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**pH AND DISSOLVED OXYGEN AS FACTORS CONTROLLING  
TREATMENT EFFICIENCIES IN WET SUBSTRATE,  
BIO-REACTORS DOMINATED BY  
SULFATE-REDUCING BACTERIA**

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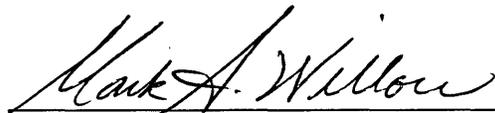
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Golden, Colorado

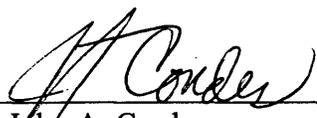
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## ABSTRACT

Four, wet-substrate, anaerobic, pilot-scale bio-reactors were constructed to examine if pH and dissolved oxygen of influent wastewaters are factors limiting the removal efficiency of heavy metals from acid-mine drainage. Previous field and laboratory studies have indicated that bacterial sulfate reduction and subsequent metal-sulfide precipitation are important processes in the removal of heavy metals by in Passive Mine Drainage Treatment Systems. The limited environmental conditions at which sulfate-reducing bacteria are active make it imperative to optimize environmental variables in the bio-reactors.

Four, column reactors in series, were fed with several formulations of synthetic mine drainages in an upflow, hydraulic configuration. Two experiments were conducted to examine the heavy metal removal rates and efficiencies utilizing a circum-neutral mine drainage containing zinc, manganese and cadmium. Two additional experiments utilized the more typical low pH synthetic mine drainage (pH = 2.7 su). One feedstock was deoxygenated by chemical deaeration, while the second was left unchanged. An additional, smaller scale reactor was constructed for use in determining the sorptive capacity of the organic substrate when being supplied with a circum-neutral, metal-laden, synthetic mine drainage. Finally, a batch reactor test was conducted on the composted

livestock manure used in the previous experiments to help quantify the sorptive capacity of the substrate.

Analysis of the results indicate that bacterial sulfate reduction in each of the experiments (and reactors) is a zero order reaction relative to sulfate concentration and may be the factor that controls the mass removal efficiencies of the reactor. The mass removal rate of metals exceeded the constant rate of sulfide production at short and long hydraulic detention times. The sorptive capacities of the reactors had not been fully exhausted during the experiments and the mass removal rates of metals exceeding the mass production of sulfide can be attributed to sorption of the metal cations to the organic substrate.

The results from the low pH, low dissolved oxygen and the normal dissolved oxygen experiments suggest that dissolved oxygen is not a limiting factor to sulfide production and metal sulfide precipitation. All dissolved oxygen is removed from the influent water within a few centimeters passage through the organic substrate. However, the reduced pH in the influent did lower the sulfate reduction capacity of the system, suggesting that the bacterial reactions producing sulfides may have been inhibited.

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## ACKNOWLEDGMENTS

A special thanks to my thesis advisor and mentor, Dr. Ronald R.H. Cohen for his scientific, technical and financial support during the course of this research. Appreciation is also extended to my thesis advisory committee: Dr. Linda Figueroa, Dr. Ronald Klusman, and Dr. Leslie Thompson.

Special appreciation is expended to Dr. Leslie Thompson and the staff at Pintail Systems, Inc., for providing technical assistance, equipment and laboratory space in which to conduct this research.

Finally, I would like to thank the Division of Environmental Science and Engineering, Mary Lanphear, Toshi Ozawa, and John Spear for their professional assistance and encouragement.

## **DEDICATION**

This thesis is dedicated to my wife, Amy, without whose unwavering support and understanding this project would not have been possible.

## 1.0 INTRODUCTION

### 1.1 Acid-Mine Drainage

Acid-mine drainage (AMD) results from the exposure of sulfide minerals, particularly pyritic and pyrrhotitic minerals, to atmospheric oxygen and water. An oxidizing environment is established in which the subsequent biological and chemical reactions generate sulfuric acid formation and mobilize heavy metals associated with the particular ore, waste rock, and/or tailings from mining operations. Both operational and abandoned mine works contribute to AMD. When insufficient calcareous materials are present to neutralize the acid-mine drainage, the rate of chemical reactions tends to increase with temperature and acidity, partially due to the catalyzing effects of naturally occurring bacteria, such as *Thiobacillus ferroxidans*, and excess ferric iron (Knapp, 1987).

Infiltration of snowmelt and rainfall into mine workings and tailings perpetuates the contamination of downstream aquatic environments. High concentrations of sulfate and ferrous iron and additional base metals such as lead, zinc, copper, cadmium and manganese may result. This aquatic impairment not only poses an immediate threat to the fauna and flora of the region, but, left unchecked, often impacts deleteriously upon

human activities such as recreation, irrigation, and livestock watering for the duration of AMD generation. These impacts could last hundreds of years (Filion et al., 1990)

### **1.1.1 Regional History and Impacts**

Acid-mine drainage directly impacts nearly 7,000 kilometers of streams and rivers and over 23,000 hectares of lakes and reservoirs throughout this country, while indirectly contributing to contamination of 20,000 kilometers and 70,000 hectares, respectively (Kleinmann, 1989).

The predominant source of AMD in the eastern United States is coal mining. The oxidation of pyrite and marcasite found in and around anthracite coal deposits has acidified thousands of kilometers of streams and rivers draining the Appalachian coal fields (Rahn, 1992). The results are waterways with less than pH 7 and a characteristic red-orange color, signifying the presence of oxidized iron. The predominant AMD generating source in the western United States is metal mine workings, whose drainage often contains metals such as cadmium, lead, nickel, copper and zinc as well as iron and manganese (Wildeman, 1991).

### **1.1.2 Impacts on Colorado**

Emerick (1988) suggests that over 2,000 kilometers of streams and rivers in Colorado may currently be exceeding water quality standards for heavy metal concentrations due to abandoned mining operations. Most of this contamination is the result of metal, or "hard-rock," mining in the Rocky Mountain region. As the ore veins are mined, the mineral surfaces become exposed to oxygen and water, especially in the waste rock and tailings piles which are discarded outside each mine portal. When the mine becomes flooded with infiltrating groundwater, additional shafts may be constructed to facilitate drainage or to lower the water table in order to expose deeper deposits. More acid-producing minerals are then exposed to oxidation (Wildeman, 1991). The resultant drainage is typically acidic with higher than normal concentrations of metals, and ultimately discharges into the closest surface and groundwater drainage systems. Typical "hard-rock" AMDs have been investigated in the Argo and Big Five Tunnels in Idaho Springs, Colorado and the Eagle Mine in Minturn, Colorado. Both drainages have been found to significantly degrade the quality of the downgradient streams and rivers.

### **1.1.3 Chalk Creek Case Study**

The Mary Murphy and Iron Chest Mines near Buena Vista, in central Colorado are sources of AMD. Both mines were in full operation from approximately 1870

through 1925, and on an intermittent basis from then until 1951 when all mining activities ceased. Sometime after operations had begun, an ore removal adit and drainage way ( the Golf Tunnel ) was constructed to facilitate ore transport from the mine workings to the mill site next to Chalk Creek. Both mines were eventually connected at this lowest level (Dings and Robinson, 1957).

The Golf Tunnel shaft offered both easier access to the mill site and a drainage adit from which meteoric infiltration and groundwater could be discharged. However, the mine was also open to discharges of metal-laden waters produced by the pyritic minerals in the raise and stopes. The extensive waste rock piles which had accumulated around the mill site and portals have leached acid and metals.

In 1986, there was a fish kill of over 800,000 trout fingerlings at the Colorado Division of Wildlife's Chalk Cliffs Fish Rearing Unit. The hatchery, located approximately twelve miles below the abandoned mine site, receives water from Chalk Creek. Chalk Creek water analysis indicated zinc and cadmium exceeding Colorado water quality standards.

The degradation resulting from this drainage has characteristics similar to that of other AMD. Closer investigation has revealed it to be of a unique character. The AMD is near neutral in pH while still maintaining a high metals loading, especially for dissolved zinc, manganese and cadmium. Table 1 is data collected on 6/13/1994 from a point in the

Golf Tunnel upstream to a seepage fracture which is contributing high alkaline, low metal laden waters.

**Table 1: Golf Tunnel Adit Drainage Chemistry.**

<b>Chemical Constituent</b>	<b>Value</b>
pH	6.05 su
Zn <sup>2+</sup>	157 mg/L
Mn <sup>2+</sup>	131 mg/L
Al <sup>3+</sup>	5.0 mg/L
Cu <sup>2+</sup>	11.6 mg/L
Fe <sup>3+</sup>	< 0.1 mg/L
Cd <sup>2+</sup>	0.86 mg/L
SO <sub>4</sub> <sup>2-</sup>	1262 mg/L
Cl <sup>-</sup>	20 mg/L

The potential threat that the contamination of Chalk Creek poses to the Arkansas River Basin, which supplies both irrigation and drinking water for a significant number of municipalities, resulted in it being included as part of Colorado's Non-point Source Program for water pollution in 1990. The United States Environmental Protection Agency, Colorado Department of Health, and the U.S. Bureau of Mines, in cooperation with the Colorado Division of Minerals and Geology, have collected data and formulated a preliminary assessment of the site. They surmise the existence of three contamination sources: leachates from the waste rock and tailings piles; drainage discharges from the

Golf Tunnel adit; and a third, as yet unidentified source. This third may be contaminated ground water (SAIC, 1993). Except for the discharge from the Golf Tunnel adit, no other identifiable point source exists, making remediation difficult. However, the adit does present an opportunity for further development of mine drainage treatment methods.

## **1.2 Acid-Mine Drainage Treatment Techniques**

Nearly \$1 million is spent each day on acid-mine drainage prevention and abatement by mining companies throughout the U.S. (Kleinmann and Hedin, 1993). The typical AMD wastewater treatment processes employed by active mines are composed of three phases (Boling et al., 1992): 1) chemical oxidation, 2) lime/caustic soda softening, and 3) sulfide addition, though the last method is infrequently used. The chemical oxidation phase primarily targets iron, manganese, and aluminum in an attempt to precipitate these metals as hydroxides through the addition of oxidizing reagents such as chlorine and potassium permanganate. The pH is raised from around 2 to 7.5.

Sedimentation and filtration are required in order to remove the solid phase precipitates prior to water discharge into receiving waters. The addition of lime/caustic soda raises the pH to nearly 10.5, thus facilitating the nearly 100% removal of dissolved cadmium, copper, lead, nickel, and zinc as hydroxides and carbonates. Again, a secondary sedimentation or filtration step is necessary. The introduction of sulfides, typically in the

form of hydrogen sulfide ( $H_2S$ ), at a pH of 8.5, results in heavy metals combining with the aqueous bisulfide ion and precipitating a solid sulfide phase. All three treatment processes are inefficient and/or potentially expensive. The addition of sulfides also requires constant monitoring to avert a low pH that could cause the release of  $H_2S$  gas, and to reduce effluent concentrations of  $H_2S$ .

The In-Line Aeration and Neutralization System (ILS) incorporates the chemical treatment processes into a functionally closed system where the treatment reactions can be more closely monitored and accelerated in order to reduce the chemical reagent costs and reaction processing times (Kleinmann, 1989). Electro-precipitation processes accomplish similar results by the precipitation of metal hydroxides or by metal ion adsorption (Jenke and Diebold, 1984). However, all of these processes, both chemical and physical, are severely limited in that they: 1) are unable to treat the sometimes excessive sulfate concentrations associated with most acid-mine drainage, 2) impart a high degree of hardness to the water, and 3) produce waste sludge which requires additional treatment and/or disposal (Grim and Hill, 1974). These methods can also require large capital costs, operations, and maintenance.

One alternative method has been investigated for the removal of manganese from circum-neutral waters. Gordon (1985) reported successful removal of manganese using a packed column reactor filled with 2-5 cm chert stones from the Duck River below Normandy Dam, Tennessee. A "black slime" developed on the stones, but the exact

nature of the removal mechanisms is yet undetermined. Possibilities range from adsorption, chemical oxidation and even bacterial mediation to achieve the near 100% removal efficiency. It was later discovered that the "black slime" could be successfully transferred to other medium, such as glass marbles. The efficiency of the system was solely a function of substrate surface area and hydraulic loading rates (Gordon and Burr, 1989; O'Neill, 1986). This system has only been tested on manganese concentrations of 2-15 mg/L, so it is uncertain if it would be equally efficient at concentrations in excess of 100 mg/L, such as those found in the Golf Tunnel discharges at Chalk Creek.

One innovative technique for the treatment of AMD has been the use of natural and artificial wetlands as a biological pollution abatement process. Kleinmann and Hedin (1993) describe the principal wetland types and relevant implementations: 1) aerobic wetlands: primarily involve the oxidation of iron and manganese and are found more frequently in conjunction with coal mining operations; they appear to be limited by dissolved oxygen concentrations and hydraulic detention times of the influent water, 2) organic substrate (compost) wetlands: commonly used where acidity of the influent water exceeds the alkalinity; wetlands may produce alkalinity through sulfate reduction and limestone dissolution; concerns exist about the bioaccumulation of toxic heavy metals, 3) anoxic limestone drains (ALDs): these can be used in conjunction with aerobic wetlands in order to raise the alkalinity and pH of the influent water prior to the oxidation reactions in the wetland; however, if the anoxic environment is breached, the iron could oxidize,

causing the resultant ferric hydroxides to coat the limestone, thus severely limiting its neutralizing capacity.

The self regenerative properties of the biological treatment systems reduce the need for continuous maintenance, offering an attractive, alternative abatement technology to the conventional systems. The biological systems also avoid the production of copious amounts of wet sludge associated with oxidative and hydrolytic processes (Silver, 1989). These Passive Mine Drainage Treatment Systems (PMDTS) (Holm and Elmore, 1986) can also operate at a fraction of the production and maintenance costs of the conventional chemical and physical treatment methods.

### **1.3 PMDTS Evolution and Limitations**

Reynolds (1991) and Machermer (1992) examined the chemical and biological processes in wetland treatment (PMDTS) systems receiving acid-mine drainage and found that the rate of sulfate reduction was, perhaps, the most crucial process involved. This conclusion has been corroborated by additional research on the utilization of natural and artificial wetlands for acid-mine drainage treatment (Cohen and Staub, 1992; Madel, 1992; Machermer and Wildeman, 1992; Staub, 1992; Tuttle et al., 1969b; Dvorak et al., 1991). Other processes in operation within these systems include the precipitation of ferric hydroxides and manganese carbonates, the subsequent adsorption of metals by the

ferric hydroxide, adsorption to the organic substrate, and the physical removal of colloidal particles through filtration by the substrate matrix. These processes contribute to the removal of contaminant metals from water, with the action of the sulfate-reducing bacteria in these systems controlling the efficiency of metal decontamination (Klusman and Macheimer, 1991).

The metal removing characteristics of the natural wetland have been improved upon with the development of PMDTSSs. Factors such as hydraulic conductivity, hydraulic detention time and loading rates can now be manipulated through a biological reactor (or bio-reactor) to provide greater removal efficiency and throughput. The generic wetland substrates have been replaced with organic materials in order to provide a more hospitable microenvironment for sulfate-reducing bacteria and other heterotrophic bacteria. The PMDTSSs have been limited by the low throughput, long contact and hydraulic detention times required to achieve higher than 95% removal rates of metals.

In order for the sulfate-reducing bacteria to thrive, they require a strict anaerobic environment (they are obligate anaerobes) with a pH in the range of 5-8 (Brown et. al., 1973). When pH and/or redox conditions are not optimum, the rate of microbial sulfate reduction declines. This, in turn, reduces metal removal capacity. The rapid influx of acidic, aerobic waters appears to drive the pH of the treatment system down and redox up, thus inhibiting bacterial sulfate reducing processes. The metal removal efficiency and

loading capacity of the treatment system then becomes a function of not only size and hydraulic conductivity, but of the acidity and oxygen content of the influent water.

#### **1.4 Hypothesis and Research Objectives**

Until PMDTS optimization research and development reduced hydraulic detention times required for 99% or better metal removal rates (Cohen and Staub, 1992), PMDTSs were constrained by their volume/discharge handling capacity. A PMDTS could treat metal waste streams effectively (99% or greater removal) for hydraulic detention times of 250-300 hours, or remove 40 - 70% of metals at shorter hydraulic detention times (Madel, 1992).

One of the limiting factors appears to be the pH of the influent water. A large influx of acidic water initiates metal contaminant breakthrough, with low treatment efficiency. The sulfate-reducing bacteria's capacity to generate bicarbonate ions to neutralize the acidic conditions cannot keep pace with the inflow rate of  $H^+$  ions, allowing the microenvironment of the system to become more acidic and thus inhibit the respiratory processes of the bacteria. These respiratory processes govern the production of hydrogen sulfide, the essential component in the treatment of metal-laden waters by a PMDTS. If the pH of the influent water were to be elevated prior to its introduction to the system, the bacteria might acquire a more optimum microenvironment, thus

permitting greater treatment efficiency. This increased efficiency due to the pre-neutralization of the mine drainage would permit the system to handle elevated influent metal loading rates while maintaining comparable treatment efficiencies to those of the longer hydraulic detention time, acidic water treatment systems.

A second possible limiting factor in the PMDTS at higher inflow rates is the introduction of dissolved oxygen to the system by the AMD. An increased loading rate of oxygen would reduce the capacity of sulfate-reducing bacteria, which require anaerobic conditions, to produce hydrogen sulfide. In an organic substrate PMDTS, microbes will consume the influent oxygen and create an anaerobic microenvironment suitable for the sulfate-reducing bacteria. With a greater influx of dissolved oxygen, it is reasonable to assume that redox potential would increase, sulfate reduction would decrease, and metals treatment efficiency would decrease. If the dissolved oxygen content of the influent mine drainage could be reduced prior to entering the treatment system, the anaerobic conditions could be maintained for a greater volume of the treatment substrate, thus allowing for an increased influent treatment capacity.

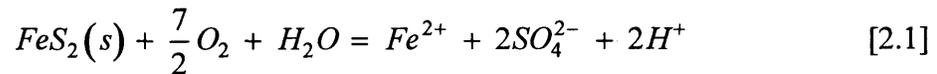
The objectives of this research are; 1. to discover if pH and dissolved oxygen concentration of the influent drainage waters are limiting or inhibiting factors for a PMDTS, and 2. by increasing the pH or decreasing the dissolved oxygen of these waters, the treatment capacity of the systems can be increased, thus allowing for greater loading

rates with shorter hydraulic detention times while maintaining 90% or higher treatment efficiencies.

