

# EVALUATION OF POTENTIAL WETLAND SUBSTRATES FOR OPTIMIZING SULFATE REDUCTION<sup>1</sup>

by

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**Abstract.** A column experiment was designed to evaluate the potential of sulfate reduction as an ameliorating process in AMD wetlands, and also to evaluate a variety of carbon-rich materials for potential use as substrates for sulfate-reducing microorganisms. Soluble sulfide was used as a direct indicator of sulfate reduction. Large differences were identified between the various materials regarding the quantity of sulfide generated. During five months of observation, clear trends were evident with respect to both vertical distribution of sulfide within the sediment columns and with time. Porewater flow rate was also shown to have significant impact on sulfide levels and distribution within the columns. Straw-Manure mixtures appear to have the greatest potential for supporting sulfate reduction as soluble sulfide levels of nearly 200 mg/L were observed in the columns. Sulfide exports from straw-manure columns were as high as 1mg/cm<sup>2</sup>/day. Ongoing observations are warranted in order to assess the long term potential of the various materials for sustaining sulfate reduction.

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## Introduction

During the last five years, there has been a surge of interest in the use of man-made wetlands as a low cost approach to the treatment of acid mine drainage (AMD). These strategies are based upon observations that AMD waters passing through wetlands often become lower in acidity and in metal content (Fe being of primary interest). Many wetlands have been constructed to treat AMD in the coal mining regions of MD, PA, OH, VA, WV, and KY (Wieder 1989). Unfortunately, the effectiveness of most of these wetlands has been below expectation. This may be due to a "black box" approach to wetland water treatment, rather than a focus on the basic biogeochemical processes.

Several potential mechanisms have been suggested to explain amelioration of AMD by wetlands. These include: sorption of metals in ionic forms; uptake of metals during the growth of plants; specific adsorption or complexation of metals with organic matter; precipitation of metals as "oxides" (oxides or oxy-hydroxides) through the activity of Fe and Mn oxidizing bacteria or simple geochemical processes; microbial reduction of sulfate to

sulfide resulting in precipitation of metals as sulfides (Kleinmann 1987; Hedin 1989).

There is a finite capacity for Fe sorption in wetland sediments which is quickly saturated, and plant uptake has been demonstrated to be of relatively minor importance (Sencindiver, and Bhumbra 1988). Some work has shown Fe to be significantly bound in an organic form, but this was usually not the primary form of Fe in the sediment and these cases also were associated with shallow, better aerated systems (Henrot and Wieder 1990; Wieder 1988; Wieder, and Lang 1986). Thus indications are that the two mechanisms with long term potential for metal removal from AMD are 1) oxidation and precipitation of metal "oxides" and 2) precipitation of metal sulfides following sulfate reduction. Because of their organic-rich nature and generally low redox potentials, wetland environments present sub-optimal conditions for Fe oxidation. Most recently, efforts at utilizing oxidation and precipitation of iron as oxy-hydroxides as a mechanism for treating acid mine drainage has focused on the use of anoxic limestone drains (Skousen 1991).

The factors required for sulfate reduction include (in addition to sulfate reducing bacteria), a source of sulfate, a source of organic matter for bacterial metabolism, and a reducing environment (Pons et al 1982; Rickard 1973). Any of these factors could potentially limit sulfidation processes in constructed wetlands (Griffin and Rabenhorst 1989; Rabenhorst and Griffin 1988.) Sulfate is generally abundant in AMD, and therefore the availability of oxidizable organic matter and a sufficiently reducing environment are conditions which tend to limit sulfate reduction in AMD wetlands. When sulfide is produced (during sulfate reduction) in the presence of soluble Fe<sup>2+</sup>,

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the Fe will be precipitated as one of several insoluble Fe sulfide minerals.

While allowing for the potential contribution of sulfate reduction processes, most workers studying AMD wetlands have largely discounted this mechanism. It appears that this is because the systems which have been studied have not provided the conditions necessary for sulfate reduction. Common design problems in experimental wetlands which interfere with sulfate reduction include: shallow aerated sediments which are not anaerobic; extraneous sources of Fe oxides (which become mobile when reduced); inadequate sediment porewater interaction (surface flow rather than sediment throughflow); and poor carbon source for microbial decomposition (Wieder 1988; Henrot, et al. 1989; Henrot and Wieder 1990; Stillings, et al 1988; Wieder and Lang 1986; Wieder and Lang 1988; Wieder et al 1990). As a result, much of the precipitated Fe in these wetlands occurs as Fe "oxides" (Wieder and Lang 1985). Only a few workers have suggested that sulfate reduction may have a more important role to play (Hedin et al. 1988; McIntire and Edenborn 1990; Wicks et al. 1988.)

