CHEMICAL AND MICROBIOLOGICAL MODIFICATION OF ACID MINE DRAINAGE USING CONSTRUCTED TYPHA WETLANDS

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ABSTRACT

Biological neutralization of acid mine drainage (AMD) in man-made cattail *(Typha)* wetlands offers an alternative to chemical treatments. Simulated *Typha*. wetlands were constructed in a controlled greenhouse environment. Wetlands were inoculated with a mixed anaerobic sediment slurry. Cattail root systems fully established both in surface and subsurface sediments and plants averaged 200 to 250 cm in height. Pore water collected from interstitial samplers showed a pronounced decrease in Eh (from -30 to -300 mV) and an increase in pH (from 2.0 to 6.5) with depth and distance respectively. Sulfate concentrations decreased with depth (ca. 200 mg kg^{-1}), due to the establishment of active sulfate reducing bacteria as indicated by $^{35}S\text{-}SO_4^{2-}$ reduction. Reduced iron sulfides accumulated as 80% acid volatile sulfides/ 20% pyrites. Iron sulfide formation could account for between 10 to 20% of the total iron removed from AMD during passage through the wetlands. Overall, the wetlands retained nearly 70% of the total applied iron over a period of one year.

INTRODUCTION

Control of AMD from both active and abandoned surface mines is one of the biggest problems facing the coal mining industry in Appalachia. Nearly 4,000 miles of streams in West Virginia alone are affected by AMD. Most mine operators chemically treat water as required by law before release to receiving streams. Chemical treatments are expensive, and some coal companies actively mining coal today are faced with treatment of water for many years to come. Biological treatment of AMD with wetlands may provide an alternative which could reduce the necessity to treat AMD chemically. Several researchers have demonstrated that iron and manganese can be removed from AMD as it moves through cattail *(Typha)* wetlands.

Both natural and man-made wetlands have been shown to improve water quality, but the exact mechanisms of iron and manganese removal are not well understood. Information about such mechanisms is vital to determining the long term utility of wetlands treating AMD.

Our study was designed to examine the chemical and microbiological mechanisms by which *Typha* wetlands remove metals from AMD. Knowledge of these mechanisms and the kinetics of the reactions will allow us to estimate the length of time a wetland will continue to remove metals. This information should assist consultants and coal operators in designing functional Typha wetlands. The process of sulfate reduction by anaerobic bacteria in wetland sediments may play a particularly important role in metal removal. Formation of iron sulfides from H_2S produced by microbial sulfate reduction, concomitant with resulting increased alkalinity, represent processes which are particularly favorable to iron removal. Investigations documenting the role of sulfate reduction in AMD-impacted wetlands will lead to a better understanding of the possible interactions of microbial and chemical processes involved in amelioration of AMD. Accordingly, the objectives of this research were the following: i) to determine the chemical composition of interstitial sediment solutions in non and AMD impacted wetlands. ii) to determine the fate of metals added to AMD impacted wetlands. iii) to determine the distribution of sulfate reducing-bacterial populations in wetland sediments, and iv) to determine sulfate reduction rates occurring in wetland sediments.

MATERIALS AND METHODS

Wetland Construction and Operation. Simulated wetlands were constructed in the green house (Figure 1). Four large wooden troughs (480 x 60 x 60 cm) were lined with plastic and fitted with three exit ports (15, 25, 35 cm) affixed with shut-off valves. Typha latifolia, collected from two different non-AMD-impacted sites (designated ecotypes A and B), were planted in each trough (32 plants) arranged in 16 rows of alternating ecotypes per row. Each system was inoculated with a mixed sediment slurry collected from an established volunteer Typha wetland currently treating AMD. Two wetland troughs receive AMD at a rate of 25 liters day-' while the remaining two troughs serve as controls and receive tap water at identical flows. Both tap water and AMD are distributed to the wetlands from holding tanks using timer- controlled peristaltic pumps. AMD is collected weekly at the emergence of an underground stream. Composition of this AMD is 438 mg 1^{-1} Fe, 4 mg 1^{-1}Mn, 2900 Mg 1^{-1} SO_4^{2-}, pH 2.75.