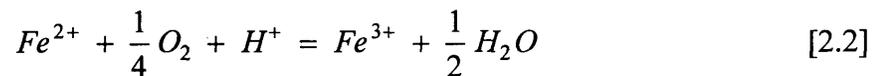
## 2.0 GENERAL BACKGROUND

### 2.1 Chemistry of Acid-Mine Drainage

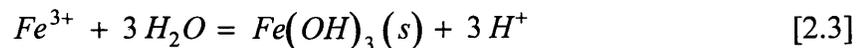
Pyrite ( $FeS_2$ ) is the primary constituent mineral in both coal and metal mining operations from which acid-mine drainage is produced. The following equations describe the two-step process by which acid-mine drainage is formed through the oxidation of pyrite (Stumm and Morgan, 1981):



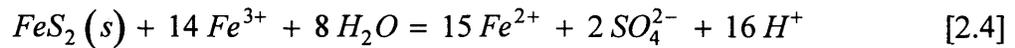
The oxidation of the solid sulfide upon exposure to oxygen and water releases ferrous iron, sulfate and acidity to the water.



The ferrous iron then oxidizes to produce ferric iron and water.

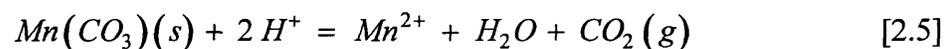


Hydrolysis of the ferric iron forms solid ferric hydroxide plus additional acidity.



The ferric iron from equation [2.2] can subsequently oxidize additional pyrite, releasing ferrous iron and acidity. Once initiated, free oxygen is not required for the oxidation of pyrite. According to Stumm and Morgan (1981), the oxidation of the pyrite is catalyzed by autotrophic iron bacteria *Thiobacillus* and *Ferrobacillus ferrooxidans* through the oxidation of ferrous iron. Because iron sulfides are the most prevalent of sulfide minerals, this catalyzed reaction becomes uniquely significant when dealing with the formation and remediation of acid-mine drainage.

The acidity produced by the oxidation of pyritic minerals can then proceed to weather additional minerals into solution. For example, the weathering of rhodochrosite ( $MnCO_3$ ) is facilitated by acidity (Wildeman, 1991):



If the  $CO_2$  gas exolves from solution, the manganese will be unable to re-precipitate under the prevailing acidity.

## **2.2 Sulfate-Reducing Bacteria**

Sulfate-reducing bacteria are both widespread and diverse in nature. However, it is significant to distinguish between assimilative and dissimilative sulfate-reducing bacteria. Most bacteria utilize sulfate as a sulfur source in the biosynthesis of amino acids, while only a few specialized, anaerobic bacteria can utilize the sulfate ion for energy generation. SRBs inhabit both terrestrial and aquatic environments and occupy at least 13 distinct taxonomic genera (Brock et al., 1994).

### **2.2.1 Dissimilatory Sulfate Reduction**

Dissimilatory sulfate reduction is the process by which sulfate acts as an electron acceptor during the oxidation of organic matter, similar to the use of oxygen by aerobic organisms. These sulfate-reducing bacteria are obligate anaerobes. They requiring strict anaerobic conditions in order to facilitate successful propagation and sulfate reduction (Nriagu, 1978). There are thirteen genera recognized as dissimilatory sulfate reducers having the ability to exploit sulfate as an electron acceptor for anaerobic respiration and subsequently excrete, as an end product of this reaction,  $H_2S$ , an important component in many biogeochemical processes (Brock et al., 1994). The sulfate anion is chemically stable and will not spontaneously reduce under ambient environmental conditions. During respiratory processes, the sulfate-reducing bacteria oxidize simple organic compounds

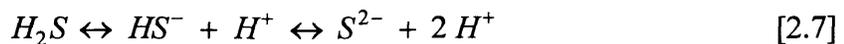
(represented by the chemical formula  $CH_2O$ ) resulting in the formation of hydrogen sulfide and bicarbonate ions at the circum-neutral pH's of "optimal" sulfate reduction (Dvorak et al., 1992):



The electron donors for this reaction can include molecular hydrogen ( $H_2$ ) and organic compounds such as acetate and lactate. The formation of bicarbonate indicates the ability of the sulfate-reducing bacteria to control the pH of their particular microenvironment (Cohen and Staub, 1992; Staub, 1992).

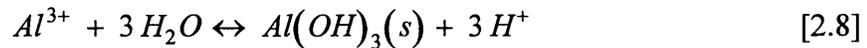
There exists evidence of competition between methanogenic bacteria and sulfate-reducing bacteria for available electron donors. If sufficient sulfate is present, and redox conditions are favorable, the sulfate-reducing bacteria appear to be favored. This dependence on organic electron donors (or  $H_2$  gas which is itself a product of organic fermentation) limits sulfate reduction to environments containing large amounts of organic matter (Brock et al., 1994). Sulfate reduction may then become carbon limited in substrates with low organic matter content, such as some marine sediments.

The form of the sulfide from the bacterially mediated sulfate reduction is dependent upon the pH of the reaction and is represented by the equilibrium:

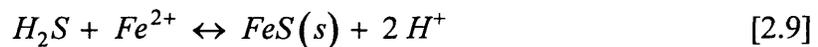


Both  $HS^-$ , which predominates at neutral pH, and  $S^{2-}$ , which occurs at high pH, are soluble in water, while  $H_2S$ , the predominant form at low pH, is not soluble and tends to exolve from solution, even at neutral pH where it is equilibrium with  $HS^-$ . Soluble sulfide concentrations, if excessively elevated, can become toxic to both indigenous aquatic lifeforms as well as the sulfate-reducing bacteria themselves.

The bicarbonate ion formed during sulfate reduction will equilibrate between  $CO_2$ ,  $HCO_3^-$  and  $CO_3^{2-}$ . However, the predominant form at the “optimal” pH range for dissimilatory sulfate reduction will be the bicarbonate ion. This rise in pH will facilitate the hydrolysis and precipitation of some contaminant metals from acidic waters as insoluble hydroxides and oxides (Stumm and Morgan, 1981):



The hydrogen sulfide created during the reduction reaction also will react with many metal species, forming insoluble metal sulfide precipitates:



These processes all contribute to the removal of contaminant metals from waste waters through the action of sulfate-reducing bacteria.

### 2.2.2 Optimization of Bacterial Sulfate Reduction

Sulfate-reducing bacteria require precise environmental conditions for both subsistence and efficient reduction of sulfate. As obligate anaerobes, it is important that their particular microenvironment remain anoxic, and that an oxidation-reduction potential, or  $E_h$ , of less than -100 mV be maintained (Brown et al., 1973; Reynolds, 1991; Tuttle et al., 1969b; Connell and Patrick, 1968). Since  $E_h$  is dependent, among other parameters, upon pH, it becomes necessary to maintain a pH of 5-8 as a condition of sulfate reduction. Wakao et al. (1979) determined that sulfate reduction actually begins around pH 4.5, but that additional alkalinity is required to facilitate more efficient sulfate reduction by the bacteria. As previously reported, the sulfate reduction reaction is capable of contributing  $HCO_3^-$  alkalinity to the system in order to maintain an “optimal” pH range. Lower pH inhibits sulfate reduction and increases the solubility of any metal sulfides that may have been formed (Widdel, 1988). High loading rates of acidic water may overwhelm the bacteria’s capacity to neutralize the influent water and maintain an “optimal” microenvironment.

Reynolds (1991) also reported an “optimal” temperature for bacterial sulfate reduction as 32 degrees celcius, while Batal (1990) observed that, at lower temperatures, the sulfate-reducing bacteria were able to compensate for decreased bacterial activity by

increasing their population size per unit area, thus maintaining a similar community reduction rate.

When all of these conditions; anoxic, organic carbon rich substrate, sulfate source and a physical matrix for metal precipitate capture, can be created and maintained, bacterial sulfate reduction can be used as an effective instrument in the treatment/removal of contaminant metals from water.

### **2.3 Passive Mine Drainage Treatment Systems**

The application of relatively maintenance free, low cost, biogeochemical water treatment at or near the source of AMD is the principal aim of Passive Mine Drainage Systems. Optimized for SRB induced precipitation of metals, they are proving to be an effective tool in AMD abatement.

#### **2.3.1 Adsorption and Complexation Processes**

Machemer (1992) and Staub (1992) examined the processes for heavy metal removal in Passive Mine Drainage Treatment Systems and concluded that, in addition to bacterial sulfate reduction, adsorption and complexation contributed significantly to removal.

Adsorption involves the interphase accumulation of particles at an interface boundary due to electrostatic forces, such as the attraction of positively charged cations like  $Zn^{2+}$  to an oppositely charged solid surface, such as the organic substrate of the reactor (Weber, 1972). Complexation refers to the reversible reaction of two species, for instance, a metal cation and an exposed, solid organic ligand surface derived from the dissociation of humic materials, which combine to create a third species (Morel and Hering, 1993). These processes effectively detain, but do not necessarily retain, the metal cation and remove it from solution. However, adsorption and complexation are transient in nature, have limited capacity, and eventually cease to contribute to additional removal of heavy metals (Machemer, 1992; Weider et al., 1988).

Experiments conducted on mine waters of the Big Five Tunnel in Idaho Springs, Colorado, revealed that, if sulfate reduction is inhibited, copper, iron, manganese and zinc were almost completely removed from the drainage water, but that the manganese and zinc reappeared in the effluent after approximately four weeks. An explanation for this phenomenon is that all of the sorption sites had become occupied during the first four weeks of operation and subsequent competition for sorption sites forced the zinc and manganese to desorb and be replaced by iron and copper. Adsorption and complexation processes for the removal of contaminant metals, though exceedingly effective in the beginning, prove limited in duration of treatment and are therefore incapable of multiple year, sustained treatment efficiency as expected for bio-reactors. It is evident that the

activity of the sulfate-reducing bacteria is responsible for the long term of treatment effectiveness.

### **2.3.2 Hydraulic Conductivity and Design**

A recurring difficulty observed in Passive Mine Drainage Treatment Systems has been the loss of substrate permeability with operating time. Hydraulic short-circuiting and pore blockage have been reported in downflow and horizontal flow reactors, principally due to gravity compaction of the substrate. Lemke (1989) examined the use of substrate additives and the removal of finer substrate fractions which could clog interstitial spaces, but both remedies failed to overcome the compaction dilemma. However, a three order of magnitude increase in permeability was achieved with the conversion of the hydraulic regime from a horizontal configuration to an upflow system. With the compaction problem resolved, substrate additives could be used to augment flow as well as contact area in order to maximize metal removing efficiencies for a particular system.

### **2.3.3 Flow Rates and Hydraulic Detention Times**

Madel (1992) and Staub (1992) both examined the significance of flow rates and hydraulic detention times in bio-reactors used to treat AMD and discovered that, at

hydraulic detention times of 50 h and less, the pH and metal removing efficiencies of the systems decreases. Greater than 50 hour hydraulic detention times yielded near 100% removal rates for most all contaminant metals while at hydraulic detention times less than 50 h, some metals, especially iron, zinc and manganese were only nominally removed. It can be speculated that these removal rates will drop even more with shorter hydraulic detention times when dealing with acidic drainages. A significant decrease in sulfate concentrations was believed to be the result of reduction processes.

The removal of manganese has proven to be difficult and is highly dependent on the pH of the system. Staub (1992) concluded that if the pH could be maintained near neutral, then complete manganese removal was probable. This inference may result from the discovery that increased flow rates (i.e. shorter hydraulic detention times) of acidic drainage not only drives the system to a lower pH, but also increases its  $E_h$ , inhibiting bacterial activities and metal removing processes.

Ozawa (1994) showed that a hydraulic detention time of 38.8 h was insufficient to remove chromium and other dissolved metals from AMD to greater than 99% removal and that longer hydraulic detention times may be required to optimize PMDTSS. At 38.8 h hydraulic detention time, 70 - 80% removal was common.

### **2.3.4 $E_h$ and pH Dependence**

$E_h$ , or the oxidation-reduction potential (ORP), is a measure of the aqueous electron activity of a system while pH is a measure of the hydrogen ion, or proton, activity. Because the electron and the proton exhibit equal yet opposite electrostatic charges, and thus neutralize each other, their activities tend to exist as converses. For example, a high positive  $E_h$  value, indicating a low electron activity, typically occurs with low pH values, or high proton activity (Langmuir, 1971).

It has been demonstrated that efficient bacterial sulfate reduction is initiated and maintained by low oxidation-reduction potentials. This is due in part by the precise anaerobic environmental requirements of dissimilatory sulfate-reducing bacteria (Postgate, 1984; Connell and Patrick, 1968; Brown et al., 1973; Tuttle et al., 1969a). Exponential bacterial growth has been observed at an  $E_h$  of -150 mV with a corresponding pH of 6.95.

The additional bacteria produced under these conditions contributed significantly to the reduction of sulfate in the system (Brown et al., 1973)(Figure 1). This ideal environment is achieved through the anaerobic conditions created by the microbial decomposition of the organic substrates and the formation of sulfides from the reduction processes, which exhibits an  $E_h$  of -75 mV (Staub, 1992; Harter and McLean, 1965). The subsequent reintroduction of oxygen into this system will raise the  $E_h$ . It is, therefore, reasonable to assume that the influent dissolved oxygen inhibits bacterial productivity and diminished

their sulfate-reducing capabilities. The accumulation of sulfides within the system, though desirable as a means to initially lower the  $E_h$ , will eventually inhibit bacterial respiration, making it necessary to remove it in order to facilitate continued growth and sulfate reduction processes (Postgate, 1984). This may be accomplished either through its removal as hydrogen sulfide gas or as a solid metal sulfide. This metal sulfide may remain within the system, however, the sulfide has been isolated from a growth inhibiting role. The equilibrium form of the sulfide is a function of the system pH, as indicated by the  $E_h$ -pH diagram for sulfur. The exsolution of hydrogen sulfide gas from the PMDTS would be undesirable due to its toxicity, so low pH should be avoided as much as possible.

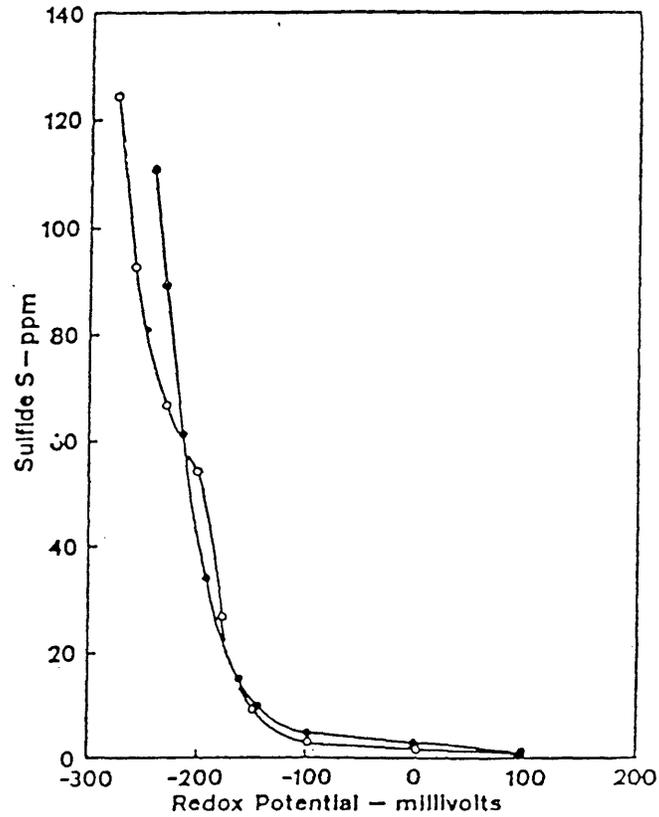


Figure 1: Reduction of sulfate to sulfide at controlled redox potentials (Connell and Patrick, 1968).

The pH of the system not only controls the equilibrium form of the sulfide ion, but also governs the growth rate of the sulfate-reducing bacteria and the solubility of the metals in the system. Connell and Patrick (1968) determined that the effective maximum growth rate of sulfate-reducing bacteria in a PMDTS occurred between a pH of 5 and 8,

though they could be sustained at a lower pH without complete mortality. Figure 2 illustrates this point.

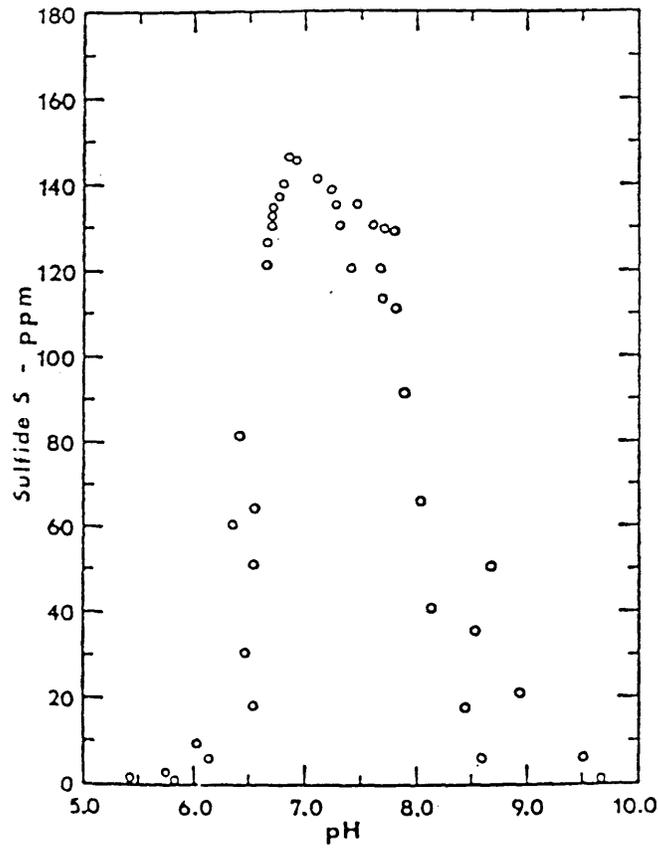


Figure 2: Reduction of sulfate to sulfite as affected by pH (Connell and Patrick, 1968).

The near neutral reactor pH values are a result of, among other things, the dissolution of carbonate materials in the substrate and the formation of bicarbonate from the sulfate reduction reactions, the production of  $NH_3$ ,  $H_2S$  and  $CH_4$ , all of which remove  $H^+$  from the system. It is believed that the rapid influx of highly acidic water could overwhelm the neutralizing capabilities of the system and the bacteria, and thus inhibit bacterial growth and sulfate reduction. Additionally, this low pH induced in the system will solublize metal-containing solids, releasing the metals once again into solution.

### **2.3.5 Substrate Selection**

The substrate not only acts as a physical matrix onto which bacterial biomass may attach and within which metal sulfide precipitates become enmeshed, but it also provides the essential organic carbon nutrient source for the sulfate-reducing bacteria. Research into the optimum organic substrates available for use in a PMDTS has been conducted for the past several years. From corn and rice wastes, to peat and mushroom compost, researchers have examined each for metal removing efficiency and hydraulic conductivity (Brodie et al., 1988; Rabenhorst et al., 1988). Peat has proven inefficient and short-lived in removing metals, while mushroom compost was partially successful at low inflow rates (Cohen et al., 1989). The ineffectiveness of the peat may be the result of the fact that peat, by its very nature, has a pH of approximately 4.5 and the organic matter has

advanced to the end of the decomposition process and has little, if any, organic carbon readily available for oxidation.

