3B) values and high sulfide concentrations (fig. 4) present in the peat. Lower effluent ferrous iron concentrations actually resulted in stage three (fig. 3A) compared to the effluent of stage two (fig. 2A) presumably due to precipitation of the iron by sulfide. Although depth profiles of ferrous iron (fig. 5) in stage two and three are similar the appearance of visible black deposits in the peat of stage three microcosms was suggestive of iron sulfide precipitation. Acid digestion of the

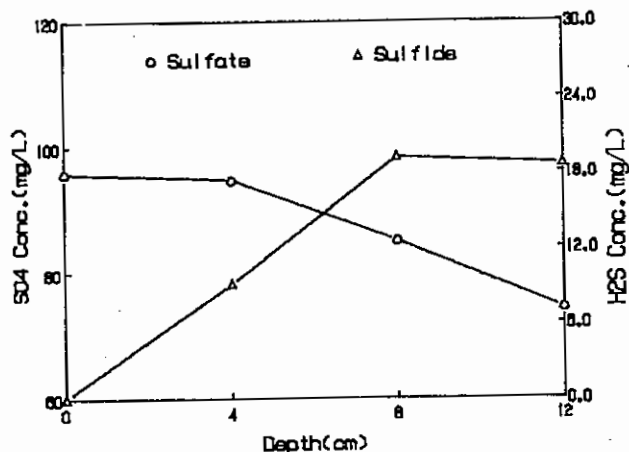


Fig. 4. Depth profile of sulfate and sulfide in stage three microcosms at the conclusion of experimental run. Values are the mean of individual measurements on three microcosms.

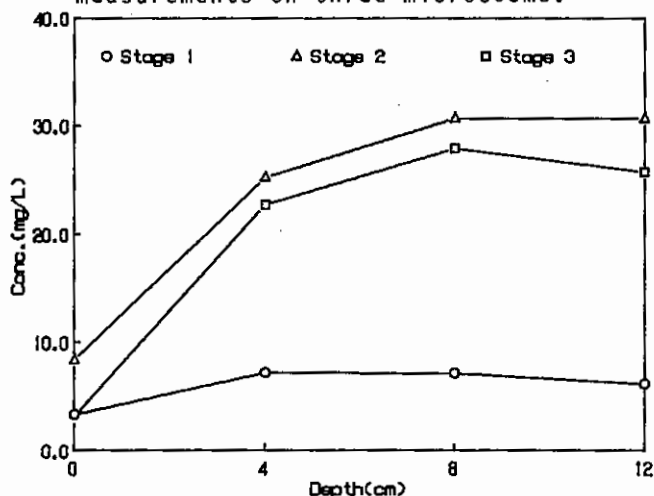


Fig. 5. Depth profile of ferrous iron for all stages of experiment as determined at conclusion of experimental run. Values are the mean of individual measurements on three microcosms.

deposits followed by ferrous iron analysis indicated the presence of ferrous iron possibly as a result of the breakdown of iron sulfides. Although only ferrous iron was intended for delivery to stage two and three microcosms, in reality, an estimated 10 to 20% of the iron in the influent became oxidized during transport from the holding tank to the microcosms. The oxidized iron would be expected to become readily solubilized in the reducing sediments of the stage three microcosm, whereas, FeS precipitates presumably would remain intact.

As noted earlier, no significant difference between effluent pH values of stage two and stage three microcosms was found over time. Although sulfate reduction might be expected to produce lower hydrogen ion concentrations in the microcosms, this did not occur, presumably owing to preferential reaction of the

Table 3. Statistical comparison of stage 2 and stage 3 results. Averages of the three experimental units.

	Statistical Comparison		
	Averages (mg/L)		Probability
	Stage 2	Stage 3	
Total Fe	18.5	10.1	<0.001
Fe(II)	8.74	2.69	<0.001
pH	3.3	3.2	1
Sulfate		91.4	

¹ Hypothesis rejected (Stage 2 > Stage 3)

sulfide ions with ferrous iron rather than hydrogen ions. Hence, only sulfide present in excess of that precipitated by iron may be available for hydrogen ion consumption. Examination of influent and effluent iron and sulfate concentrations of stage three units clearly demonstrated that sulfate losses did not exceed ferrous iron removal. In the present study, an excess of sulfide was never observed and the pH profile of the microcosm (stage three units) which exhibited sulfate reduction was not significantly different than that of stage two where only minor sulfate reduction took place. Although the pH was not increased as a result of sulfate reduction in this experiment, increased pH may still be a benefit of sulfate reduction. The design of this experiment may have prevented the occurrence of pH improvement due to low pH and high iron concentrations of the influent and the shallow depth of the tank. Further investigations are needed to determine whether sulfate reduction in a peat environment can improve the pH of acid mine water.

CONCLUSIONS

The results of the laboratory study support the contention that ferric oxides deposited in *Sphagnum* microcosms are subject to microbial reduction and solubilization. Under these circumstances, the fate of iron oxide deposits in organically rich sediments must become a serious consideration in wetlands developed for acid mine drainage treatment.

Sulfate reduction was demonstrated at the low pH of the peat material of the experimental units with as much as 10% of influent sulfate concentrations being reduced. The sulfide produced through sulfate reduction had a significant effect on effluent iron concentrations in that more than 50% greater iron removal occurred in the sulfate-reducing microcosms as compared to sulfate-limited units. The iron removed was retained in the form of iron sulfides.

The important implications of the study for the development of wetlands earmarked for acidic mine drainage treatment lie with peat-producing plants, such as *Sphagnum*, *Typha*, *Carex*, and

Scirpus. An artificially constructed system, in which the reducing zone is sufficient to solubilize and reduce oxidized iron species, but lacks the presence of sulfide for precipitation of the iron, may eventually release soluble iron at levels in equilibrium with that of the influent. Construction of deep cells for the collection of fermentable plant matter should assure adequate reducing potential for microbial sulfate reduction in the wetland bed since acidic mine waters normally contain high sulfate concentration environment.

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