Sample Collection. Interstitial water samples were collected in 6.5 cm increments with depth from the influent, midpoint, and effluent of each wetland trough. High density polyethylene samplers were assembled by filling wells with sterile degassed Milli-Q water, placing a 0.4 11 m (pore size) polyester membrane over wells and securing the membrane by attachment of a retaining plate with nylon screws. Samplers were installed in the wetlands and allowed to equilibrate for a minimum of 2 weeks prior to pore water analysis. Sediment/slurry samples were collected under anaerobic conditions at various depths using permanently installed PVC sampling tubes installed at the influent, midpoint, and effluent of each wetland. Anoxic conditions were maintained by continuously flushing the sample tube and collection flask with oxygen-free argon gas.

Pore Water Chemistry. Water samples removed from the interstitial water sampler wells were maintained under anaerobic conditions for Eli and pH determinations using a platinum

electrode and glass H" sensing electrodes paired with a Ag/AgC1 double junction electrode. Total Fe, Mn, and Ca were determined by atomic absorption spectroscopy. Sulfate was determined by single column ion chromatography using 5 mM pthalic acid as a mobile phase.

Reduced Sulfur Pools. Anaerobic sediment slurries were used to determine reduced sulfur pools. Acid volatile sulfide (AVS) and pyritic sulfur (PS) were determined by digestion and distillation of samples under an atmosphere of oxygen free nitrogen using a modified Nishita apparatus. Liberated H 2S was retained in two 20 nil traps containing either sulfide antioxidant buffer (SAOB) or 2M NaOH. Total sulfide (TS=AVS+PS) was determined as Cr (II) reducible sulfur (CPS) in a hot acid digestion. PS was determined as CRS after AVS was first removed by addition of acid. AVS was determined by difference, i.e. AVS=TS-PS.

Sulfate Reduction. Sulfate-reducing bacteria were enumerated by a MPN procedure using a modified Starkey medium. Incubations were at 30 C for 2 weeks prior to enumeration. All manipulations were performed utilizing anaerobic procedures. Anaerobic sediment slurries were utilized in sulfate reduction rate analyses. Samples were transferred to an anaerobic gove bag where they were distributed to replicate plastic cups. Subsamples were used for SO_4^{2-} determination (HPLC), dry matter determination, and SO_4^{2-} reduction assays. Replicate cups received 2 | Ci of $^{35}SO_4^{2-}$, were capped and placed at 25 C in anaerobic Torbal jars for 24 to 40 hours with subsequent freezing -20 C. Samples were thawed under 2M NaOH and transferred to digestion flasks. Total sulfides were determined as described above. Four ml from each NaOH trap was transferred to vials for determination of ^{35}S-TS by scintillation counting.

RESULTS

Patterns of Eh and pH with depth were similar in control troughs, irrespective of sampling location (Figure 2). Positive Eh values were evident in surface sediments, but clearly exhibited development of reducing anaerobic conditions within 10 cm below the surface. Concentrations of soluble Fe, Mn, SO_4^{2-}, and Ca also exhibited similar patterns through the profile (Figure 2). Levels of Fe were always low (< 50 mg ml^{-1}) and increased slightly at lower depths. Calcium concentrations also exhibited an increase with depth, whereas the opposite was true of SO_4^{2-} levels.

Patterns of Eh and pH with depth varied considerably with respect to sampling location in AMD treated troughs (Figure 3). Overall, pH was significantly lower and Eh higher when compared with control troughs. At the influent, pH values ranged from < 3.0 at the surface to 4.1 at 40 cm. Eh values were poised high by the large iron load, ranging from +500 mV in surface sediments to +225 mV at 40 cm. Midpoint samples exhibited similar trends in Eh, although values dropped to ca. + 10 mV at 40 cm. Effluent Eh values were more similar to control wetlands as reducing sediments were clearly evident below 20 cm. At this location, within the upper 10 cm, pH values remained low but approached circumneutrality below this depth.