Two substrates which have exhibited exceptional potential for supporting high rates of sulfate reduction are decomposed wood chips and composted livestock manure (Reynolds, 1991; Bolis, 1992; Cohen and Staub, 1992). Both have shown an ability to buffer the inflow and system to near neutral pH more effectively than the aforementioned substrates. This is especially important when attempting to treat highly acidic drainages.

Bolis (1992) and Reynolds (1991) also found that composted livestock manure amended with hay or hay extract yielded the highest sulfate reduction rates yet encountered.

Additionally, the manure is of such high organic carbon content that the system does not require frequent recharging.

### 3.0 EXPERIMENTAL METHODOLOGY

#### 3.1 Wet-Substrate, Anaerobic Bio-reactor Design

The experimental apparatus consisted of four upflow, wet-substrate, anaerobic bio-reactors in parallel. Columns A<sub>1</sub> and A<sub>2</sub> were constructed of clear PVC with an inside diameter of 16.5 cm. Columns B<sub>1</sub> and B<sub>2</sub> were of opaque PVC with an inside diameter of 15.2 cm (Figures 3 and 4).

Because all four columns were not identical in total length, a substrate depth of 143 cm was chosen for each. By standardizing the length of the reactor beds, columns A<sub>1</sub> and A<sub>2</sub> had 4.4 L larger volumes than columns B<sub>1</sub> and B<sub>2</sub>. This difference in reactor volume, and the variation in flow rates delivered to the four reactor columns, allowed us to operate the reactors at different discrete hydraulic detention times while they were supplied with an identical synthetic mine drainage. See Table 2 for column specifications.

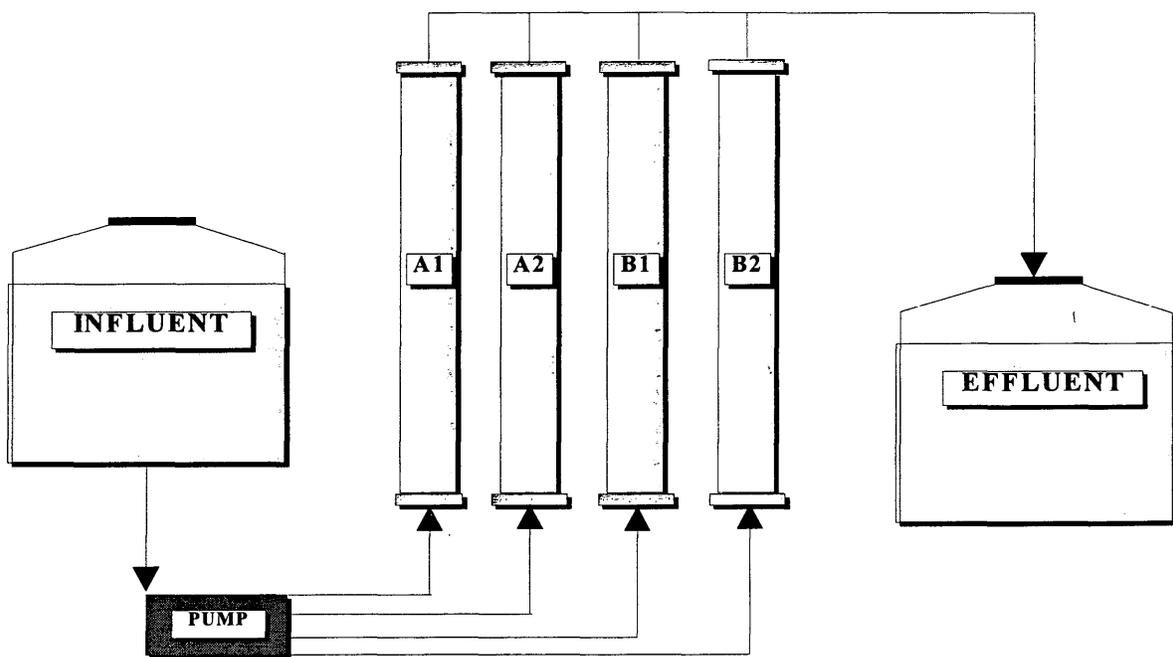


Figure 3: Wet-substrate bio-reactor system design. Reactors A<sub>1</sub> and A<sub>2</sub> had an operating, substrate bed volume of 29 L. Reactors B<sub>1</sub> and B<sub>2</sub> had volumes of 25 L.

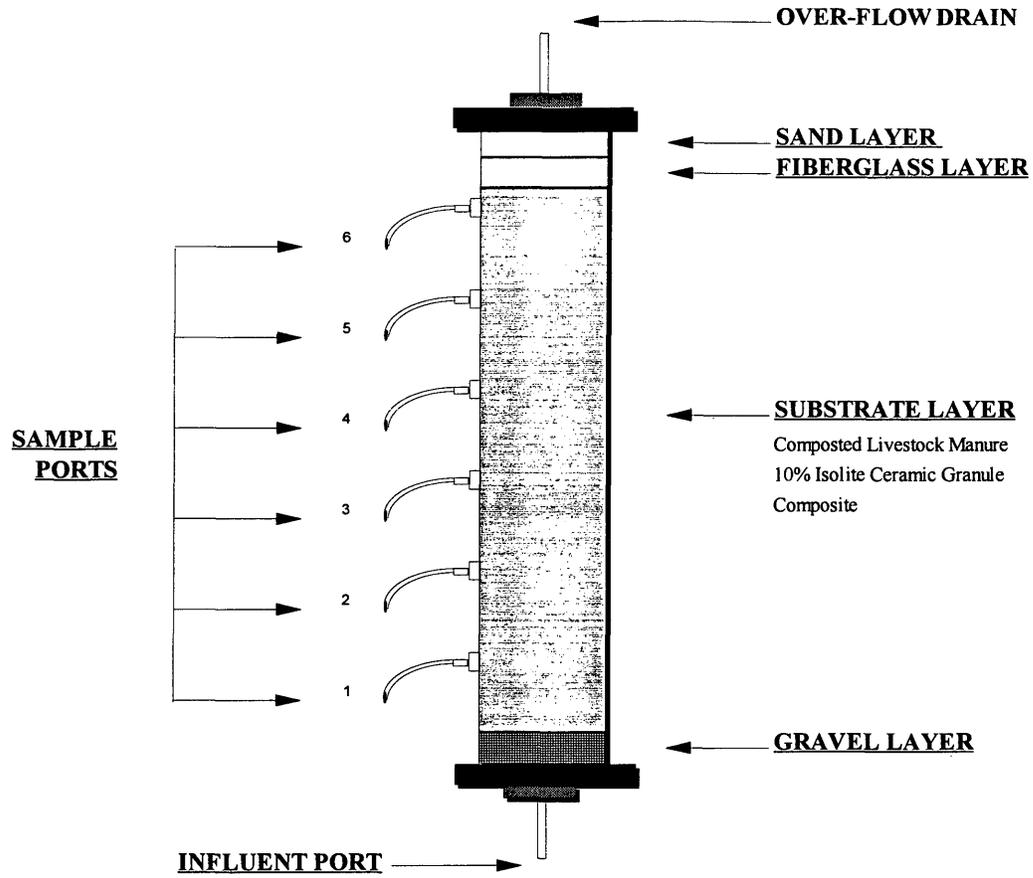


Figure 4: Experimental reactor design. The spacing between the ports was; port #1 at 25 cm from the bottom of the column, port #2 at 48 cm, port #3 at 71 cm, port #4 at 95 cm, and port #5 at 118 cm, with the effluent port #6 at 141 cm.

**Table 2: Reactor Column Design and Specifications for Experiments 1-4.**

Column Specifications	Reactor Columns			
	A <sub>1</sub>	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>
Total Height (cm)	178	170	159	163
Inside Diameter (cm)	16.5	16.5	15.2	15.2
Height of Gravel Layer (cm)	4	4	4	4
Height of Substrate (cm)	143	143	143	143
Height to Top Effluent Port (cm)	141	141	141	141
Total Volume of Reactor (L)	38	36	29	30
Volume of Gravel Layer (L)	0.8	0.8	0.7	0.7
Volume of Substrate (L)	30	30	25	25
Volume of Substrate to Port #6 (L)	29	29	25	25

The effluent was recovered from a point 2.5 cm below the standardized bed level for all of the reactors (identified as port #6 on all columns), (Figure 4). The two primary columns, A<sub>1</sub> and B<sub>1</sub>, had additional sample ports attached at regular intervals along their height; port #1 at 25 cm from the bottom of the column, port #2 at 48 cm, port #3 at 71 cm, port #4 at 95 cm, and port #5 at 118 cm, with the effluent port #6 at 141 cm. These sampling ports consisted of perforated HDPE tubing which spanned the inside diameter of the column and were covered with water permeable geo-membrane to prevent passage of solids from the substrate. The HDPE tubing was connected to a hose barb mounted in the column casing, to which an external tube was attached and from which liquid samples could be drawn. The total internal volume of each sample port was approximately 10

mL. Therefore, 10 mL was purged from each port prior to sample collection to insure a fresh sample.

Once constructed, the columns were cleaned using a 0.2 N *HCl* solution as a rinse, preceded and followed by a tap water rinse.

Each column was packed with a composite of composted livestock manure and porous ceramic pellets. The pellets were used as a bulking agent to ensure adequate permeability. The porous ceramic pellets, known by their trademark name Isolite™, are composed of furnace fired diatomaceous earth. Table 3 details the specifications of Isolite™:

**Table 3: Technical Specifications of Isolite™  
Porous Granular Ceramic Pellets Used as  
Bulking Agent in Substrate Composite Mixture.**

Porosity	74%
Pore Size	0.1 - 1.0 micron
Bulk Density	< 0.7 g/cm <sup>3</sup>
Bulk Weight *	32 lb./ft <sup>3</sup>
Particle Density	2.27 g/cm <sup>3</sup>
Cation Exchange Capacity	< 2 meq/100g
Chemical Composition	78% <i>SiO</i> <sub>2</sub> 12% <i>Al</i> <sub>2</sub> <i>O</i> <sub>3</sub> 5% <i>Fe</i> <sub>2</sub> <i>O</i> <sub>3</sub>
Electrical Conductivity	< 0.5 μmhos/cm

\* Specifications and bulk weight in English units obtained from Sumitoma Technical Bulletin (1993).

Isolite™ was chosen as a bulking agent for several reasons. First, the granule pores are continuous with no ‘dead-end’ pore spaces. Permeability of the substrate is enhanced and the synthetic mine drainage flows around and through the ceramic pellets. Secondly, Isolite™ is reported to resist compaction and will not shrink, swell or deteriorate. Finally, Isolite™ has been found to be chemically inert (Sumitoma Technical Bulletin, 1993). The particular form chosen for this experiment was CG-6 (Ceramic Granules of 6 millimeter diameter). Isolite™ was mixed uniformly with the composted livestock manure and was 10% by volume of this mix.

A layer of pea gravel, approximately 4 cm thick, was loaded into the base of each column and was covered with permeable geo-membrane. The pea gravel disperses the influent across the reactor. The manure/ Isolite™ substrate mixture was wetted with water in a 113 L vessel and then loaded as a slurry into the columns. Each column was placed at a 45° angle during loading in order to prevent the separation of the ceramics from the manure and to minimize damage to the internal sample port tubing from the heavy, wet substrate.

In order to maintain an anaerobic system once the reactor was packed, it was essential that the substrate not be exposed to the atmosphere. Washed fiberglass insulation was used as a head space fill material. It is light, porous, and does not contribute significantly to compaction of the substrate. Sand was then added for the remaining 5 cm of each column. Each alternating layer; substrate, fiberglass, and sand,

was separated by a disc of permeable geo-membrane to reduce migration of solid materials between layers.

### 3.2 Synthetic Mine Drainage

Due to the enormous volumes of mine drainage required for the experiments, an artificial drainage was synthesized to resemble the composition of the drainage from the Golf Tunnel adit at the Mary Murphy Mine site near Buena Vista, Colorado. The synthetic mine drainage was stored in a 1136 L (300 gallon) plastic vessel. The effluent from each reactor was collected in a similar vessel. The influent vessel was cleaned using 5.7 L (1.5 gallons) of chlorine bleach in 200 L (50 gallons) of tap water, followed by a 1.0 N *HCl* rinse. Both solutions were brushed over all surfaces and rinsed thoroughly. The synthetic mine drainage composition is presented in Table 4:

**Table 4: Experimental Synthetic Mine Drainage Chemical Constituents and Desired Concentrations Used for Experiments 1-4.**

Constituen	Reagent	Desired	Amount Used
$Zn^{2+}$	$ZnCl_2$	100.0 mg/L	0.21 g/L
$Mn^{2+}$	$MnCl_2 \cdot 4H_2O$	50.0 mg/L	0.18 g/L
$Cu^{2+}$	$CuSO_4 \cdot 5H_2O$	8.0 mg/L	$3.14 \times 10^{-2}$ g/L
$Al^{3+}$	$AlCl_3 \cdot 6H_2O$	5.0 mg/L	0.045 g/L
$Cd^{2+}$	$3CdSO_4 \cdot 8H_2O$	0.5 mg/L	$1.14 \times 10^{-3}$ g/L
$SO_4^{2-}$	$Na_2SO_4$	1000 mg/L	1.48 g/L
$HCO_3^-$	$NaHCO_3$	50 mg/L	$6.88 \times 10^{-2}$ g/L

Ion composition and pH of the synthetic mine drainage resembled that of the Golf Tunnel. Most cations in the synthetic mine drainage were added as their chloride complexes (Weast et al., 1990). Therefore, chloride concentration exceeded that found in the Golf Tunnel drainage water (193.2 mg/L compared to 20 mg/L). The excess chloride could be used as a conservative tracer. Carbonate and sulfate concentrations were maintained independent of the chloride. Typical rates of sulfate reduction by SRBs has been reported to be approximately  $600 \times 10^{-9}$  moles of  $SO_4^{2-}$  /cm<sup>3</sup> of organic substrate/day (Reynolds, 1991; Cohen and Staub, 1992). To support that level of sulfate reduction, a minimum of 170 mg-  $SO_4^{2-}$ /L- was needed in the influent drainage at a hydraulic detention time of  $\tau = 70$  h. Saturating conditions with respect to sulfate concentration (1000 mg/L-  $SO_4^{2-}$  ) were used to ensure that sufficient sulfate was available to the bacteria.

A mixed-bed, ion exchange, deionization column was utilized to remove potential contaminants from the domestic water supply source.

### **3.3 Reactor Preparation**

All four reactors were inoculated with a consortium of sulfate-reducing bacteria. The inoculum speeds the development of bacterial biomass in the bio-reactors (Bolis, 1992). The SRBs were prepared for the reactors by incubating the seed for four days in a

culture medium broth (Appendix A). One gallon of the SRB culture media was pumped into each column. The cell density of the culture medium was not measured.

A 300 gallon (1135 L) HDPE carboy was filled with a solution containing 50 mg/L as  $SO_4^{2-}$  and 33 mg/L as  $Cu^{2+}$  cupric sulfate ( $CuSO_4 \cdot 5H_2O$ ), and the solution was pumped to the reactors with a peristaltic pump. The pump was adjusted to deliver 8.7 mL/min. to each column for 18 days. The result was an empty bed reactor hydraulic detention time ( $\tau = \frac{V}{Q}$ ) of 50 h in the smallest columns ( $B_1$  and  $B_2$ ) and 60 h in the larger columns ( $A_1$  and  $A_2$ ), where  $V$  = the total volume of the substrate, and  $Q$  = the flow rate of the influent solution. The cupric sulfate solution supplied the SRBs with their obligatory electron acceptor -- sulfate. Copper was used to saturate sorption sites on the organic substrate surfaces, so that removal of metals as sulfides would be the predominant removal mechanism instead of adsorption.

### **3.4 Experimental Design**

The research was divided into six experiments: 1. Two experiments with circum-neutral pH, mine drainage water; 2. One experiment with low pH - low dissolved oxygen; and 3. One experiment with low pH - and water saturated with dissolved oxygen. Two

additional experiments were conducted to measure the sorptive capacity of the substrate. All of the experiments were run indoors and at approximately 20 degrees celsius.

#### **3.4.1 Experiment #1 - Series 1: Circum-neutral Drainage pH**

In experiment #1, with circum-neutral pH, metal-leaden synthetic mine drainage, we expect to see an increase in flow rates and decrease in hydraulic detention times while maintaining 90% or greater removal efficiency of the metal contaminants. Aluminum and copper were omitted from the synthetic mine drainage due to their propensity to precipitate at neutral pH ( Moore and Ramamoorthy, 1984). Three of the constituent metal ions were used; zinc, manganese and cadmium. These were also the metals of highest concentration in the Golf Tunnel adit drainage. The synthetic mine drainage was fed through the columns for fourteen days.

The use of the four columns of different dimensions ( $A_1$ ,  $A_2$ ,  $B_1$  and  $B_2$ ), and two different pump tubing sizes, permitted four hydraulic detention times to be tested concurrently. The experiments could then test several detention times while minimizing the duration of each individual experiment. The flow rates were adjusted to produce the following approximate hydraulic detention times; 30 h for  $A_1$  and  $A_2$  and 85 h for  $B_1$  and  $B_2$ . The system flowed for an additional seven days during which time periodic measurements were made on samples obtained from each effluent port (#6) and the

influent tank. Data were collected on pH (su),  $E_h$  (mV), and conductivity ( $\mu\text{mhos/cm}$ ). These variables were used to monitor the approach to steady state conditions for the reactor and to determine an appropriate time at which to sample. Steady state conditions were decided to exist when these variables were stable for three or more days.

After twenty-one days of flow of the original synthetic mine drainage, 100 mL samples were drawn from each of the fourteen separate sample ports and the influent tank. Physical parameters (pH (su),  $E_h$  (mV), and conductivity ( $\mu\text{mhos/cm}$ ) were measured on all samples. The samples were filtered through  $0.45\mu\text{m}$  glass fiber filters. Anions were analyzed using a Dionex™ ion chromatograph. The remaining samples were acidified with 1 mL concentrated nitric acid and stored until cation analysis could be performed.

#### **3.4.2 Experiment #1 - Series 2: Circum-neutral Drainage pH**

Identical drainage to experiment #1 - series 1 was used for this experiment. We combined tubing sizes and pump speeds to obtain four different flow rates to the four reactors, simultaneously. The influent flowed for ten days at the following approximate hydraulic detention times:  $A_1 = 20$  h,  $A_2 = 55$  h,  $B_1 = 15$  h and  $B_2 = 50$  h. Physical measurements were made on the effluents to confirm steady state conditions when pH

(su),  $E_h$  (mV), and conductivity ( $\mu\text{mhos}$ ) ceased fluctuating. After 10 days, samples were again collected. Analysis was conducted as was done in series 1.

During the interim between experiment #2 and #3, the influent tank was drained, cleaned, and refilled with 757 L (200 gallons) of 1000 mg/L sulfate solution in water. DI water was not used during this phase because we were sustaining the SRB populations while preparations were made for the next two experiments. Flows were adjusted to achieve hydraulic detention times of 90 h in  $A_1$  and  $A_2$  and 75 hours in  $B_1$  and  $B_2$ . This drainage and these hydraulic detention times were maintained for 29 days.