In the last year, several workers have begun to identify the important potential role of sulfate reduction in wetlands designed to treat AMD. Sulfate reduction was shown to be an effective mechanism in removing Ni from acid influent passing through columns in a laboratory study (Hammack and Edenborn 1991). Others have built enclosed "reactors" in order to enhance sulfate reduction in treating AMD (Dvorak et al. 1991). These researchers consistently use a "mushroom compost" as the wetland substrate. Because mushroom compost is highly heterogeneous, and because it commonly contains limestone, interpretations of water chemistry may be confounded. In addition, the partially decomposed nature of the compost should make it less easily oxidized by sulfate reducing organisms.

The objectives of this project were: 1) to develop laboratory scale model wetlands to evaluate the potential of sulfate reduction as an ameliorating process in AMD wetlands; and 2) to evaluate a variety of carbon-rich materials for potential use in wetland construction with respect to their suitability as a substrate for sulfate-reducing microorganisms.

### Methodology

This experiment was designed to evaluate six different materials as potential substrates for man-made wetlands. The following materials were used in the experiment, and specific properties are given in Table 1.

1. Timothy hay
2. Barley straw
3. Leaf compost (from the composting facility in Dickerson, MD)
4. Straw & manure mixture (1:2 on dry weight basis of straw and composted, and partially dehydrated manure)

5. BOM compost (provided by the Maryland Bureau of Mines, Frostburg, MD)
6. Hay & manure mixture

These materials were placed in 10cm diameter columns, to a thickness of 50 cm, and were prepared in duplicate. Packing densities and other properties of the columns are provided in Table 2. Because the purpose of this experiment was to test the suitability of the materials as a substrate for sulfate reduction, and because sulfate reducing bacteria are usually adversely affected by low pH (Widdell 1988), the solution passed through these columns was not strongly acidified (the pH was adjusted to 6.5 and was largely unbuffered). Because of the insolubility of metals (Fe, Al, or Mn) at near neutral pH, they were not added to the solution. The solution that was used had a concentration of 10 mM sulfate (9mM  $K_2SO_4$  plus 1mM  $MgSO_4$ ) and the initial flow rate was 1 cm/day (80mL/day per 10cm diameter column). After approximately 3 weeks the flow rate on half of the columns was increased to 5cm/day (400mL/day per column). The pH of the porewater was monitored over the course of the experiment. Soluble sulfide was measured in the porewaters using the methylene blue procedure (Cline 1969; APHA 1985).

## Results and Discussion

### Measurements of pH

There were no apparent differences in pH values of porewater between the two flow regimes for the same materials, and only small differences as a function of depth. For the purpose of comparing materials, data are presented for the porewater collected from the 30cm depth in the columns flowing at 5cm/day (Fig. 1). There are major differences in pH between materials, which reflect the nature of the materials themselves. The two composted materials have pH values which begin and remain above 7 suggesting the presence of free lime ( $CaCO_3$ ). The pH of hay is lower than that of the straw, perhaps due to a higher level of soluble organic acids in the hay. Similarly, the pH of the hay-manure mixture was consistently below that of the straw-manure mixture. The addition of manure to the hay or straw, however, raised the pH by approximately one unit. Those materials with an initial pH below 7 (those containing hay or straw), demonstrated a gradual increase of 0.7 to 1.5 units over the period of observation. This is generally consistent with observations that reducing soil systems tend toward a neutral pH (Ponnamperuma 1972). This may also represent the leaching of organic acids from the columns.

### Soluble Sulfide

During the first few weeks of the experiment, the columns were tested for the presence of sulfide in a qualitative manner by adding 0.25mL of Cline's solution B to approximately 5mL of the sediment porewater and looking for the development of a blue color (Cline 1969). Because we postulated that the sulfides would first

Table 1. Properties of the materials used in preparing wetland substrates in the column experiment.