Concentrations of metals and sulfate also varied with location in the treated troughs (Figure 3). Generally, soluble Fe and Mn increased with depth, however concentrations at a given

depth decreased from influent to effluent sampling locations. Effluent Fe averaged 150 mg 1^{-1} exiting the wetland. Although high sulfate concentrations were evident throughout the treated wetland, a 200 mg 1^{-1} reduction from surface to deep sediments was exhibited. Dissolved Ca increased dramatically with depth which is indicative of limestone dissolution occurring in acidic, anaerobic sediments of AMD treated troughs.

Populations of sulfate-reducing bacteria (SRB) were initially reduced upon exposure to AMD (Table 1). Higher numbers persisted initially near the effluent of the treated troughs. Subsequent analyses showed that SRB numbers increased substantially in the AMD-treated troughs and that establishment of these populations were evident throughout the depth profile at different locations. SRB populations in control troughs, although fluctuating slightly, also increase with higher numbers generally found in upper sediments.

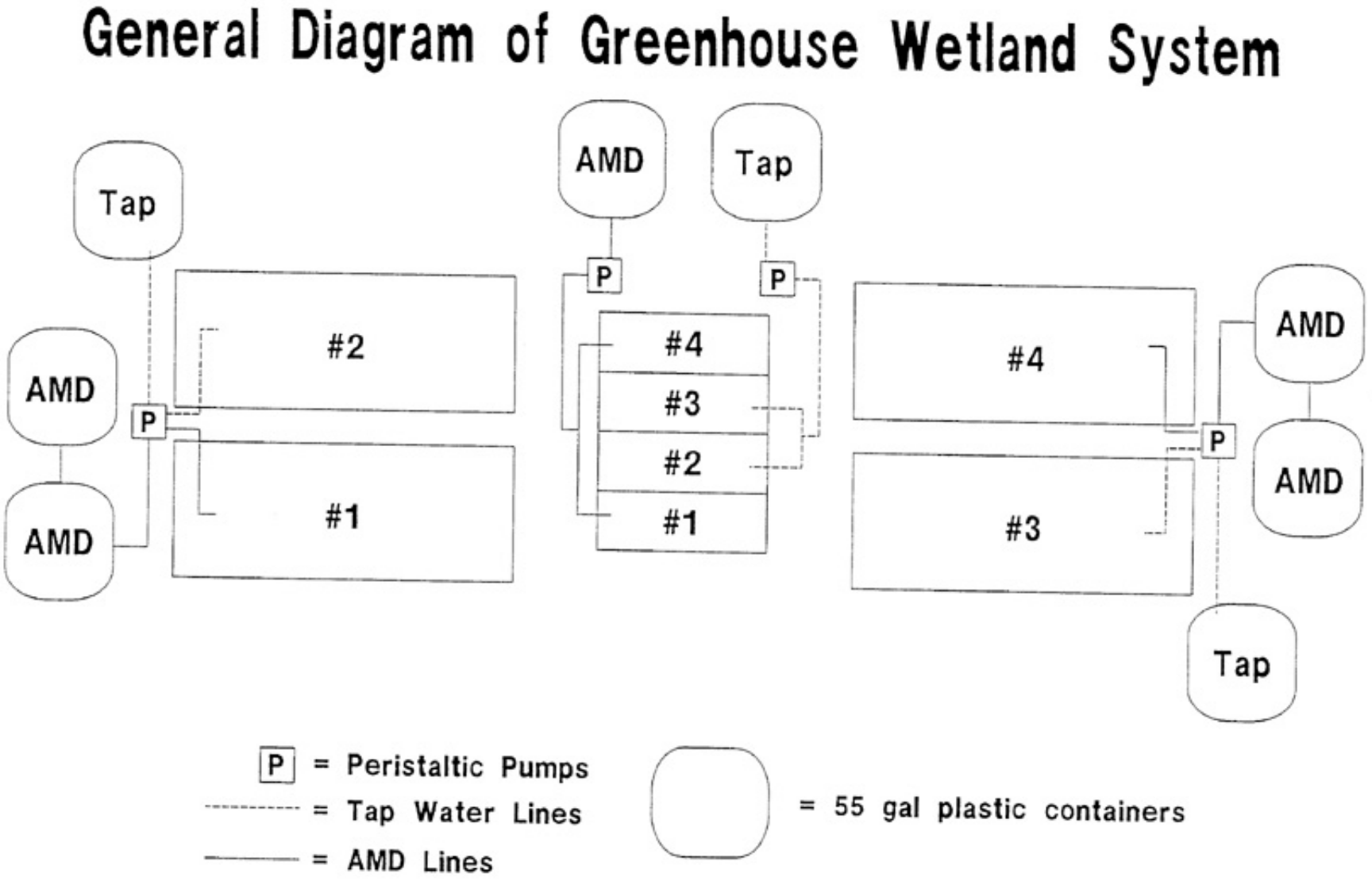
Direct measurements of sulfate reduction activity were made using radiolabelled $^{35}SO_4^{2-}$. Sulfate reduction appeared to be more active in AMD impacted wetlands, despite similar enumerable populations of SRB compared with controls (Table 2). Rates were generally higher at 12 and 22 cm compared with 32 cm, in both control and AMD-treated troughs. Relatively high rates of sulfate reduction in control sediments are attributable to nonlimiting sulfate in the tap water used to feed these wetlands. Rates in both systems were much higher than would be expected in non-sulfate impacted freshwater sediments and are comparable to rates normally observed in coastal marine sediments where sulfate reduction is a favored process for C mineralization.