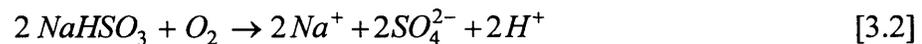
### **3.4.3 Experiment #3: Low pH and Reduced Dissolved Oxygen Drainage**

Dissolved oxygen may reduce metal removal efficiencies of the reactors due to the increase of  $E_h$  beyond the level at which SRBs can optimally function. The examination of the effect of dissolved oxygen on treatment efficiencies was conducted using drainage containing all of the metal constituents, including aluminum and copper. The water then was purged of dissolved oxygen. The drainage was maintained at pH 2.7. The drainage was allowed to flow at 75+ h hydraulic detention times for five days prior to deoxygenating the influent tank.

Traditional techniques for oxygen removal include simple methods, such as purging with an inert gas and vacuum degasification, to more elaborate catalytic,

industrially applied methods (Rollie et al., 1987; Harhay and Wolfe, 1987). However, due to the high cost of most of these methods, we removed dissolved oxygen using sodium bisulfite.

Suitable oxygen scavengers are strongly reducing agents that have an exothermic heat of reaction with  $O_2$  and a kinetic requirement of reasonable reactivity (Cotton, 1987). Reagents which fulfill these requirements include chromous sulfate, vanadous sulfate, sodium sulfite(s), and hydrazine. Of these reagents, the sulfite compounds, which include sodium sulfite ( $Na_2SO_3$ ), sodium bisulfite ( $NaHSO_3$ ), sodium metabisulfite ( $Na_2S_2O_5$ ), and ammonium sulfite [ $(NH_4)_2SO_3 \cdot H_2O$ ], were the most suitable for this experiment, though the use of the latter was restricted due to the possible formation of ammonia gas. Sodium bisulfite reacts according to the following equation (Rollie et al., 1987):



Though this reaction contributes to the total dissolved solids of the solution, it also yields an additional sulfate source which could be utilized by the SRBs. The reaction would also contribute protons to the solution

The rate of the sulfite-oxygen reaction is dependent upon both temperature and pH. An increase in temperature decreases reaction time. A pH in the range of 9.0 to 10.0 yields the most rapid reactions (BETZ, 1980). We allowed the drainage water to

reach thermal equilibrium with the atmosphere and maintained a circum-neutral pH until deoxygenating was complete. The addition of catalyzing compounds, as illustrated by Figure 5, speed the reaction.

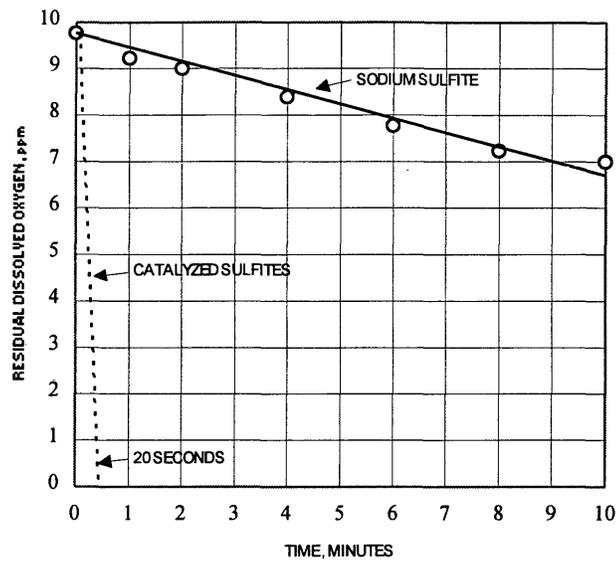
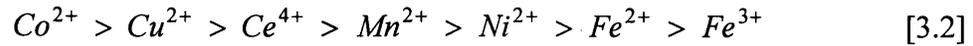


Figure 5: Sodium sulfite vs. catalyzed sodium sulfite as a chemical dissolved oxygen scavenger (BETZ, 1980).

Several transition metal species are known to speed the sulfite-oxygen reactions.

The following order illustrates the magnitude of catalytic activity among these metals

(Hobson et al., 1987):



Because both copper and manganese, two of the listed catalysts, were original constituents of the synthetic mine drainage, their presence would facilitate a more rapid deoxygenating of the influent tank.

Stoichiometric calculations using a ratio of 2 moles  $NaHSO_3$  : 1 mole  $O_2$  and assuming a saturation of 8.0 mg/L -  $O_2$  at a temperature of 28° C (Vesilind et al., 1994) required the use of 49.3 grams of sodium bisulfite to deoxygenate the remaining 946 L (250 gallons) of synthetic acid-mine drainage. In order to maintain low dissolved oxygen through the experiment, 147.7 grams of sodium bisulfite (or 3 times the calculated value) was used. The concentration of dissolved oxygen dropped to 0.0 mg/L almost instantaneously after the addition of the sodium bisulfite. Plastic bubble wrap covered the water surface in the synthetic mine drainage feed tank to minimize reaeration.

The column flow rates were adjusted to establish the following hydraulic detention times:  $A_1 = 30$  h,  $A_2 = 90$  h,  $B_1 = 25$  h and  $B_2 = 75$  h. These flow rates were maintained for sixteen days. Physical measurements were made on the effluents and influent to confirm steady state conditions when pH (su),  $E_h$  (mV), and conductivity ( $\mu$ mhos) ceased fluctuating. Dissolved oxygen (DO) was now included among the measurements. Samples were taken after sixteen days and analyzed according to the same procedures as in experiments #1. Instead of 100 mL samples, two 30 mL samples

were taken from each sample port. The second sample was sent to an outside laboratory for dissolved aluminum analysis.

#### **3.4.4 Experiment #4: Low pH and Elevated Dissolved Oxygen Drainage**

The influent feed tank was washed and rinsed with DI water. All of the constituent metals were dissolved in the feed water and the pH was maintained throughout the experiment at 2.7 su. The sulfate concentration was decreased to 500 mg/L in order to conserve chemical usage. This concentration was still in excess of the earlier calculated minimum requirement of 170 mg/L- $\text{SO}_4^{2-}$  needed by the SRBs. The tank was allowed to remain open to atmospheric oxygen. The solution flowed for seventeen days at the following reactor hydraulic detention times:  $A_1 = 30$  h,  $A_2 = 95$  h,  $B_1 = 30$  h and  $B_2 = 80$  h, after which samples were collected. Measurements on pH, Eh, DO and conductivity were made throughout the seventeen day period. Once the pH, Eh and conductivity stabilized, 2-30 mL samples were drawn for analysis.

#### **3.4.5 Experiment #5: Bench-scale Substrate Sorption Test**

Because adsorption to the solid phase can remove a large mass of metal ions in these wet-substrate bio-reactors, it was important to quantify the sorptive capacity of the composted manure substrate. Two, one-liter solutions, containing 75 mg/L copper, were

created using 0.4 grams cupric chloride, dihydrate ( $CuCl_2 \cdot 2H_2O$ ) dissolved in DI water. The two beakers were stirred on magnetic stir plates. At time = 0 ( $t = 0$ ), a two mL sample was drawn from each beaker. Once this sample was taken, 10 grams of dry manure used in the previous experiments was added to the stirring solution. The two samples taken prior to introduction of the manure were filtered through a  $0.8 \mu m / 0.2 \mu m$  syringe-tip filter. A 1 mL sample was drawn from this solution and added to 9 mL of DI water to create a 1:10 dilution. Sample collection was repeated at the following time intervals:  $t = 10, 20, 35, 60, 180, 1440, 2880, 4320$  and 5760 minutes. The samples were analyzed for copper.

#### **3.4.6 . Experiment #6: Composite Substrate Adsorption Column Test**

To measure the effective adsorption capacity of the larger columns ( $A_1, A_2, B_1$  and  $B_2$ ), a smaller version was constructed. The following table contains the technical specifications for this column:

<b>Table 5: Adsorption Test Column Technical Specifications.</b>	
<b>Total Height</b>	139.7 cm
<b>Diameter</b>	5.08 cm
<b>Gravel Layer</b>	
Height	15.24 cm
Volume	308.9 mL
<b>Substrate Layer</b>	
Height	109.22 cm
Volume	2213.7 mL
<b>Sand Layer</b>	
Height	15.24 cm
Volume	308.9 mL

The feed stock synthetic mine drainage solution consisted of zinc, manganese and cadmium in concentrations stated in experiment #1. Sulfate and bicarbonate similarly were added such that the feed solution had a pH of 6.2. A concentration of 1 mmol/L (0.065 g/L) of sodium azide ( $NaN_3$ ), an SRB inhibitor, was added to the influent and used when wetting the substrate mixture for initial packing. The sodium azide added permitted sorption to be favored as the predominant metal removal mechanism (Postgate, 1979).

A single channel, variable speed, peristaltic pump was used to deliver the influent drainage to the column. Hydraulic detention time was not constant at 40 h during the experiment. Hydraulic detention times of 30 to 36 hours were measured on three

occasions. The average hydraulic detention time, however, was maintained above the 40 hour threshold.

The experiment ran for 60 days. 30 mL samples were collected approximately every three days. pH,  $E_h$  and conductivity were measured. The samples were filtered (0.45  $\mu\text{m}$ ), acidified using 0.3 mL concentrated nitric acid, and stored until metals could be analyzed.

### **3.5 Analysis of Influent and Effluent Samples**

The influent synthetic drainage for each experiment was analyzed for metals and anions to provide confirmation of the calculate concentrations.

Dissolved metal concentrations were measured using an *Instrumentation Laboratory* model 457 flame atomic absorption/atomic emission spectrophotometer according to standard instrument methods (APHA-AWWA-WEF, 1992). Analysis was performed on each liquid sample for zinc, manganese, cadmium, and copper. Aluminum was analyzed by XRAL Laboratories, Golden, Colorado.

Analysis of influent and effluent anion concentrations was performed ion chromatography (Dionex Co., Sunnyvale, CA) according to APHA-AWWA-WEF (1992) prescribed methodology. The analysis yielded additional results on sulfate, chloride, nitrate, nitrite and phosphate concentrations. The following parameters were also

analyzed according to APHA-AWWA-WEF (1992) methods: pH using an Orion ionalyzer/specific ion meter (model no. 401) and Orion combination electrode (model no. 91-55);  $E_h$  using a Beckman field pH/ $E_h$  meter and a Corning Redox Combination probe (Cat. no. 476080); conductivity using a VWR automatic temperature compensating digital conductivity meter; and dissolved oxygen using a Yellow Springs Instrument Co. (YSI) dissolved oxygen meter (model no. 50B) and dissolved oxygen probe (model no. 5718).

## 4.0 RESULTS AND DISCUSSION

### 4.1 Metal Loading Rates and Hydraulic Detention Times at Near Neutral pH

Experiments were performed to determine if the inflow of metal laden water at circum-neutral pH increases metal removal efficiency at reduced hydraulic detention times. The flow rates to the reactors during the first series of runs were:  $A_{1/1} = 14.5$  mL/min.,  $A_{2/1} = 15.6$  mL/min.,  $B_{1/1} = 4.9$  mL/min., and  $B_{2/1} = 4.8$  mL/min. These flow rates yielded empty bed hydraulic detention times of 34 h, 31 h, 85 h and 86 h, respectively. During the second series, the following flow rate and hydraulic detention times were examined:  $A_{1/2} = 24.7$  mL/min.,  $A_{2/2} = 9.1$  mL/min.,  $B_{1/2} = 26$  mL/min., and  $B_{2/2} = 8.6$  mL/min. These flow rates yielded empty bed hydraulic detention times of 20 h, 54 h, 16 h and 48 h, respectively.

Zinc and cadmium, at 98 mg/L and 0.62 mg/L for the first series and at 96 mg/L and 0.54 mg/L in the second series influent solution, respectively, were almost entirely removed (>98%) at all of the hydraulic detention times (Table 6). The removal efficiency of manganese in two of the high flow reactors,  $A_{1/1}$  and  $A_{2/1}$ , was 68.0% and 66.2% respectively. Manganese was removed at near 100% for hydraulic detention times greater than 34 h, with the exception of column/series  $A_{2/2}$ , which had a hydraulic detention time

of 54 h, yet removed manganese with only 68% efficiency. Manganese removal efficiency was similar in reactors  $A_{1/2}$  and  $A_{2/2}$ , even though there almost was a three fold higher detention time in  $A_{2/2}$ . This phenomenon may be an artifact due to column  $A_2$  having been operated at an increased flow rate during the first series. The adsorptive capacity of column  $A_2$  may have been compromised with respect to manganese sorption, allowing more of that metal to escape the system, even at the reduced flow rate used in the second series. This phenomenon will be addressed in a later section.  $B_{1/2}$ , which was at a lower flow rate for series 1 and achieved a manganese removal efficiency of 99.9 %, achieved only 73.3 % removal efficiency for manganese during series 2, while  $B_{2/2}$ , which remained at a low flow rate throughout both series, was still removing manganese at near 100% efficiency.

**Table 6: Metal Removal Efficiencies and Hydraulic Detention Times for the Wet-Substrate Bio-Reactors Under Circum-neutral pH Conditions.**

Reactor/ Series	Hydraulic Detention Time (hours)	Zinc Removal (%)	Cadmium Removal (%)	Manganese Removal (%)
$B_{2/1}$	86	99.9	100	99.7
$B_{1/1}$	85	99.9	100	99.8
$A_{2/2}$	54	99.6	100	67.9
$B_{2/2}$	48	99.9	100	99.9
$A_{1/1}$	34	99.1	100	68.0
$A_{2/1}$	31	99.8	100	66.2
$A_{1/2}$	20	99.3	98.1	66.9
$B_{1/2}$	16	99.9	100	73.3

Based solely on metal removal efficiencies, the systems were not losing significant treatment capacity at hydraulic detention times of less than 34 h, except for the removal of manganese. Both zinc and cadmium were almost entirely removed, while manganese was removed at a greater than 65% efficiency.

There appeared to be a direct relationship between metal removal capacities and metal loading rates. Based on daily molar metal removal amounts, a three times increase in flow yielded a three times increase in moles of metals removed between the two sets of reactors.

A comparison of the mass of metals removed per day (moles/day) and the mass of sulfate lost to reduction (moles/day) indicates that sorption processes may have contributed to the high metal removal efficiencies. For example, 0.045 moles of metals were being removed from reactor A<sub>1/1</sub> per day (hydraulic detention time = 34 h), while only 0.023 moles/day of sulfate were being removed in this reactor. Manganese (removed at 0.014 moles/day in A<sub>1/1</sub>) was most likely removed as a carbonate species ( $MnCO_3$ ) (Morel and Hering, 1993). The 0.014 moles/day was subtracted from the total metal mass removal rates. There was a combined 0.031 moles of zinc and cadmium removed and only 0.023 moles of sulfate to convert to sulfides to be available to form sulfide precipitates. Metal removal rates were higher than could be accounted for by assuming that sulfide precipitation was the predominant removal mechanism. It is reasonable to assume that the additional removal was the result of adsorption and

complexation processes between the metals and the organic substrate of the reactors.

This conclusion led to further experiments to test the sorptive capacity of the organic substrate (Experiments #5 & #6).

The molar sulfate loss per day appeared independent of the loading rate of sulfate for all hydraulic detention times examined (Figure 6).

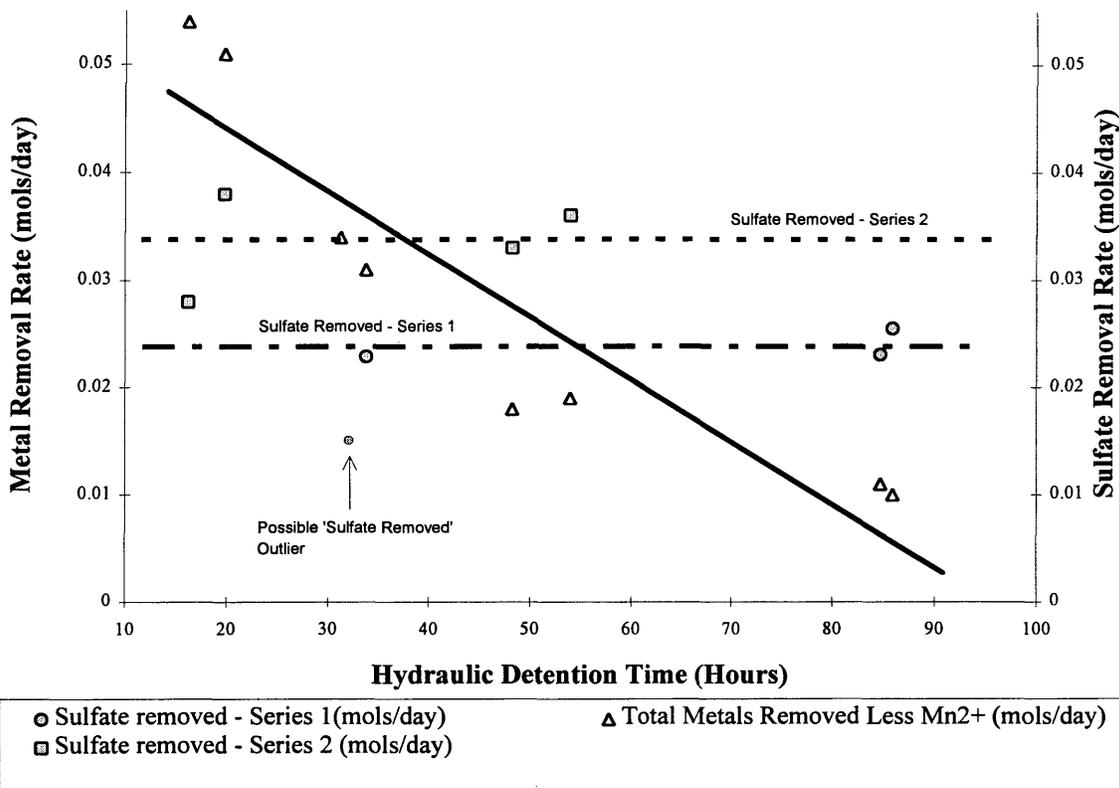


Figure 6: Comparison of sulfate removal average with metals removal as a function of hydraulic detention time for circum-neutral pH experiment (flow series 1 and 2). Intersection between total metals less Mn<sup>2+</sup> line and sulfate removal line indicates maximum possible removal rate as metal sulfide precipitates during each series. The possible ‘sulfate removed’ outlier is 1.5 standard deviations from the mean.