Material	% Carbon	% Nitrogen	C:N ratio	% Moisture <sup>a</sup>
Timothy Hay	44.5	1.27	35.0	9.4
Barley Straw	45.4	0.54	84.1	8.1
Leaf Compost	31.9	1.95	16.3	41.3
Manure	33.4	1.96	17.0	48.3
BOM Compost	26.3	2.76	9.5	57.9

<sup>a</sup>Moisture content calculations based on the moist weight of the sample.

Table 2. Density of packing and carbon content of the six potential wetland substrate materials as arranged in columns for use in the experiment.

Column Nos.	Material	Moist Density <sup>b</sup> Mg/m <sup>3</sup>	Dry Density Mg/m <sup>3</sup>	C Content Kg/m <sup>3</sup>
1 & 2	Timothy Hay	0.100	0.092	41
3 & 4	Barley Straw	0.075	0.068	31
5 & 6	Leaf Compost	0.500	0.294	94
7 & 8	Straw & Manure (2:1 dry wt.)	0.286 <sup>c</sup>	0.171 <sup>c</sup>	64
9 & 10	BOM Compost	0.551	0.232	61
11 & 12	Hay & Manure (2:1 dry wt.)	0.412 <sup>c</sup>	0.248 <sup>c</sup>	92

<sup>b</sup>Materials were packed to a firm density. The densities of straw and hay were intended to reflect the density of baled materials.

<sup>c</sup>These densities are estimated based upon the quantities of each of the materials added.

develop near the bottom of the columns, only the porewater from the lowest depth (50cm) was tested in this manner. A slight green color was detected at the base of columns 7 and 8 (straw-manure mixture) after approximately one week, with the other materials showing no indications of sulfide. This persisted for over three weeks, at which time, we decided to test the porewaters at all of the depths. To our surprise, sulfides were detected in the upper portions of many of the columns. Quantitative sulfide determinations were then initiated. While sulfide will be discussed in detail below, it is of interest that sulfide was generated in the straw substrate while the pH was approximately 5. This is considerably more acid than the reported optimal conditions for sulfate reducing organisms (Widdell 1988).

**Effect of material type.** The levels of sulfides measured in the sediment porewaters of each of the materials after 8, 16 and 22 weeks are shown in Figures 2 through 7. Several observations can be made. During the first 8 weeks (Figs 2 and 3), the quantity of soluble sulfide was much greater in the straw-manure mixture, straw, and the

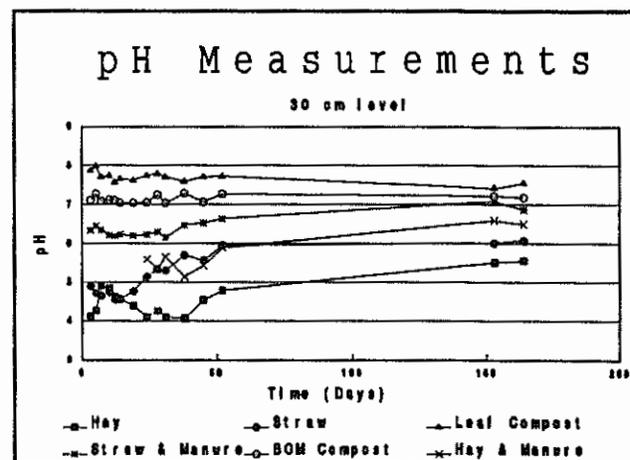


Figure 1. Measured pH of porewater over time collected at a depth of 30 cm from 6 different wetland substrate materials.

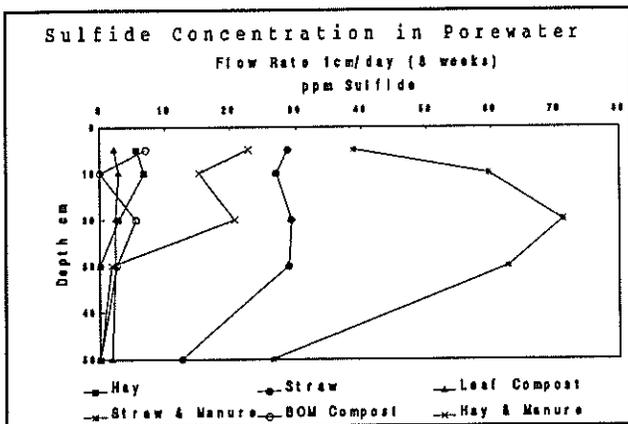


Figure 2. Concentration of sulfide in porewaters of various wetland substrates after 8 weeks. Flow rate is 1cm/day.