We investigated the distribution of reduced iron sulfides in anaerobic slurries o AMD impacted sediments. Formation of FeS and to a lesser extent $FeSe_2$ from H_2S produced by microbial sulfate reduction was an important mechanism of iron removal from these sediments (Figure 4). Reduced iron sulfides increased with depth at all sampling points, and increased 10x in effluent compared with influent sediments. Sulfides accumulated primarily as acid volatile monosulfides (AVS), although production of pyritic disulfides (PS) was also noted in all samples.

CONCLUSIONS

Our data confirm that sulfate reduction plays an important role in removing iron during passage of AMD through anaerobic sediments of constructed Typha wetlands. Examination of influent and effluent water chemistry shows that the wetlands are retaining > than 66% of the iron applied in AMD over a period of one year. Using a mass balance approach, based on the distribution of total sulfides in the wetland (Figure 4) and assuming that all iron was removed as an iron monosulfide, sulfate reduction can account for between 15 to 20% of the total iron removal. Direct fractionation of both oxidized and reduced Fe species (data not reported in this paper) suggest that iron sulfides account for at least 16% of the total iron retained by the wetlands, confirming that our mass balance estimates of iron retention as sulfides are reasonable.

Figure 1.


General Diagram of Greenhouse Wetland System
Tap
AMD
AMD
P
#2
#1
AMD
Tap
P
P
#4
#3
#2
#1
#4
#3
P
AMD
AMD
Tap
P = Peristaltic Pumps
= Tap Water Lines
= AMD Lines
= 55 gal plastic containers