**Table 7 : Circum-neutral pH Drainage Experiment Sulfate Removed Averages and Metals Removed Line Equation Parameters.**

Parameter	Sulfate Removed (series 1)	Sulfate Removed (series 2)	Total Metals Removed less Mn <sup>2+</sup>
Slope	--	--	-0.0006
Y-Intercept	0.02380	0.03375	0.0558
R <sup>2</sup>	--	--	0.8516

At 85 h and 86 h hydraulic detention times, reactors B<sub>1/1</sub> and B<sub>2/1</sub> had 0.023 moles/day and 0.026 moles/day of sulfate removed, respectively. Even though reactors A<sub>1/1</sub> and A<sub>2/1</sub> had flow rates three times greater than B<sub>1/1</sub> and B<sub>2/1</sub>, and the sulfate loading rate was three times greater, the sulfate loss was similarly 0.023 moles/day for A<sub>1/1</sub> and 0.016 moles/day for reactor A<sub>2/1</sub>. We anticipated that, with 1/3 of the hydraulic detention time, and therefore triple the sulfate loading rates, the sulfate reduction in A<sub>1/1</sub> and A<sub>2/1</sub> would be 3 times that of B<sub>1/1</sub> and B<sub>2/1</sub>. This, however, did not occur. The results indicate that the SRBs may be at zero order reaction rates with respect to sulfate (in enzyme kinetics, this would be at saturation with sulfate).

Any additional sulfate will not enhance the rate of sulfate reduction. SRB activity even may be reduced at high sulfate loading rates due to the high influx of oxygenated water. The removal rates per unit volume of the reactor substrates were (Table 8):

<b>Flow Series</b>	<b>A<sub>1</sub></b>	<b>A<sub>2</sub></b>	<b>B<sub>1</sub></b>	<b>B<sub>2</sub></b>
1	779	549	928	1024
2	1283	1228	1137	1315

These removal rates, if predominantly due to sulfate reduction, are higher than those reported by Hedin and Nairn (1988), 300 nanomoles- $\text{SO}_4^{2-}/\text{cm}^3$  substrate/day, and Reynolds (1991) of 600 nanomoles- $\text{SO}_4^{2-}/\text{cm}^3$  substrate/day. These rates are near or higher than the rate of those previously reported and indicate that either there are larger numbers of SRBs per unit volume of reactor than in previously reported systems, resulting in higher sulfate reduction, or that the SRB activity rates have increased.

An alternative sink for sulfate is the formation of gypsum ( $\text{CaSO}_4$ ). Though no calcium was added directly, the manure could have provided a source of  $\text{Ca}^{2+}$ . Gypsum is formed under oxidizing conditions (Garrels and Christ, 1990) and is manifested by white crystalline precipitates. All four reactors remained in relatively reducing conditions, top to bottom, during both flow series. No white crystalline scale was observed. Thus, most of the sulfate loss can be presumed to be attributed to reduction to sulfide.

If the rate of sulfate removal is interpreted as reduction to sulfide, then the moles  $\text{S}^{2-}/\text{cm}^3/\text{day}$  can be assumed to react with metal cations on a mole to mole basis. With a four reactor average of 820 nmols/ $\text{cm}^3/\text{day}$  of sulfide produced during the first flow series, a loading rate of 820 nmols/ $\text{cm}^3/\text{day}$  of cationic metals could be precipitated as metal sulfides (or acid volatile sulfides (AVS), Reynolds, 1991). During the second flow series, this average increased to 1241 nmols/ $\text{cm}^3/\text{day}$  of sulfide produced.

If metal loading rates exceed  $S^{2-}$  production rates per unit volume of substrate, then there are two potential fates of the excess metals under the reducing conditions found in the reactors (Ozawa, 1994): 1. excess metals are mobilized and are transferred through the reactor to represent breakthrough; or 2. excess metals might adsorb to the extensive solid surfaces. If metal loading rates are less than  $S^{2-}$  production rates, much of the excess  $S^{2-}$  may be given off as  $H_2S$ .

The results indicate that all measured metals, except for manganese were consistently removed at near 100% removal efficiency at all hydraulic detention times. In reactors with detention times greater than 54 h, excess  $S^{2-}$  was detected as  $H_2S$ .

Figure 6 shows metal and sulfate removal rates as a function of flow rates (the higher the flow rate, the shorter the hydraulic detention time). The regressed sulfate lines are almost horizontal, demonstrating the independence of sulfate reduction rate to flow rate (and, therefore, sulfate loading rate). Metal removal is proportional to flow rate (Figure 6, Table 7). Metal removal rates that fall below the horizontal sulfate reduction rate lines can be assumed to be removed as metal sulfide precipitates (although potential removal by sorption cannot be discounted). Metal removal rate data points in Figure 6 that plot above the sulfate reduction rate lines can be assumed to be removed predominantly by sorption (there are no more sulfides to account for metal removal). Experiments on and discussion of sorption capacity will follow. Intersection points between the metals removed and sulfate reduction lines are shown in Table 9. The

hydraulic detention times corresponding to the intersection points can be considered an “optimal” hydraulic detention time. For higher flow rates and thus shorter detention times, metals loading rates will exceed  $S^{2-}$  production rates and the residual metals must be removed by sorption. Sorption capacity is finite, and when exceeded, will cause metal breakthrough. If a bio-reactor is used as a metals stream treatment system, the effective unit lifespan will be dictated by the length of time sorption capacity lasts, if hydraulic detention times are lower than “optimal.” At “optimal” hydraulic detention times, metal sulfide precipitation can presumably maintain metal removal efficiencies even after sorption capacity is exhausted.

**Table 9: Hydraulic Detention Time Points of Intersection of Sulfate and Metal Removal Rate Lines for Circum-neutral pH Drainage Experiment.**

Flow Series	Hydraulic Detention Time (h)
1	53.3
2	36.8

Empty bed hydraulic detention times less than 53 h exceeded the “optimal” hydraulic detention times during series one and those less than 37 h exceeded the “optimal” hydraulic detention time for series two. The hydraulic detention times, as determined by the intersection of metal and sulfate removal curves (Table 9) are very

close to the hydraulic detention times of 40 h, below which metal removal efficiency declines (Madel, 1992).

The results indicate that optimization of the reactor environment for SRB sulfate reduction activity will generate the best, long-term, reactor efficiency. As the systems matured under “optimal”, circum-neutral pH conditions for SRBs, sulfate reduction capacity appeared to increase, as evidenced by the increase in the sulfate reduction curves from series 1 to series 2. The “optimal” hydraulic detention times decreased with the increase in sulfate reduction rates. From Figure 6, it appears that a hydraulic detention time of 26 h could be achieved with complete metal removal rates, assuming sulfide precipitation was the primary removal mechanism. Influent metal loading rates could then be adjusted up or down to match the sulfate reduction rates and maintain long-term metal removal.

$E_h$  data indicates that short-circuiting may have been occurring in reactor  $A_1$  during the first series of experiments. The trend of decline from +200 mV of the influent synthetic drainage to -43 mV at effluent port #4 was broken by a 100+ mV spike at effluent port #5 (Figure 7), supporting this assumption. The short-circuiting does not seem to have impaired the treatment capacity of the system. The suspicion that short-circuiting was occurring in reactor  $A_1$ , as indicated during the first series of flows, however, was not supported during the second series of flows (Figure 8). Both  $E_h$  and pH,

as well as the metal removing efficiency of this reactor did not change between effluent ports #3 and #5.

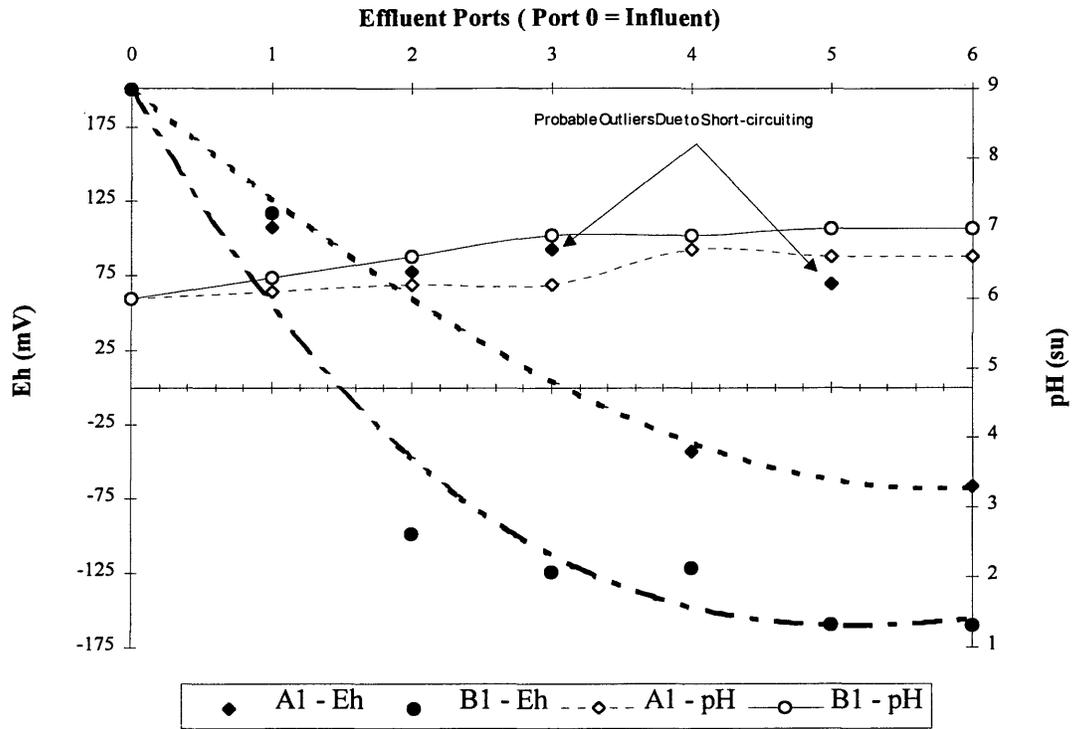


Figure 7:  $E_h$  and pH measurements at end of experimental run from reactors  $A_{1/1}$  and  $B_{1/1}$  during first series of flows.  $E_h$  in reactor  $A_{1/1}$  deviates from port to port trend at ports #3 and #5.  $A_{1/1}$  had a hydraulic detention time of 34 h while  $B_{1/1}$  had a hydraulic detention time of 85 h.

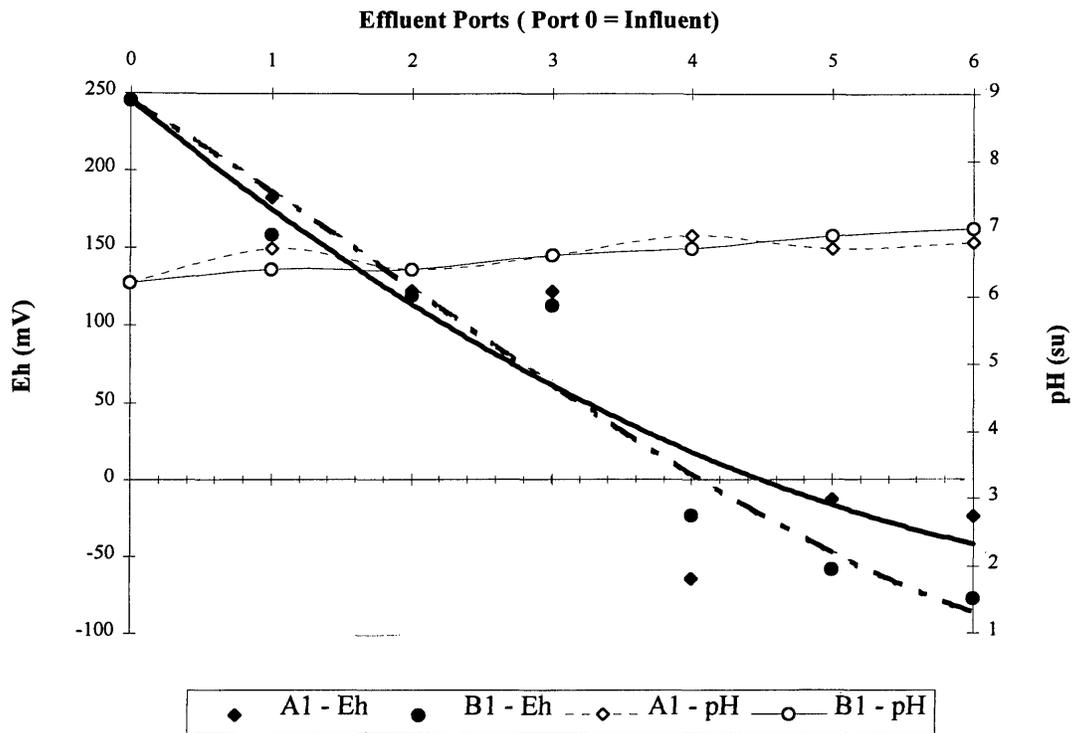


Figure 8:  $E_h$  and pH measurements at end of experimental run from reactors  $A_{1/2}$  and  $B_{1/2}$  during second series of flows. Short-circuiting is not evident. The hydraulic detention times for reactors  $A_{1/2}$  and  $B_{1/2}$  were 20 h and 16 h, respectively.

#### 4.2 Metal Loading Rates and Hydraulic Detention Times Under Acidic pH and Reduced Dissolved Oxygen Conditions

The pH of the influent drainage was decreased from 6.2 to 2.7 su. To examine the effect of dissolved oxygen and elevated  $E_h$  on the activity of the SRBs and metal removal efficiency, the DO was driven to near 0.0 mg/L using sodium bisulfite. We expected low

pH to inhibit sulfate reducing activities and low oxygen to enhance activity (Connell and Patrick, 1968; Postgate, 1984)

The flow rates to each column were:  $A_1 = 17$  mL/min.,  $A_2 = 5.4$  mL/min.,  $B_1 = 16.9$  mL/min., and  $B_2 = 5.5$  mL/min. The flow rates yielded empty bed hydraulic detention times of 29 h, 90 h, 25 h and 76 h, respectively.

The first important phenomenon observed was that sulfate reduction was lower than the levels observed in the circum-neutral pH drainage experiment, even though the hydraulic detention times were longer (Table 10).

**Table 10: Sulfate Removal Rates (nanomoles- $\text{SO}_4^{2-}$ /cm<sup>3</sup>/day) for the 4 Bio-reactors Using Acidic/Reduced Dissolved Oxygen Influent.**

$A_1$	$A_2$	$B_1$	$B_2$
911	615	640	715

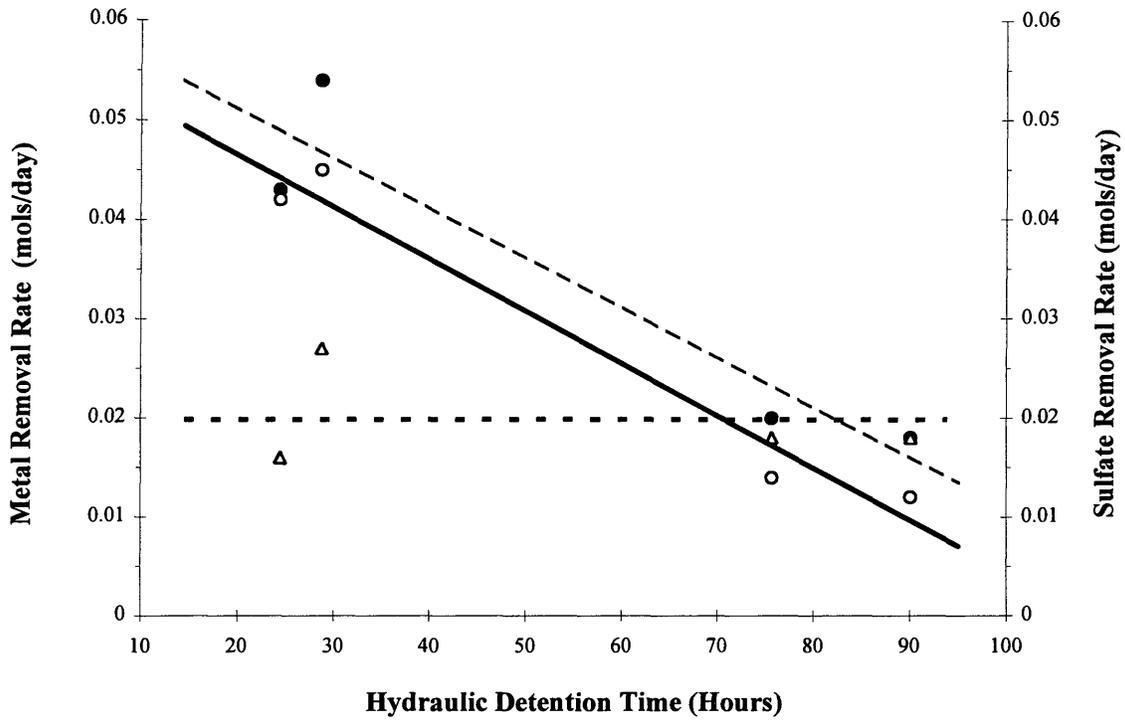
In reactors  $A_2$ ,  $B_1$  and  $B_2$ , the sulfate reduction rates were half of those in the circum-neutral pH experiment. Sulfate reduction was reduced approximately 41.5 % on average compared to previous experiment using the same reactors, yet metal removal rates were still directly proportional to flow rate and inversely proportional to hydraulic

detention time (Figure 9, Table 12). The fact that sulfate reduction fell off is all the more remarkable considering that prior to this experiment, all of the reactors had been maintained for 29 days on a 1000 mg/L sulfate solution at a pH of near 7.0 and hydraulic detention times of 75- 90 h, providing the most “optimal” environment for SRB proliferation. It appears that the reduction in pH directly impacted sulfate utilization. It is unclear whether the lower pH physically reduced the bacterial biomass by destroying organisms or whether the reduced pH simply slowed the sulfate-reduction reaction kinetics per unit organism. SRBs have been shown to survive at lower pH, though their individual activity may be reduced (Reynolds, 1991; Connell and Patrick, 1968).

The “optimum” flow rates were lower, and hydraulic detention times were higher compared to the results in the circum-neutral pH experiments (Table 11).

**Table 11: Hydraulic Detention Time Points of Intersection of Sulfate and Metal Removal Rate Lines for Acidic/Reduced Dissolved Oxygen Experiment.**

<b>Curve</b>	<b>Hydraulic Detention Time (h)</b>
Total Metals	83.1
Total Metals Less Mn <sup>2+</sup>	74.7



● Total Metals Removed (mols/day) ○ Total Metals Less Mn<sup>2+</sup> (mols/day) △ Sulfate Removed (mols/day)

Figure 9: Comparison of sulfate removal average with metals removal as a function of hydraulic detention time for the acidic/reduced dissolved oxygen drainage experiment. The sulfate removal line has decreased from that observed in the circum-neutral pH drainage experiment.