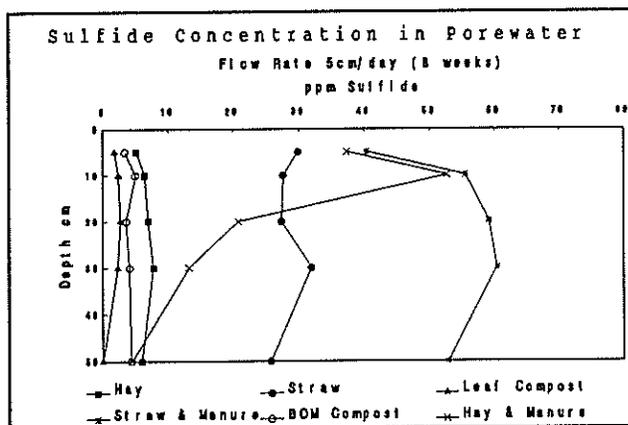


Figure 3. Concentration of sulfide in porewaters of various wetland substrates after 8 weeks. Flow rate is 5cm/day.

hay-manure mixture, than in the other three materials, with the levels in the straw-manure mixture approximately twice those of the next highest material.

While both of the composted materials produced small amounts of sulfide, neither produced sulfide in levels near that produced by the straw or straw-manure mixture. The sulfide production was low even though the composted materials had pH values which were substantially higher than the others and were approximately seven, the range considered optimal for sulfate reducing bacteria. Observations of sulfide levels conformed to our predictions that these sulfate reducing systems may be carbon limited, and that given an adequate supply of readily decomposable carbon, sulfate-reduction would occur. In fact, the sulfide levels in these materials were greater than those observed in tidal marsh soils of Chesapeake Bay (Haering 1986). Because the composted materials have already been substantially decomposed in their formation, they would be expected to have relatively little readily decomposable carbon, which is in contrast to straw or hay which represent fresh organic matter.

One puzzling observation which ran counter to our original hypothesis was that the hay did not initially appear to be functioning well as a substrate for sulfate reduction. Comparisons of sulfide levels during successive measurements between weeks 5 and 8 indicated that sulfide levels were beginning to increase, especially in the hay-manure mixture. It was also noted that the sulfide levels were increasing in the uppermost portions of the columns, and this was especially evident where the flow rate was lower. This may suggest that there are certain compounds produced in the hay columns which inhibit sulfate-reducing bacteria (perhaps even alcohol produced during early fermentation), and that as these are removed by leaching, sulfide generation can proceed.

After 16 weeks, sulfide levels in the straw-manure mixture were still substantially greater than any of the other substrates, while levels in the straw generally remained greater than those of the hay or composts (Figs 4 and 5). Sulfide levels in the hay-manure mixture, however, had increased substantially and were at (1cm/day) or above (5cm/day) the levels in the straw. By the 22nd week, some marked changes were beginning to be evident (Figs 6 and 7). The straw-manure mixture still had very high levels of sulfide, which were as great or greater than the other materials, but the relative ranking of the other substrates was changing. The rate and magnitude of these changes appeared to be related, in part, to the flow regime.

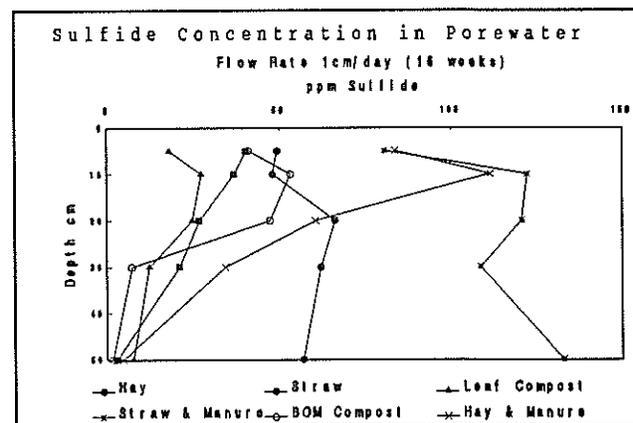


Figure 4. Concentration of sulfide in porewaters of various wetland substrates after 16 weeks. Flow rate is 1cm/day.