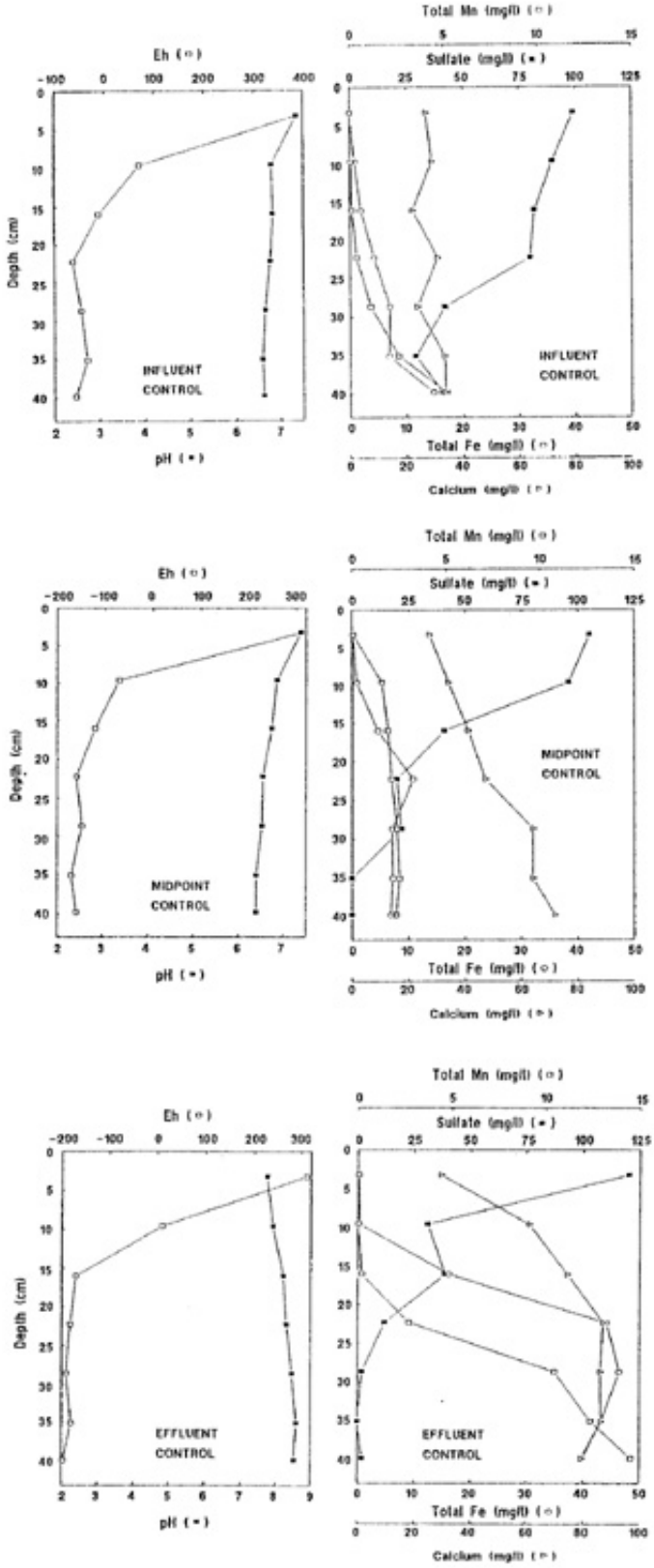

Eh (○)
pH (•)
Depth (cm)
INFLUENT
CONTROL
Total Mn (mg/l) (○)
Sulfate (mg/l) (•)
Total Fe (mg/l) (○)
Calcium (mg/l) (▷)
INFLUENT
CONTROL
MIDPOINT
CONTROL
MIDPOINT
CONTROL
EFFLUENT
CONTROL
EFFLUENT
CONTROL

Figure 2.