<b>Table 12 : Acidic/Reduced Dissolved Oxygen Experiment Sulfate Removed Average and Metals Removed Regression Line Equation Parameters.</b>			
<b>Parameter</b>	<b>Sulfate Removed</b>	<b>Total Metals Removed</b>	<b>Total Metals Removed less Mn<sup>2+</sup></b>
<b>Slope</b>	--	$-5.0 \times 10^{-4}$	$-5.0 \times 10^{-4}$
<b>Y-Intercept</b>	0.01975	0.0613	0.0571
<b>R<sup>2</sup></b>	--	0.8908	0.9677

Metal removal efficiency decreased with the lower pH influent. Cadmium was removed at nearly 100% efficiency (except in reactor A<sub>1</sub> where efficiency dropped from 100% to 96.9%). Zinc removal dropped to the following levels: A<sub>1</sub> = 96.9 %, A<sub>2</sub> = 80.3%, B<sub>1</sub> = 90.1 %, and B<sub>2</sub> = 93.4 %. These results were not unexpected for the two higher flow reactors, A<sub>1</sub> and B<sub>1</sub>, because their metal loading rates exceed sulfide production rates. The removal efficiency reduction observed in reactors A<sub>2</sub> and B<sub>2</sub>, at 90.1 and 75.7 h hydraulic detention time, was unexpected. The decrease in metal removal efficiency was observed in spite of the fact that sulfate reduction rates exceeded metal loading rates. Lower pH may have increased the formation of H<sub>2</sub>S, causing the loss of S<sup>2-</sup> that otherwise would be used for metals precipitation. The lower pH could have re-dissolved sulfides which were created and precipitated during the previous two experiments.

Manganese removal efficiency declined the most dramatically of all of the metals. The manganese removal efficiencies for reactors A<sub>1</sub> and B<sub>1</sub> were 36.3 % and 2.8 %, and 72.4 % and 71.3 % for reactors A<sub>2</sub> and B<sub>2</sub>, respectively. Both A<sub>2</sub> and B<sub>2</sub> attained a system pH of 7.1 su and 7.0 su, which were more favorable to the formation of rhodochrosite than those of A<sub>1</sub> and B<sub>1</sub>, at 6.7 and 6.3 su respectively. Because of the assumption that manganese would be removed in the form of rhodochrosite (*MnCO<sub>3</sub>*), it follows that by reducing the pH of the system, the formation of *MnCO<sub>3</sub>* was inhibited.

pH-p $\epsilon$  diagrams show that  $MnCO_3$  is stable only above pH 7.1 (Garrels and Christ, 1990), though the activity of  $Mn^{2+}$  and  $HCO_3^-$ , as well as the ionic strength of the solution, also control its stability. The manganese passed unimpeded through the system, except for adsorption. Even adsorption is not expected to remove manganese under low pH conditions and competition with zinc, cadmium and copper for sorption sites (Kerndorf and Schnitzer, 1980).

Considering the fact that dissolved oxygen fluctuated within the columns around 1.0 mg/L in the circum-neutral pH drainage experiment, while sulfate reduction remained high, and was approximately 0.0 mg/L in the acidic/reduced dissolved oxygen experiment, it is reasonable to assume that this variable played a minor role in the treatment efficiency of these systems. It certainly appears that the pH of the influent drainage was the predominant controlling variable.

The slight decrease in the pH at port #5, compared to ports 1,2,3,4 and 6 in reactor A<sub>1</sub>, and the elevation of the E<sub>h</sub> at that port may indicate short-circuiting of influent through the reactor was occurring (Figure 10). This conclusion was supported by the fact that both manganese and zinc concentrations were also elevated at this port. Reactor B<sub>1</sub> did not show the pH, E<sub>h</sub> and metal concentration deviation from a uniform trend through the reactor, and it is impossible to know if it was occurring in A<sub>2</sub> and B<sub>2</sub>, since they only had one sampling effluent port each.

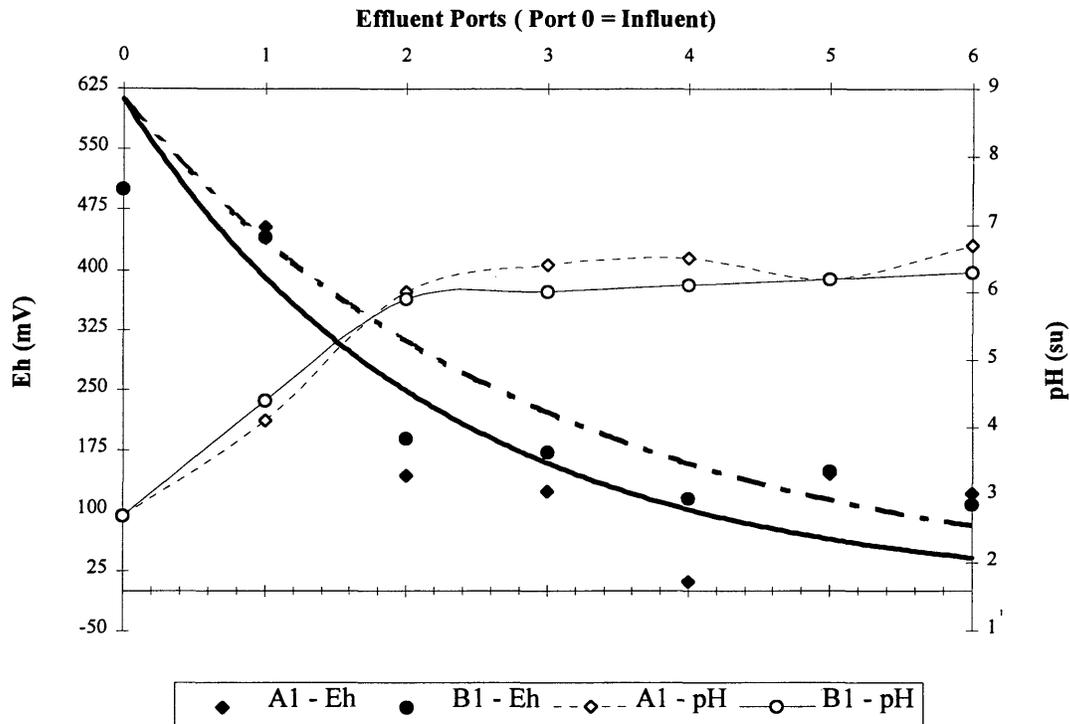


Figure 10:  $E_h$  and pH measurements from reactors  $A_1$  and  $B_1$  for acidic/reduced dissolved oxygen experiment. Decrease in pH and increase in  $E_h$  at port #5 for reactor  $A_1$  suggests short-circuiting.

The hypothesis that near neutral mine drainage water can be treated more efficiently than acidic waters is supported by the neutral pH experiment and the low pH, low dissolved oxygen experiment.

### 4.3 Metal Loading Rate Capacity Under Acidic and Elevated Dissolved Oxygen Influent Conditions

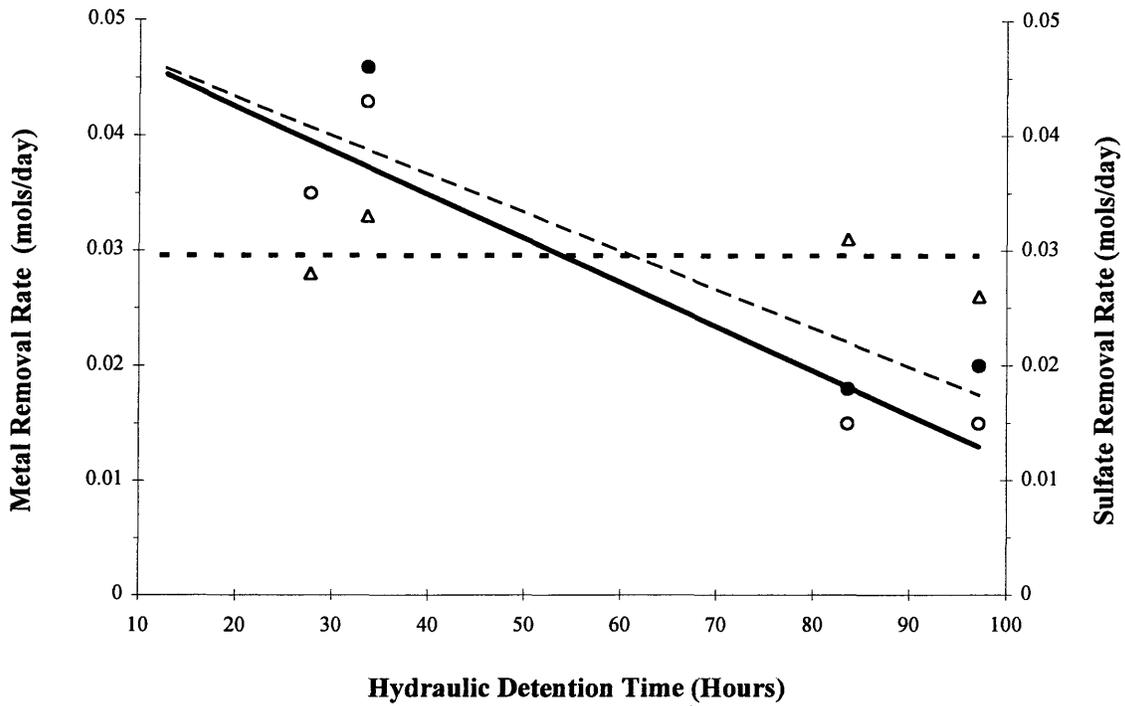
The influent solution was not deoxygenated as in experiment #3. The flow rates to each reactor were slightly higher:  $A_1 = 14.5$  mL/min.,  $A_2 = 5$  mL/min.,  $B_1 = 14.9$  mL/min., and  $B_2 = 5$  mL/min. The flow rates corresponded to empty bed hydraulic detention times of 34 h, 97 h, 28 h and 84 h, respectively. The influent contained zinc, manganese, cadmium, aluminum, and copper.

There was removal of cadmium, aluminum and copper at nearly 100% removal efficiency. Zinc removal efficiency increased from acidic/reduced dissolved oxygen experiment for reactor  $A_2$ ; from 80.3 % to 98.6 %; remained similar from 97% and 93.4% to 94% and 93% for reactors  $A_1$  and  $B_2$ ; and decreased for reactor  $B_1$  from 90.1 % to 69.2%.

The removal of manganese was increasingly inefficient from acidic/reduced dissolved oxygen experiment to experiment #4, suggesting perhaps that adsorption could no longer fully remove it from solution and that the reduced pH of the system was inhibiting the formation of  $MnCO_3$ . Adsorption is as much a function of pH as it is of the specie's affinity to sorb, for which manganese has perhaps the weakest affinity of all of the constituent metal cations.

The mass loss of sulfate was higher in the acidic/elevated dissolved oxygen experiment compared to the acidic/reduced dissolved oxygen experiment (Figure 11,

Table 13), yet still remained constant over the four reactors. Anion samples acidic/elevated dissolved oxygen experiment were stored for two months. There was evidence of evaporation. Sulfate was added to the influent to a concentration of 500 mg/L. The analysis of the influent yielded a concentration of 922 mg/L. Accuracy may have been poor for the sulfate measurement, but precision may have been preserved. The total moles of sulfate lost per day appears to have remained consistent from experiment to experiment and reactor to reactor. The actual values for sulfate mass removal seem high with respect to the acidic/reduced dissolved oxygen experiment.



● Total Metals Removed (mols/day) ○ Total Metals Less Mn<sup>2+</sup> (mols/day) Δ Sulfate Removed (mols/day)

Figure 11: Comparison of sulfate removal average with metals removal as a function of hydraulic detention time. The sulfate removal average line has increased respective to the acidic/reduced dissolved oxygen experiment.

**Table 13: Acidic/Elevated Dissolved Oxygen Drainage Experiment Sulfate Removed Average and Metals Removed Line Equation Parameters.**

Parameter	Sulfate Reduction	Total Metals Removed	Total Metals Removed less Mn <sup>2+</sup>
Slope	--	-3.0x10 <sup>-4</sup>	-4.0x10 <sup>-4</sup>
Y-Intercept	0.0295	0.0502	0.0503
R <sup>2</sup>	--	0.7942	0.8890

**Table 14: Hydraulic Detention Time Points of Intersection of Sulfate and Metal Removal Rate Lines for the Acidic/Elevated Dissolved Oxygen Experiment.**

<b>Reactor</b>	<b>Hydraulic detention Time (h)</b>
Total Metals	69
Total Metals Less Mn <sup>2+</sup>	52

The mass loss rate of sulfate, assumed to be converted to sulfide, varies little from reactor to reactor and hydraulic detention time to hydraulic detention time. If metal loading rates exceed sulfate reduction rates, then there are two possible outcomes: 1. metal treatment efficiency will decrease; or 2. sorption processes will remove metals that have not been converted to sulfide precipitates. Previous research (Machemer and Wildeman, 1992) suggests that sorption capacity is short-lived in reactors similar to those described above. It would be useful to quantify the sorption life for wet-substrate bio-reactors. If sorption capacity is exceeded, and metal breakthrough occurs, then the solution is either to decrease the metal loading rates, increase reactor size and hydraulic detention times, or increase the rate of sulfate reduction.

As observed in the acidic/reduced dissolved oxygen experiment, dissolved oxygen does not appear to be affecting the removal efficiency of the heavy metals. The dissolved oxygen was removed almost immediately upon entering the reactor systems.

The  $E_h$  and pH data indicate that, similar to the acidic/reduced dissolved oxygen experiment, short-circuiting may have occurred in column A<sub>1</sub> (Figure 12). The deviation from port to port trend of concentration of zinc, manganese, and cadmium at port #5 support this conclusion.

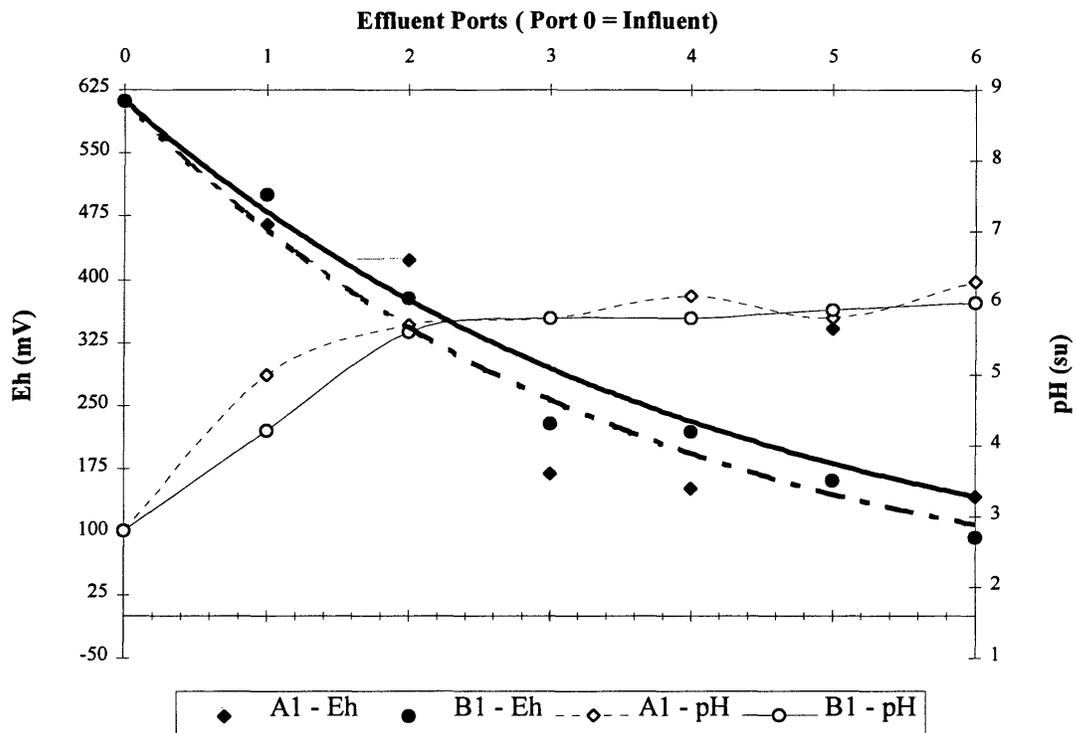


Figure 12:  $E_h$  and pH measurements during sampling from reactors A<sub>1</sub> and B<sub>1</sub> in acidic/elevated dissolved oxygen drainage experiment.  $E_h$  in reactor A<sub>1</sub> deviates from the port to port trend at port #5.

#### **4.4 Adsorption Capacity Experiments: Metal Removal Capacities of Substrate**

A 75 mg/L copper sulfate solution was added to a batch reactor that contained 1 liter of the copper solution and 10 grams of composted livestock manure. 10 samples were collected over a five days and were analyzed for copper on the fifth day. Sodium azide was added to the solution to inhibit the activity of the SRBs such that sorption was the predominant copper removal process. The concentrations of copper (*ppm*) were plotted against time to create the following figure (Figure 13):

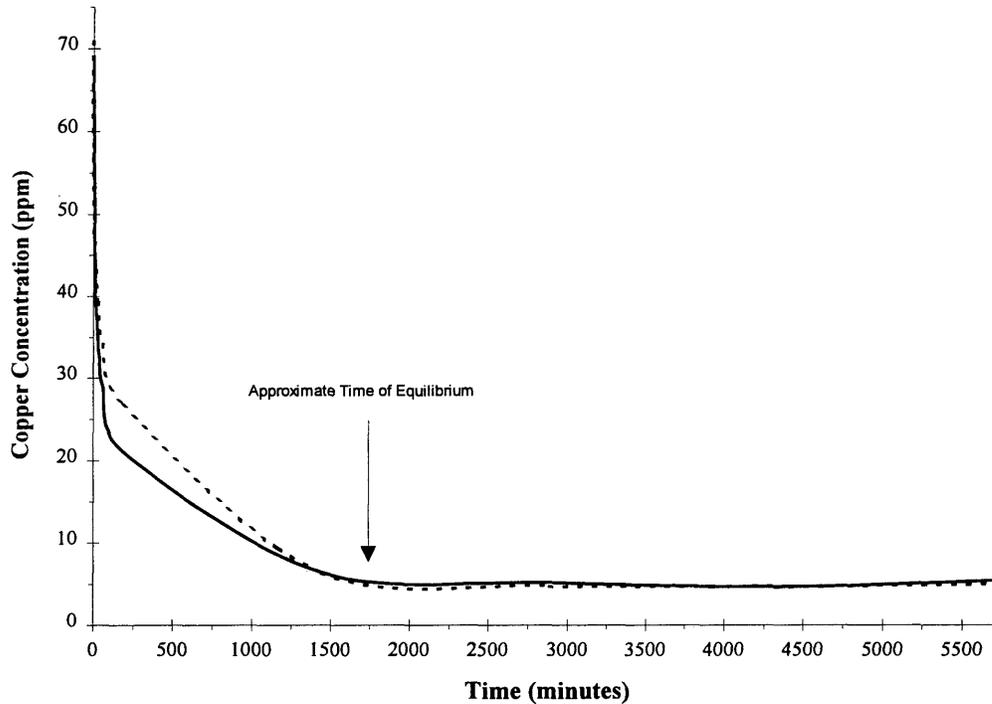


Figure 13: Removal of dissolved copper ions from a 1 liter solution containing 10 grams of composted livestock manure. Two experimental runs yielded similar results.

Equilibrium between the copper solution and the organic substrate the batch reactor was achieved at approximately 1750 minutes (1.22 days). Analysis of the data yielded an average sorption capacity for the organic substrate of 0.205 milliequivalents per gram from the two tests. This calculation was arrived at by converting the mass of copper removed from solution to milliequivalents.