There are at least two possible explanations for the strong performance of the mixtures containing manure relative to that of straw or hay alone. We hypothesize that the manure, having its origin in the anaerobic digestive tract of a cow, provides a higher number of sulfate-reducing bacteria and serves as an inoculum to the system. Alternatively, the manure may be providing some nutrient, or other component which favors the activity of sulfate reducing bacteria. In either case, it might be expected that given sufficient time (months or years), the manure-mixtures and the unamended straw or hay may

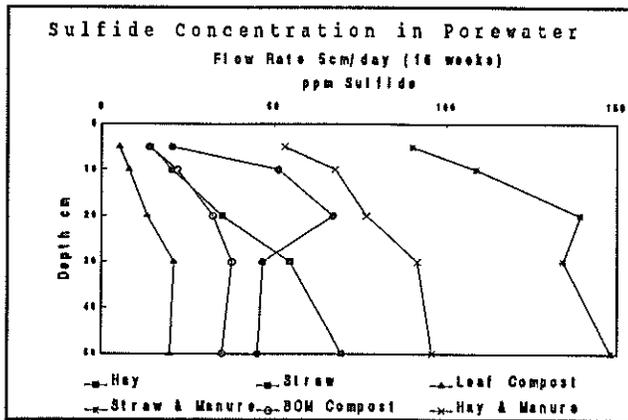


Figure 5. Concentration of sulfide in porewaters of various wetland substrates after 16 weeks. Flow rate is 5cm/day.

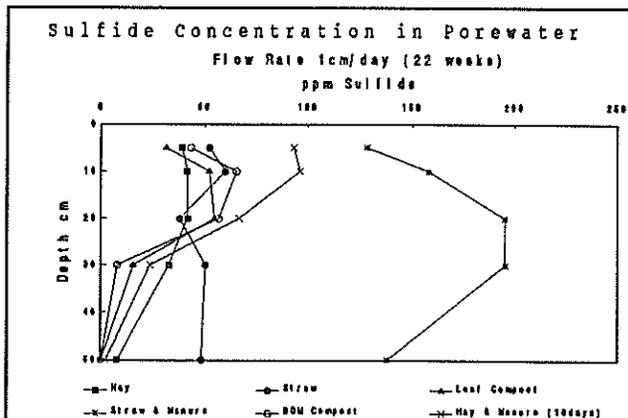


Figure 6. Concentration of sulfide in porewaters of various wetland substrates after 22 weeks. Flow rate is 1cm/day.

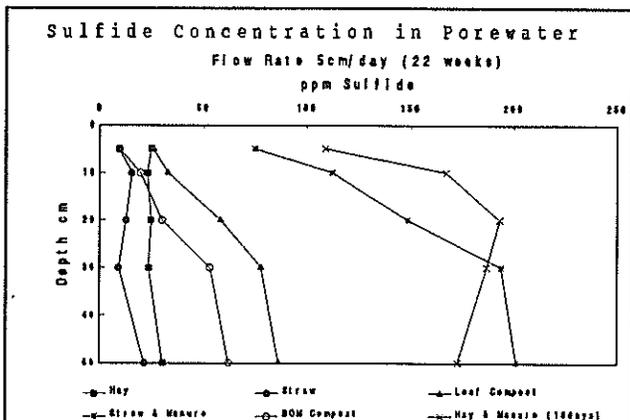


Figure 7. Concentration of sulfide in porewaters of various wetland substrates after 22 weeks. Flow rate is 5cm/day.

prove to function similarly. This would not be true if the manure functioned only in a catalytic capacity.

**Effect of flow rate.** By assuming piston type flow and a porosity of approximately 80%, the estimated porewater

residence time under the two flow regimes specified in this experiment (1cm/day and 5cm/day), would be approximately 40 and 8 days, respectively. In other words, with a flow rate of 1cm/day, the porewater is fully exchanged from above once every 40 days (or once every 8 days, with a flow rate of 5cm/day.) In general, the sulfide concentration of the porewater for a given substrate under the faster flow regime was equal to or greater than the concentration under the slower flow regime. Contrary to our expectation, the higher flow did not have a negative impact on the sulfide production, and in fact may actually have enhanced the sulfate reduction.