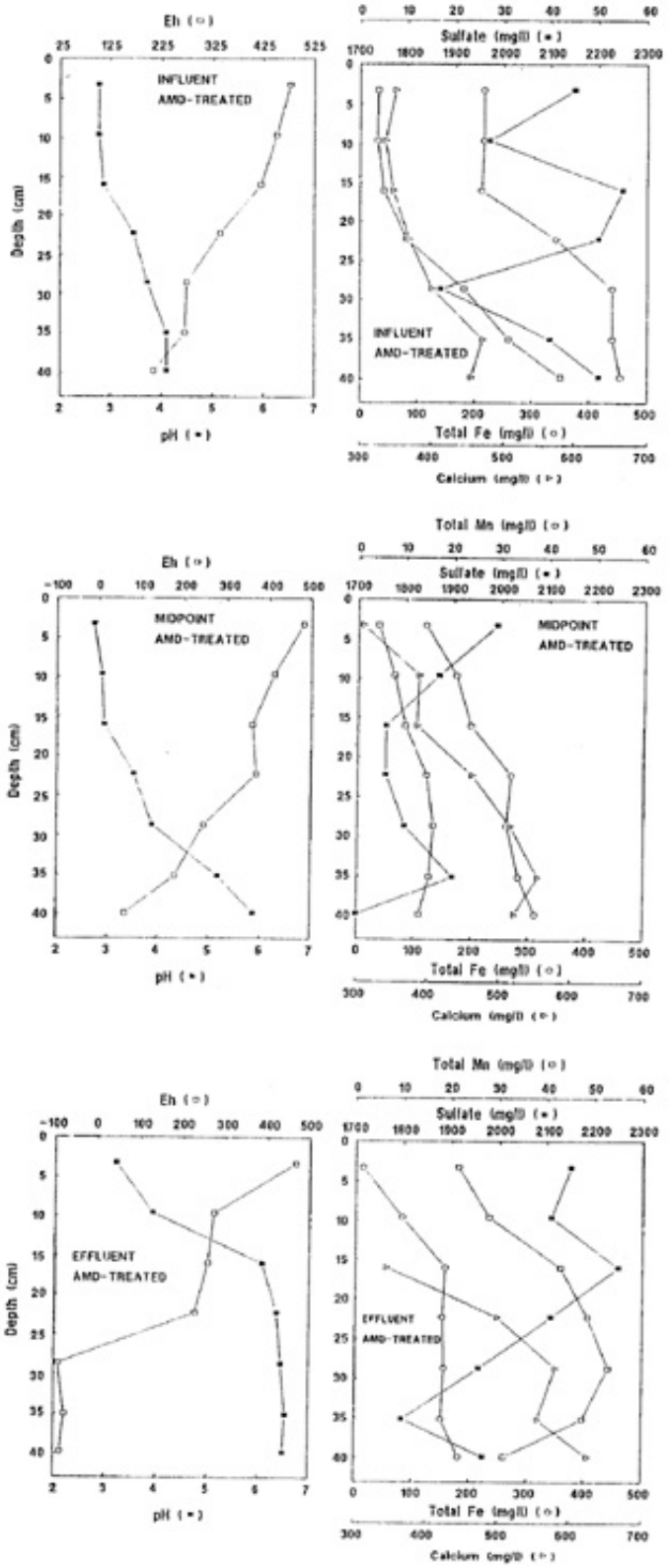

Eh (○)
Sulfate (mg/l) (●)
INFLUENT
AMD-TREATED
Depth (cm)
pH (●)
Total Fe (mg/l) (○)
Calcium (mg/l) (▷)
Total Mn (mg/l) (○)
MIDPOINT
AMD-TREATED
EFFLUENT
AMD-TREATED

Figure 3.

Figure 4.