To examine the sorptive characteristics of the substrate under conditions resembling those of the first experiments, a solution of 106.5 mg/L zinc, 63.9 mg/L manganese, and 0.37 mg/L cadmium was pumped continuously to a 2.7 L reactor filled with 2.2 L of the organic substrate mixture. The SRB inhibitor, sodium azide, was added to insure that adsorption of the metals was the predominant removal mechanism.

Analysis of the data obtained from this adsorption column test is presented in Figure 14. The results confirm previous reports in which zinc and manganese were the first elements to breakthrough a wet-substrate bio-reactor (Machemer and Wildeman, 1992). Kerndorf and Schnitzer (1980) determined that the strength of adsorption to organic matter varied with each metal, with zinc and manganese most loosely sorbed. Elements such as cadmium were more strongly sorbed and tended to compete with and subsequently displace zinc and manganese once the sorptive capacity of the system was exhausted. The batch reactor experiment demonstrated that the substrate has finite sorption sites, while this experiment demonstrated that the metals will eventually breakthrough the column according to their individual sorption affinities. This phenomenon is represented in Figure 14 in which manganese appears in the effluent before zinc. Cadmium is strongly sorbed and should undergo cation exchange with manganese and zinc.

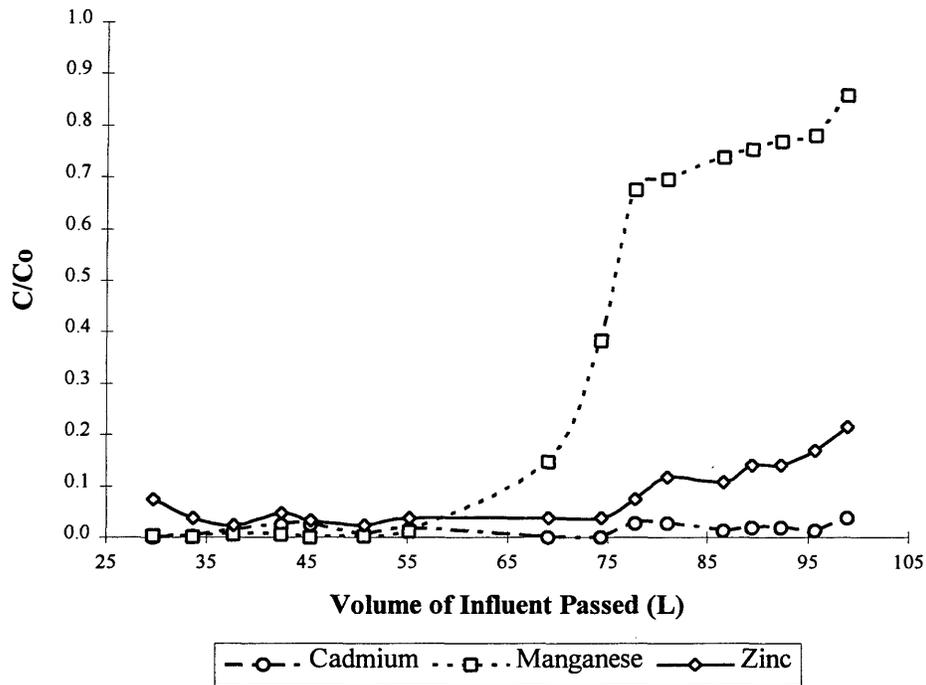


Figure 14: Adsorption column effluent concentration vs. volume of influent passed. Manganese is the first constituent metal to appear in the effluent.

These two experiments support the premise that adsorption is a transient metal removal mechanism. Without active sulfide precipitation, the treatment capacity of the systems is severely limited.

## 5.0 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Metal Removal Efficiencies

Analysis of the relationship of sulfate and metal mass removal to volume throughput demonstrates that, though we did achieve nearly 100% removal of the contaminant metals, a significant component of the removal was the result of sorption processes. Manganese was least effectively removed of all of the metals. Manganese is both weakly sorbed and is unstable as a metal sulfide under reducing conditions. The manganese that was removed may have precipitated as a carbonate species, rhodochrosite, in locations where pH was 7.1 or higher.

As the experiments progress, we observe a decrease in metal removal capacities of the reactors. At high flow rates and short hydraulic detention times, metal loading rates may exceed the combination of sulfate reduction rates and sorption. As sorption sites approach capacity, strongly sorbing metals like copper will displace those that do not have as strong an affinity for surface bonding. This concept was supported by the results of the experiments in which SRB activity was suppressed and sorption acted unimpeded. Manganese appeared in the effluent first, followed by zinc, while cadmium remained completely removed.

## **5.2 Sulfate Removal and Sulfide Production Balance**

Regardless of the loading rate of sulfate to each of the reactors, the data clearly supports the conclusion that the mass removal rate of sulfate was constant during each experiment and from reactor to reactor. The zero-order removal rate delineates the maximum level at which sulfide precipitation can function as the principal removal mechanism. The metal loading rates would need to be adjusted to match this sulfate reduction level in order to avoid contaminant breakthrough once the transient effects of adsorption removal processes were exhausted.

## **5.3 pH and Dissolved Oxygen as the Limiting Factors**

The experiments have shown that pH is more critical to reactor efficiency than dissolved oxygen. The metal loading rate capacity of a wet-substrate bio-reactor can be enhanced, and hydraulic detention times reduced, by modifying the pH of the influent to near neutral. The neutral pH permits enhanced activity of SRBs, the production of sulfide, and the removal of metals as metal sulfide precipitates. The dissolved oxygen was almost completely removed upon entering the reactors, while the pH required a greater proportion of the substrate in order to reach suitable levels. Since the SRBs are not only obligate anaerobes, but also require a narrow pH range near neutral for “optimal” sulfate reduction, it is reasonable to assume that pH is the predominant controlling

factor. Optimization and enhancement of the reactors may be accomplished through the modification of mine drainage to circum-neutral pH. Perhaps the use of anoxic limestone drains (ALDs) prior to the SRB bio-reactor would raise the pH to levels more acceptable to the bacteria. The ALDs could simultaneously decrease the dissolved oxygen content of the influent as well.

#### **5.4 Permeability and Sustainability**

During high flow experiments (i.e. short hydraulic detention time), there was evidence that a small amount of short-circuiting was occurring up to effluent port #5 in reactor A<sub>1</sub>. Data for E<sub>h</sub> and pH, as well metals concentrations at this port, support this conclusion. The phenomenon does not appear to have developed in any of the remaining reactors, including B<sub>1</sub>, the second high flow system.

With the occurrence of short-circuiting, which reduces the effective volume of the reactor, the treatment efficiency of that reactor decreases. The metal contaminated drainage bypasses the substrate. It is possible that the ceramic granules used as a bulking agent were too few in proportion to the composted livestock manure. The high flow of water, impeded by hydraulic conductivities of approximately 10-30 cm/s, and under 202 mmHg (as measured on reactor B<sub>1</sub>), would seek a path of least resistance -- the short-circuit.

In order to reduce the possibility of short-circuiting in this particular substrate mixture, it is recommended that the volume proportion of ceramic granules be increased from 10% to 25 - 30%. Doing so would increase the substrate permeability, and still provide a carbon source and accumulation areas for the sulfate-reducing bacteria.

### **5.5 Adsorption Removal**

It appears that a significant proportion of the short-term removal of metal ions was due to adsorption onto the organic manure substrate. Experimental results indicate that adsorption was limited by the sorptive capacity of the substrate. Different substrate materials will have different sorptive capacities. Combined with the fact that adsorption in these systems is also a function of pH and the particular metal species being removed, each system will be unique in character. A universal sorption capacity cannot be assigned to Passive Mine Drainage Systems as a whole, due to the unique character of each treatment system and the quality of the water being treated. Thus, most attention should be focused on optimizing conditions for sulfate reduction.

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**APPENDIX A:**  
**Bacterial Culture Medium**

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**SULFATE-REDUCING BACTERIA  
INOCULANT NUTRIENT BROTH MEDIA**

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<b>Constituent</b>	<b>grams per 1 Liter</b>	<b>grams per 5 Gallons</b>
<i>K<sub>2</sub>HPO<sub>4</sub></i>	0.5	9.0
<i>NH<sub>4</sub>Cl</i>	1.0	18.0
<i>NaSO<sub>4</sub></i>	1.0	18.0
<i>CaCl<sub>2</sub></i>	0.1	1.8
<i>MgSO<sub>4</sub></i>	2.0	36.0
<i>Na</i> -Lactate	3.5 mL	63.0 mL
yeast	1.0	18.0
<i>FeSO<sub>4</sub></i>	0.5	9.0
Ascorbic Acid	0.1	1.8
<i>Na</i> -Thioglycolate	0.1	1.8

\* Nutrient broth developed and prepared by Pintail Systems laboratory.

**APPENDIX B:**  
**Experiment #1 Data**

## INFLUENT SAMPLE DATA

Experiment: **pH-1**  
Date: **9/20/94**

Sample Time: 1430

<u>Variables</u>	<u>Units</u>	<u>Tank</u>
Eh (observed)	mV	-8.4
Eh (corrected)	mV	200.6
DO	mg/L	6.3
pH	su	6
SpC	umhos	1440
Chloride	ppm	184.863
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	0
Sulfate	ppm	454.214

<b>Zn2+</b>	<i>ppm</i>	<b>98</b>
Std. Dev.		0.06
RSD		1.55
<b>Mn2+</b>	<i>ppm</i>	<b>55.6</b>
Std. Dev.		0.07
RSD		1.21
<b>Cd2+</b>	<i>ppm</i>	<b>0.62</b>
Std. Dev.		0.01
RSD		0.92

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-1**  
Date: **9/20/94**

Column: **A1**

Flow:	1	2	3	<i>Average</i>		
	14	14.6	14.8	14.5	ml/min	
Volume:					29364	ml
Residence Time:					<b>33.8</b>	hrs

<b>PORT SAMPLES</b>		<b>A1-1</b>	<b>A1-2</b>	<b>A1-3</b>	<b>A1-4</b>	<b>A1-5</b>	<b>A1-6</b>
Sample Time:		1400	1330	1255	1230	1130	1015
<u>Variables</u>	<u>Units</u>						
Eh (observed)	mV	-101.3	-130.9	-116.2	-252.1	-139.4	-275.5
Eh (corrected)	mV	107.7	78.1	92.8	-43.1	69.6	-66.5
DO	mg/L	1.9	1.1	1.1	1	0.9	0.9
pH	su	6.1	6.2	6.2	6.7	6.6	6.6
SpC	umhos	1442	1463	1495	1613	1564	1560
Chloride	ppm	183.852	193.525	179.969	182.586	213.734	194.543
Nitrite	ppm	0	0	0	0	0	0
Nitrate	ppm	0	0	0	0	0	0
Phosphate	ppm	0	0	29.205	41.614	35.003	36.541
Sulfate	ppm	403.689	424.745	380.433	328.222	369.025	349.012

<b>Zn2+</b>	<i>ppm</i>	<b>32.1</b>	<b>17.7</b>	<b>17.3</b>	<b>0.72</b>	<b>0.35</b>	<b>0.88</b>	
		Std. Dev.	0.07	0.03	0.02	0.01	0.02	0.02
		RSD	2.02	1.72	1.43	1.46	4.29	2.35
<b>Mn2+</b>	<i>ppm</i>	<b>42.9</b>	<b>33.6</b>	<b>35.2</b>	<b>17.6</b>	<b>17.7</b>	<b>17.8</b>	
		Std. Dev.	0.01	0.03	0.03	0.01	0.01	0.01
		RSD	0.31	0.8	0.85	0.85	0.51	0.61
<b>Cd2+</b>	<i>ppm</i>	<b>0.09</b>	<b>0.01</b>	<b>0.01</b>	<b>0</b>	<b>0</b>	<b>0</b>	
		Std. Dev.	0	0	0	0	0	0.01
		RSD	3.08	76.07	84.24	<i>error</i>	<i>error</i>	<i>error</i>

## SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-1**  
Date: **9/20/94**

Column: **A2**

Flow:	1	2	3	<i>Average</i>	
	15.5	15.8	15.6	15.6	ml/min
Volume:				29364	ml
Residence Time:				31.3	hrs

## PORT SAMPLES

A2-6

Sample Time:

1020

VariablesUnits

Eh (observed)	mV	-288
Eh (corrected)	mV	-79
DO	mg/L	0.5
pH	su	6.75
SpC	umhos	1623
Chloride	ppm	196.703
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	38.371
Sulfate	ppm	385.286

<b>Zn2+</b>	<i>ppm</i>	<b>0.15</b>
Std. Dev.		0.01
RSD		7.79
<b>Mn2+</b>	<i>ppm</i>	<b>18.8</b>
Std. Dev.		0.01
RSD		0.53
<b>Cd2+</b>	<i>ppm</i>	<b>0</b>
Std. Dev.		0
RSD		<i>error</i>

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-1**Date: **9/20/94**Column: **B1**

Flow:	1	2	3	<i>Average</i>		
	5	4.9	4.8	4.9	ml/min	
Volume:					24904	ml
Residence Time:					<b>84.7</b>	hrs

**PORT SAMPLES**

Sample Time:	<b>B1-1</b>	<b>B1-2</b>	<b>B1-3</b>	<b>B1-4</b>	<b>B1-5</b>	<b>B1-6</b>
	1410	1335	1300	1235	1140	1045

<u>Variables</u>	<u>Units</u>					
------------------	--------------	--	--	--	--	--

Eh (observed)	mV	-91.9	-307.8	-333.7	-330.9	-368.8	-369.3
Eh (corrected)	mV	117.1	-98.8	-124.7	-121.9	-159.8	-160.3
DO	mg/L	1.5	0.4	0.5	0.1	0.1	0.4
pH	su	6.3	6.6	6.9	6.9	7	7
SpC	umhos	1452	1462	1570	1615	1665	1675
Chloride	ppm	188.498	191.675	181.354	221.669	199.807	197.952
Nitrite	ppm	0	0	0	8.749	0	0
Nitrate	ppm	0	0	0	0	0	0
Phosphate	ppm	29.246	31.219	39.25	30.657	42.335	50.111
Sulfate	ppm	358.755	360.752	266.518	215.711	163.964	139.378

<b>Zn<sup>2+</sup></b>	<i>ppm</i>	<b>7.67</b>	<b>0.82</b>	<b>0.09</b>	<b>0.06</b>	<b>0.07</b>	<b>0.06</b>
Std. Dev.		0.07	0.02	0.02	0.01	0.01	0
RSD		0.87	2	16.92	17.28	14.79	8.31
<b>Mn<sup>2+</sup></b>	<i>ppm</i>	<b>28.9</b>	<b>26.1</b>	<b>5.65</b>	<b>2.92</b>	<b>0.25</b>	<b>0.08</b>
Std. Dev.		0.03	0.01	0.05	0.02	0	0.01
RSD		1.04	0.57	0.8	0.67	1.34	6.19
<b>Cd<sup>2+</sup></b>	<i>ppm</i>	<b>0</b>	<b>-0.01</b>	<b>-0.01</b>	<b>-0.01</b>	<b>0</b>	<b>-0.01</b>
Std. Dev.		0.01	0	0	0.01	0	0
RSD		<i>error</i>	-34.82	-17.44	-74.18	<i>error</i>	-63.83

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-1**  
Date: **9/20/94**

Column: **B2**

Flow:	1	2	3	<i>Average</i>	
	4.9	5	4.6	4.8	ml/min

Volume: 24904 ml

Residence Time: 85.9 hrs

#### PORT SAMPLES

**B2-6**

Sample Time: 1050

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-361.1
Eh (corrected)	mV	-152.1
DO	mg/L	0.3
pH	su	7.1
SpC	umhos	1751
Chloride	ppm	174.27
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	59.133
Sulfate	ppm	99.806

<b>Zn<sup>2+</sup></b>	<i>ppm</i>	<b>0.07</b>
Std. Dev.		0.01
RSD		17.08
<b>Mn<sup>2+</sup></b>	<i>ppm</i>	<b>0.15</b>
Std. Dev.		0.01
RSD		3.61
<b>Cd<sup>2+</sup></b>	<i>ppm</i>	<b>0</b>
Std. Dev.		0
RSD		<i>error</i>

### SULFATE UTILIZATION AND METAL REMOVAL

Experiment: **pH-1**  
Date: **9/20/94**

Column:		<b>A1</b>	<b>A2</b>	<b>B1</b>	<b>B2</b>
Flow Rate:	ml/min	14.5	15.6	4.9	4.8
Residence Time:	hrs	33.8	31.3	84.7	85.9
<b>Influent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		98	98	98	98
Mn <sup>2+</sup>		55.6	55.6	55.6	55.6
Cd <sup>2+</sup>		0.62	0.62	0.62	0.62
SO <sub>4</sub> <sup>2-</sup>		454.2	454.2	454.2	454.2
<b>Effluent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		0.88	0.15	0.06	0.07
Mn <sup>2+</sup>		17.8	18.8	0.08	0.15
Cd <sup>2+</sup>		0	0	0	0
SO <sub>4</sub> <sup>2-</sup>		349	385.3	139.4	99.8
<b>Net Loss</b>					
	moles/day				
Zn <sup>2+</sup>		0.031	0.034	0.011	0.010
Mn <sup>2+</sup>		0.014	0.015	0.007	0.007
Cd <sup>2+</sup>		0.000	0.000	0.000	0.000
SO <sub>4</sub> <sup>2-</sup>		0.023	0.016	0.023	0.026
Total Moles Metals:		0.045	0.049	0.018	0.017
Total Moles Sulfate:		0.023	0.016	0.023	0.026
Total Moles Metals: (less moles Mn <sup>2+</sup> )		0.031	0.034	0.011	0.010
Total Moles Sulfate:		0.023	0.016	0.023	0.026

**APPENDIX C:**  
**Experiment #2 Data**

## INFLUENT SAMPLE DATA

Experiment: **pH-2**  
Date: **9/30/94**

Sample Time: 1400

<u>Variables</u>	<u>Units</u>	<u>Tank</u>
Eh (observed)	mV	36.5
Eh (corrected)	mV	245.5
DO	mg/L	4.4
pH	su	6.2
SpC	umhos	1890
Chloride	ppm	171.574
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	0
Sulfate	ppm	807.11

<b>Zn2+</b>	<i>ppm</i>	<b>96</b>
Std. Dev.		0.03
RSD		0.86
<b>Mn2+</b>	<i>ppm</i>	<b>52.9</b>
Std. Dev.		0.05
RSD		0.89
<b>Cd2+</b>	<i>ppm</i>	<b>0.54</b>
Std. Dev.		0
RSD		0.77

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-2**  
Date: **9/30/94**

Column: **A1**

Flow:	1	2	3	<i>Average</i>		
	25.2	25.2	23.6	24.7	ml/min	
Volume:					29364	ml
Residence Time:					<b>19.8</b>	hrs