Under the lower flow regime of 1cm/day, most of the materials contained higher levels of sulfide in the upper portion of the columns, whereas under conditions of faster flow, the sulfide maxima tended to be toward the bottom of the columns. One explanation for the higher concentrations near the surface under the slower flow conditions, is that there may be certain soluble compounds present within the materials which inhibit sulfide generation and which must be leached before sulfide production can reach an optimum. There was, for example, evidence of fermentation in some of the columns during the first week of operation, which presumably resulted in the generation of alcohol. This might have an inhibitory effect on sulfate reducing organisms. In this case, sulfide generation would be expected sooner, and more evenly distributed throughout the columns, under faster flow conditions.

A less likely explanation for these trends might be that because the pKa of  $H_2S$  is around 7, and most of the materials have pHs below this, we would expect much of the sulfide to be in the  $H_2S$  form. While  $H_2S$  is a highly soluble gas (Henry's law constant of 102mmol/L/atm compared to  $O_2$  with a Henry's law constant of 1.3 mmol/L/atm) (Sposito 1989), the equilibrium with the gas phase might result in an upward migration of the sulfide. Where the flow rate is slow (ie. 1cm/day), this might cause the sulfide to be concentrated in the upper portion of the column, whereas a faster flow (ie. 5cm/day) might mask any such effects.

**Trends with time.** The average sulfide concentration within the columns over time is shown in figures 8 and 9. The materials generally show a roughly linear increase with time, and the trends are similar under both flow regimes. After 156 days (22 weeks), the sulfide content of most of the columns was continuing to increase. The sulfide levels in the straw columns, however, appeared to have reached a maximum two to four weeks before this. Continued observations are warranted in order to identify longer term trends.

**Sulfide Export.** While the mean sulfide concentration within the columns is one parameter which can be used for making comparisons between substrate materials, another approach for comparison is measurement of the quantity of sulfide discharged from the columns. Therefore, the daily sulfide export from the columns

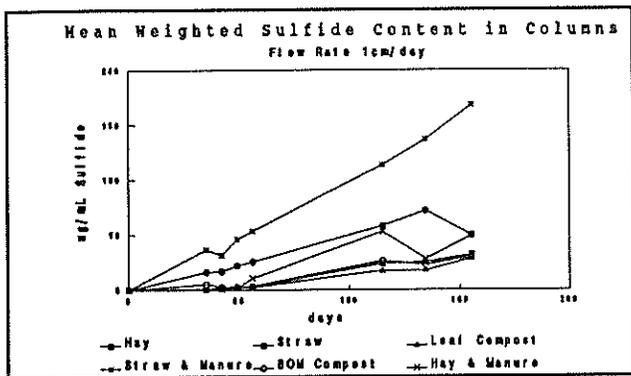


Figure 8. Weighted average sulfide concentration (ug/mL) in the substrate porewaters over time. Flow rate 1cm/day.

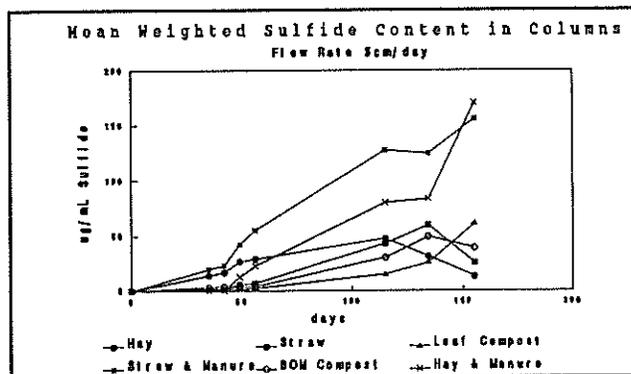


Figure 9. Weighted average sulfide concentration (ug/mL) in the substrate porewaters over time. Flow rate 5cm/day.

(Figures 10 and 11) was calculated using the sulfide concentration at the outflow port (50cm) and the daily flow rate. To account for the diameter of the columns, these data are reported as  $\mu\text{gS}^-/\text{cm}^2$  (surface area) of substrate.