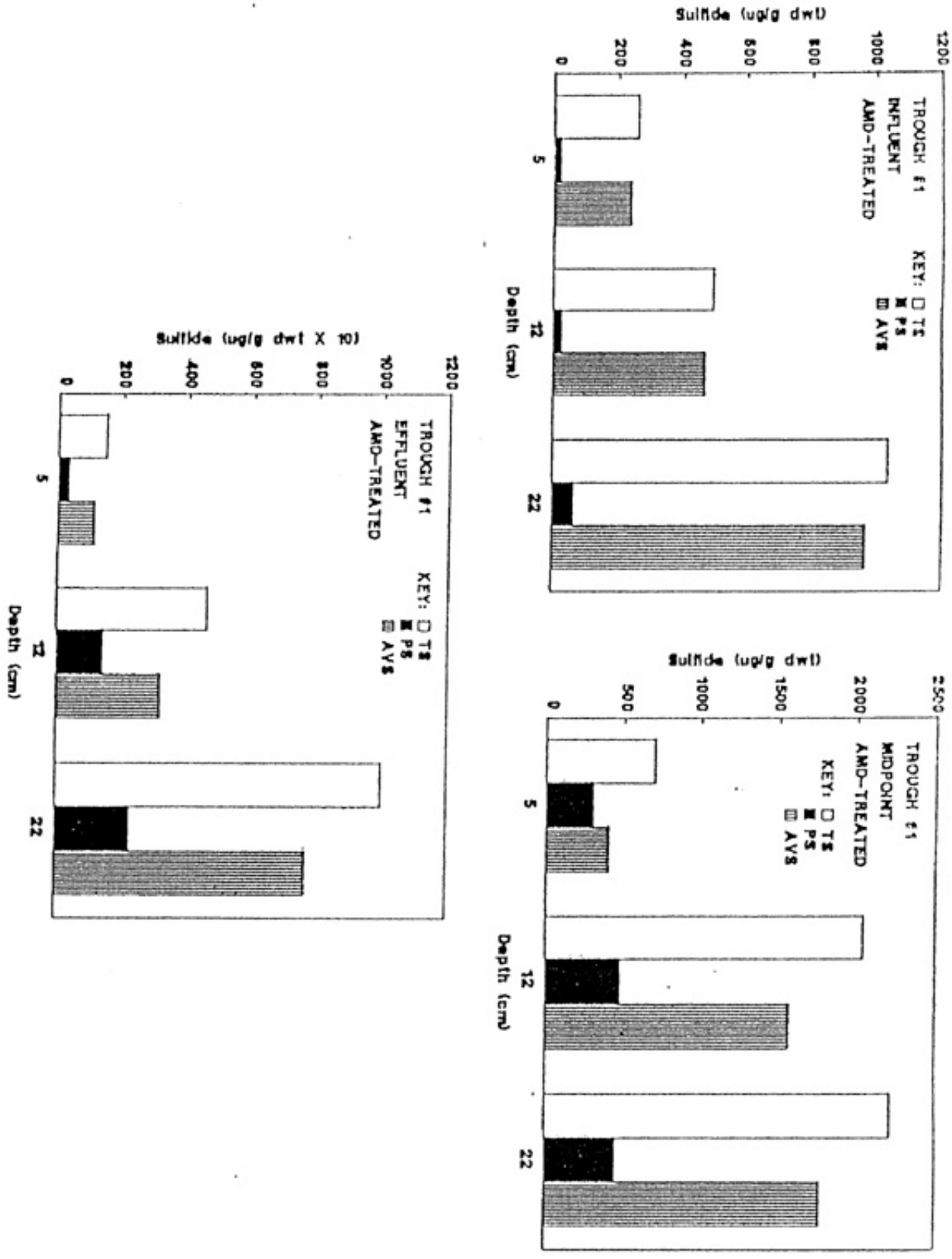

Sulfide (ug/g dwt)
0
200
400
600
800
1000
1200
TROUGH #1
INFLUENT
AMD-TREATED
KEY: TS PS AVS
5
12
22
Depth (cm)
Sulfide (ug/g dwt X 10)
TROUGH #1
EFFLUENT
AMD-TREATED
KEY: TS PS AVS
Sulfide (ug/g dwt)
0
500
1000
1500
2000
2500
TROUGH #1
MIDPOINT
AMD-TREATED
KEY: TS PS AVS

Table 1. Quantitation of Sulfate-reducing Bacteria in Control and AMD-treated Greenhouse Typha Wetland Systems.

		MPN (x 10^2) / g dwt							
		Baseline		3 Months		7 Months		10 Months	
Wetland	Depth	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Control	12cm	890	27	310	2600	140000	28000	15000	17000
	22cm	7600	42	1100	230	12000	17000	7990	17000
	32cm	3800	210	1900	4700	990	8000	2210	1300
AMD	12cm	69	580	3	9	1400	46000	12000	5560
	22cm	1600	190	72	870	3100	16000	6900	770
	32cm	1600	230	260	1300	89000	31000	15000	2300

Table 2. $^{35}SO_4^{2-}$- Reduction In Greenhouse Wetland Sediments

	Control		AMD-Treated	
Sample	SO_4^{2-} Reduction (n moles gwm^{-1} day^{-1})	[SO_4^{2-}] (n moles gwm^{-1})	SO_4^{2-} Reduction (n moles gwm^{-1} day^{-1})	[SO_4^{2-}] (n moles gwm^{-1})
Influent:				
12cm	74	251	247	16,589
22cm	43	573	169	18,215
32cm	67	398	73	19,663
Midpoint:				
12cm	38	685	218	16,437
22cm	88	340	133	17,006
32cm	25	163	89	20,911
Effluent:				
12cm	67	371	186	11,640
22cm	38	199	121	15,829
32cm	30	156	60	19,397