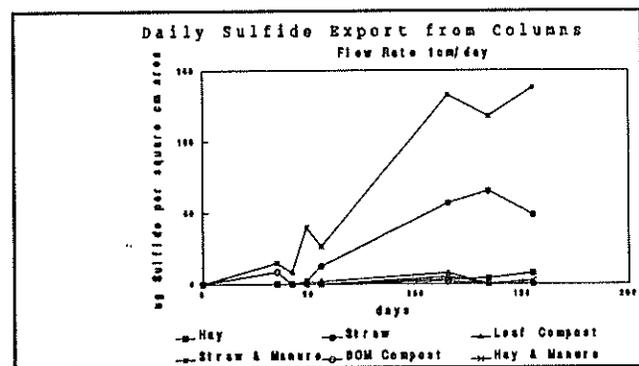


Figure 10. Daily sulfide export over time from the columns flowing at 1cm/day.

The daily sulfide export is clearly much greater under the higher flow conditions. This is due to both the greater volumes of solution passing through the columns and to the higher concentrations at lower depths in these columns. Under the lower flow rate, only the straw and straw-manure mixture show any significant degree of sulfide export. When these daily rates are integrated as

cumulative sulfide exports (Figures 12 and 13), the differences are even more pronounced.

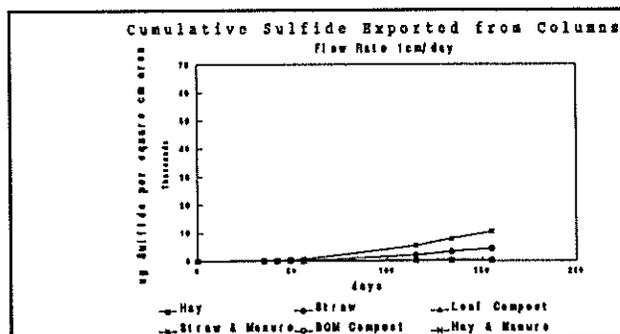


Figure 11. Cumulative sulfide export over time from the columns flowing at 1cm/day.

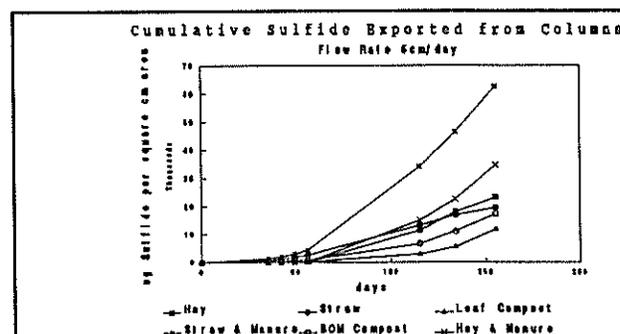


Figure 12. Cumulative sulfide export over time from the columns flowing at 5cm/day.

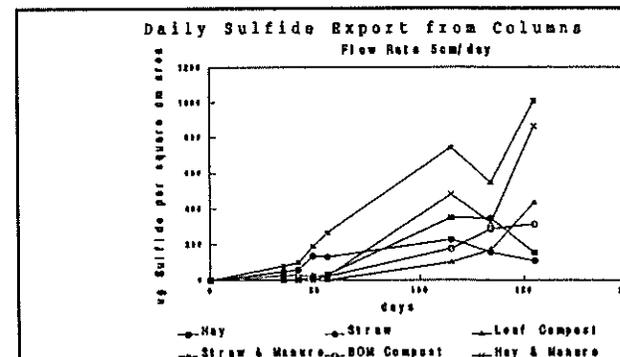


Figure 13. Daily sulfide export over time from the columns flowing at 5cm/day.

## Conclusions

Results to date lead to the judgment that, provided certain constraints, sulfate reduction processes can be important in the biogeochemistry of constructed AMD wetlands. It is also clear that, regarding the potential for sulfide generation, there are significant differences between the types of organic materials which may be used as wetland substrates. Following nearly six months of operation, the straw-manure and hay-manure mixtures appear to be the best substrates for promoting sulfate reduction. Because sulfide generation within some of the materials has changed markedly during this period of observation,

longer term observations should be made. The rates of solute flow through the sediment also appear to significantly affect the quantity and distribution of sulfide. Nevertheless, because of the inherent complexity of these systems and the added difficulty associated with studying dynamic flowing systems, the factors governing the vertical distribution of sulfide within the various substrates is not fully understood at this time.

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