<b>PORT SAMPLES</b>		<b>A1-1</b>	<b>A1-2</b>	<b>A1-3</b>	<b>A1-4</b>	<b>A1-5</b>	<b>A1-6</b>
Sample Time:		1420	1315	1300	1220	1200	1015
<u>Variables</u>	<u>Units</u>						
Eh (observed)	mV	-26.7	-86.8	-87.6	-273.6	-222	-233.2
Eh (corrected)	mV	182.3	122.1	121.4	-64.6	-13	-24.2
DO	mg/L	1.3	1.0	1.1	0.7	1.0	1.0
pH	su	6.7	6.4	6.6	6.9	6.7	6.8
SpC	umhos	1919	1979	2010	2070	2040	2070
Chloride	ppm	174.283	173.624	180.616	175.249	180.399	182.258
Nitrite	ppm	0	0	0	0	0	0
Nitrate	ppm	0	0	0	0	0	0
Phosphate	ppm	0	0	31.484	40.226	35.509	37.202
Sulfate	ppm	802.267	770.022	788.127	711.948	737.211	705.398

<b>Zn2+</b>	<i>ppm</i>	<b>51.6</b>	<b>7.21</b>	<b>2.85</b>	<b>0.64</b>	<b>0.55</b>	<b>1.67</b>	
		Std. Dev.	0.11	0.12	0.04	0.01	0.01	0.02
		RSD	2.06	1.69	1.38	2.3	2.5	1.24
<b>Mn2+</b>	<i>ppm</i>	<b>47.3</b>	<b>29</b>	<b>19.5</b>	<b>11.6</b>	<b>19.1</b>	<b>17.5</b>	
		Std. Dev.	0.06	0.02	0.02	0.01	0.02	0.02
		RSD	1.28	0.82	1.03	0.79	0.93	0.92
<b>Cd2+</b>	<i>ppm</i>	<b>0.08</b>	<b>0</b>	<b>0</b>	<b>0.01</b>	<b>0</b>	<b>0.01</b>	
		Std. Dev.	0.01	0.01	0	0	0	0.01
		RSD	7.27	<i>error</i>	<i>error</i>	52.19	-20.2	81.56

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-2**  
Date: **9/30/94**

Column: **A2**

Flow:	1	2	3	<i>Average</i>		
	9.2	9	9	9.1	ml/min	
Volume:					29364	ml
Residence Time:					<b>54.0</b>	hrs

#### PORT SAMPLES

**A2-6**

Sample Time: 1025

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-247
Eh (corrected)	mV	-38
DO	mg/L	0.9
pH	su	6.8
SpC	umhos	2010
Chloride	ppm	174.631
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	41.283
Sulfate	ppm	542.688

<b>Zn2+</b>	<i>ppm</i>	<b>0.43</b>
Std. Dev.		0
RSD		0.91
<b>Mn2+</b>	<i>ppm</i>	<b>17</b>
Std. Dev.		0
RSD		0.25
<b>Cd2+</b>	<i>ppm</i>	<b>0</b>
Std. Dev.		0.01
RSD		<i>error</i>

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-2**  
Date: **9/30/94**

Column: **B1**

Flow:	1	2	3	<i>Average</i>		
	25.6	25.8	25.6	25.7	ml/min	
Volume:					24904	ml
Residence Time:					<b>16.2</b>	hrs

<b>PORT SAMPLES</b>		<b>B1-1</b>	<b>B1-2</b>	<b>B1-3</b>	<b>B1-4</b>	<b>B1-5</b>	<b>B1-6</b>
Sample Time:		1430	1320	1310	1235	1210	1035
<u>Variables</u>	<u>Units</u>						
Eh (observed)	mV	-50.6	-89.7	-96.1	-232.4	-267.3	-286.6
Eh (corrected)	mV	158.4	119.3	112.9	-23.4	-58.3	-77.6
DO	mg/L	0.8	1.0	1.1	0.8	0.7	0.3
pH	su	6.4	6.4	6.6	6.7	6.9	7.0
SpC	umhos	1909	1927	1955	1973	1997	2020
Chloride	ppm	176.641	176.634	173.116	177.197	175	171.885
Nitrite	ppm	0	0	0	0	0	0
Nitrate	ppm	0	0	0	0	0	0
Phosphate	ppm	0	0	0	0	29.138	32.144
Sulfate	ppm	811.956	807.507	777.084	775.297	750.65	733.585

<b>Zn2+</b>	<i>ppm</i>	<b>37</b>	<b>18.3</b>	<b>3.51</b>	<b>0.43</b>	<b>0.3</b>	<b>0.13</b>
Std. Dev.		0.04	0.03	0.04	0.01	0.01	0.01
RSD		1.07	1.91	1.11	3.17	4.78	6.97
<b>Mn2+</b>	<i>ppm</i>	<b>46.5</b>	<b>42.8</b>	<b>31.9</b>	<b>28.4</b>	<b>20.6</b>	<b>14.1</b>
Std. Dev.		0.04	0.06	0.03	0.05	0.01	0.01
RSD		0.84	1.33	0.93	1.72	0.59	0.68
<b>Cd2+</b>	<i>ppm</i>	<b>0.01</b>	<b>0.01</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0.01</b>
Std. Dev.		0	0	0	0.01	0	0
RSD		76.9	78.5	-91.3	<i>error</i>	<i>error</i>	15.24

### SAMPLE ANALYSIS DATA SHEET

Experiment: **pH-2**  
Date: **9/30/94**

Column: **B2**

Flow:	1	2	3	<i>Average</i>		
	8.4	8.2	9.2	8.6	ml/min	
Volume:					24904	ml
Residence Time:					<b>48.3</b>	hrs

#### PORT SAMPLES

**B2-6**

Sample Time: 1040

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-359.1
Eh (corrected)	mV	-150.1
DO	mg/L	0.1
pH	su	7.3
SpC	umhos	2100
Chloride	ppm	174.727
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	46.49
Sulfate	ppm	553.123

<b>Zn<sup>2+</sup></b>	<i>ppm</i>	<b>0.05</b>
Std. Dev.		0.01
RSD		23.13
<b>Mn<sup>2+</sup></b>	<i>ppm</i>	<b>0.18</b>
Std. Dev.		0.01
RSD		2.87
<b>Cd<sup>2+</sup></b>	<i>ppm</i>	<b>0</b>
Std. Dev.		0.01
RSD		<i>error</i>

### SULFATE UTILIZATION AND METAL REMOVAL

Experiment: **pH-2**  
Date: **9/30/94**

Column:		<b>A1</b>	<b>A2</b>	<b>B1</b>	<b>B2</b>
Flow Rate:	ml/min	24.7	9.1	25.7	8.6
Residence Time:	hrs	19.8	54	16.2	48.3
<b>Influent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		96	96	96	96
Mn <sup>2+</sup>		52.9	52.9	52.9	52.9
Cd <sup>2+</sup>		0.54	0.54	0.54	0.54
SO <sub>4</sub> <sup>2-</sup>		807.11	807.11	807.11	807.11
<b>Effluent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		1.67	0.43	0.13	0.05
Mn <sup>2+</sup>		17.5	17	14.1	0.18
Cd <sup>2+</sup>		0.01	0	0	0
SO <sub>4</sub> <sup>2-</sup>		705.4	542.7	733.6	553.1
<b>Net Loss</b>					
	moles/day				
Zn <sup>2+</sup>		0.051	0.019	0.054	0.018
Mn <sup>2+</sup>		0.023	0.009	0.026	0.012
Cd <sup>2+</sup>		0.000	0.000	0.000	0.000
SO <sub>4</sub> <sup>2-</sup>		0.038	0.036	0.028	0.033
<b>Total Moles Metals:</b>		<b>0.074</b>	<b>0.028</b>	<b>0.081</b>	<b>0.030</b>
<b>Total Moles Sulfate:</b>		<b>0.038</b>	<b>0.036</b>	<b>0.028</b>	<b>0.033</b>
<b>Total Moles Metals: (less moles Mn<sup>2+</sup>)</b>		<b>0.051</b>	<b>0.019</b>	<b>0.054</b>	<b>0.018</b>
<b>Total Moles Sulfate:</b>		<b>0.038</b>	<b>0.036</b>	<b>0.028</b>	<b>0.033</b>

**APPENDIX D:**  
**Experiment #3 Data**

## INFLUENT SAMPLE DATA

Experiment: **DO-1**  
Date: **11/22/94**

Sample Time: 1430

<u>Variables</u>	<u>Units</u>	<u>Tank</u>
Eh (observed)	mV	291.1
Eh (corrected)	mV	500.1
DO	mg/L	0.0
pH	su	2.7
SpC	umhos	2800
Chloride	ppm	221.714
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	0.679
Sulfate	ppm	996.003

<b>Zn<sup>2+</sup></b>	<i>ppm</i>	<b>103.1</b>
Std. Dev.		0.12
RSD		11.65
<b>Mn<sup>2+</sup></b>	<i>ppm</i>	<b>55.48</b>
Std. Dev.		0.039
RSD		0.7
<b>Cd<sup>2+</sup></b>	<i>ppm</i>	<b>0.54</b>
Std. Dev.		0.01
RSD		1.39
<b>Al<sup>3+</sup></b>	<i>ppm</i>	<b>5.3</b>
ICP DL = 0.5		
<b>Cu<sup>2+</sup></b>	<i>ppm</i>	<b>8.23</b>
Std. Dev.		0.1
RSD		1.17

## SAMPLE ANALYSIS DATA SHEET

Experiment: **DO-1**  
Date: **11/22/94**

Column: **A1**

Flow: 1 2 3 *Average*  
17.2 16.8 17 17.0 ml/min  
Volume: 29364 ml  
Residence Time: 28.8 hrs

**PORT SAMPLES**

		<b>A1-1</b>	<b>A1-2</b>	<b>A1-3</b>	<b>A1-4</b>	<b>A1-5</b>	<b>A1-6</b>
Sample Time:		1415	1340	1320	1310	1250	1123
<u>Variables</u>	<u>Units</u>						
Eh (observed)	mV	244.5	-66.2	-86.0	-198.0	-63.4	-87.8
Eh (corrected)	mV	453.5	142.8	123.0	11.0	145.6	121.2
DO	mg/L	0.0	0.0	0.0	0.0	0.2	0.4
pH	su	4.1	6.0	6.4	6.5	6.2	6.7
SpC	umhos	2120	2130	2230	2230	2170	2250
Chloride	ppm	224.345	230.686	226.187	225.373	229.523	216.289
Nitrite	ppm	0.0	0.0	0.0	0.0	0.0	0.0
Nitrate	ppm	0.0	0.0	0.0	0.0	0.0	0.0
Phosphate	ppm	0.335	0.0	0.0	8.809	1.029	16.788
Sulfate	ppm	976.319	952.612	892.013	901.157	886.418	890.976

<b>Zn2+</b>	<i>ppm</i>	<b>83.4</b>	<b>37.5</b>	<b>5.9</b>	<b>3.7</b>	<b>27.3</b>	<b>3.2</b>
Std. Dev.		0.045	0.007	0.003	0.003	0.013	0.002
RSD		5.37	1.97	4.8	8.29	4.59	7.53
<b>Mn2+</b>	<i>ppm</i>	<b>56.91</b>	<b>49.61</b>	<b>46.21</b>	<b>34.19</b>	<b>41.81</b>	<b>35.34</b>
Std. Dev.		0.048	0.027	0.021	0.022	0.026	0.027
RSD		0.84	0.54	0.46	0.63	0.63	0.75
<b>Cd2+</b>	<i>ppm</i>	<b>0.26</b>	<b>0.02</b>	<b>0.0</b>	<b>0.01</b>	<b>0.01</b>	<b>0.02</b>
Std. Dev.		0.0	0.0	0.0	0.0	0.0	0.0
RSD		1.13	16.0	73.45	27.48	31.2	25.17
<b>Al3+</b>	<i>ppm</i>	<b>2</b>	<b>&lt; 0.5</b>				
ICP DL = 0.5							
<b>Cu2+</b>	<i>ppm</i>	<b>2.07</b>	<b>0.01</b>	<b>0.0</b>	<b>0.01</b>	<b>0.0</b>	<b>0.02</b>
Std. Dev.		0.05	0.0	0.0	0.01	0.0	0.0
RSD		2.28	16.94	error	50.06	error	24.0

## SAMPLE ANALYSIS DATA SHEET

Experiment: **DO-1**  
Date: **11/22/94**

Column: **A2**

Flow:	1	2	3	<i>Average</i>	
	5.3	5.5	5.5	5.4	ml/min
Volume:				29364	ml
Residence Time:				<b>90.1</b>	hrs

## PORT SAMPLES

A2-6

Sample Time:

1140

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-222.3
Eh (corrected)	mV	-13.3
DO	mg/L	0
pH	su	7.1
SpC	umhos	2320
Chloride	ppm	218.745
Nitrite	ppm	0
Nitrate	ppm	0
Phosphate	ppm	31.88
Sulfate	ppm	772.972

<b>Zn2+</b>	<i>ppm</i>	<b>0.2</b>
Std. Dev.		0.01
RSD		62.71
<b>Mn2+</b>	<i>ppm</i>	<b>15.34</b>
Std. Dev.		0.007
RSD		0.44
<b>Cd2+</b>	<i>ppm</i>	<b>0.01</b>
Std. Dev.		0.0
RSD		38.02
<b>Al3+</b>	<i>ppm</i>	<b>&lt; 0.5</b>
ICP DL = 0.5		
<b>Cu2+</b>	<i>ppm</i>	<b>0.01</b>
Std. Dev.		0.01
RSD		41.38

## SAMPLE ANALYSIS DATA SHEET

Experiment: **DO-1**  
Date: **11/22/94**

Column: **B1**

Flow:	1	2	3	Average	
	16.8	17.2	16.8	16.9	ml/min
Volume:	24904				ml
Residence Time:	24.5				hrs

PORT SAMPLES		B1-1	B1-2	B1-3	B1-4	B1-5	B1-6
Sample Time:		1420	1345	1330	1315	1300	1155
<u>Variables</u>	<u>Units</u>						
Eh (observed)	mV	231.6	-19.8	-36.9	-94.5	-60.5	-101.8
Eh (corrected)	mV	440.6	189.2	172.1	114.5	148.5	107.2
DO	mg/L	0.0	0.0	0.0	0.0	0.3	0.6
pH	su	4.4	5.9	6.0	6.1	6.2	6.3
SpC	umhos	2090.0	2130.0	2130.0	2150.0	2180.0	2220.0
Chloride	ppm	216.294	216.115	220.925	219.517	214.918	219.793
Nitrite	ppm	0.0	0.0	0.0	0.0	0.0	0.0
Nitrate	ppm	0.0	0.0	0.0	0.0	0.0	0.0
Phosphate	ppm	0.0	0.406	0.624	2.446	3.202	1.221
Sulfate	ppm	963.587	952.523	968.046	955.815	925.593	933.075

<b>Zn2+</b>	ppm	<b>113.5</b>	<b>78.7</b>	<b>61.5</b>	<b>40.2</b>	<b>14.8</b>	<b>10.2</b>
Std. Dev.		0.044	0.036	0.017	0.008	0.002	0.003
RSD		3.89	4.46	2.78	2.07	1.67	3.02
<b>Mn2+</b>	ppm	<b>56.05</b>	<b>52.12</b>	<b>62.31</b>	<b>62.92</b>	<b>53.13</b>	<b>53.91</b>
Std. Dev.		0.034	0.028	0.04	0.037	0.032	0.025
RSD		0.61	0.55	0.65	0.58	0.6	0.46
<b>Cd2+</b>	ppm	<b>0.42</b>	<b>0.02</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>
Std. Dev.		0.01	0.0	0.0	0.0	0.0	0.0
RSD		1.85	9.7	35.3	28.41	5.52	15.16
<b>Al3+</b>	ppm	<b>1.7</b>	<b>&lt; 0.5</b>				
ICP DL = 0.5							
<b>Cu2+</b>	ppm	<b>1.2</b>	<b>0.0</b>	<b>0.01</b>	<b>0.01</b>	<b>0.02</b>	<b>0.0</b>
Std. Dev.		0.02	0.0	0.0	0.0	0.0	0.0
RSD		2.04	error	23.16	26.81	11.25	97.3

## SAMPLE ANALYSIS DATA SHEET

Experiment: DO-1  
Date: 11/22/94

Column: B2

Flow:	1	2	3	Average	
	5.45	5.5	5.5	5.5	ml/min
Volume:				24904	ml
Residence Time:				75.7	hrs

## PORT SAMPLES

B2-6

Sample Time: 1200

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-268.8
Eh (corrected)	mV	-59.8
DO	mg/L	0.0
pH	su	7.0
SpC	umhos	2210
Chloride	ppm	218.9
Nitrite	ppm	0.0
Nitrate	ppm	0.0
Phosphate	ppm	14.0
Sulfate	ppm	780.0

<b>Zn<sup>2+</sup></b>	<i>ppm</i>	<b>6.8</b>
Std. Dev.		0.003
RSD		5.06
<b>Mn<sup>2+</sup></b>	<i>ppm</i>	<b>15.91</b>
Std. Dev.		0.012
RSD		0.73
<b>Cd<sup>2+</sup></b>	<i>ppm</i>	<b>0.01</b>
Std. Dev.		0.0
RSD		31.61
<b>Al<sup>3+</sup></b>	<i>ppm</i>	<b>&lt; 0.5</b>
ICP DL = 0.5		
<b>Cu<sup>2+</sup></b>	<i>ppm</i>	<b>0.01</b>
Std. Dev.		0.0
RSD		21.96

### SULFATE UTILIZATION AND METAL REMOVAL

Experiment: **DO-1**  
Date: **11/22/94**

Column:		<b>A1</b>	<b>A2</b>	<b>B1</b>	<b>B2</b>
Flow Rate:	ml/min	17	5.4	16.9	5.5
Residence Time:	hrs	28.8	90.1	24.5	75.7
<b>Influent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		103.1	103.1	103.1	103.1
Mn <sup>2+</sup>		55.48	55.48	55.48	55.48
Cd <sup>2+</sup>		0.54	0.54	0.54	0.54
Al <sup>3+</sup>		5	5	5	5
Cu <sup>2+</sup>		8.23	8.23	8.23	8.23
SO <sub>4</sub> <sup>2-</sup>		996	996	996	996
<b>Effluent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		3.2	20.3	10.2	6.8
Mn <sup>2+</sup>		35.34	15.34	53.91	15.91
Cd <sup>2+</sup>		0.02	0.01	0.01	0.01
Al <sup>3+</sup>		0	0	0	0
Cu <sup>2+</sup>		0.02	0.01	0	0.01
SO <sub>4</sub> <sup>2-</sup>		891	773	933.1	780
<b>Net Loss</b>					
	moles/day				
Zn <sup>2+</sup>		0.037	0.010	0.035	0.012
Mn <sup>2+</sup>		0.009	0.006	0.001	0.006
Cd <sup>2+</sup>		0.000	0.000	0.000	0.000
Al <sup>3+</sup>		0.005	0.001	0.005	0.001
Cu <sup>2+</sup>		0.003	0.001	0.003	0.001
SO <sub>4</sub> <sup>2-</sup>		0.027	0.018	0.016	0.018
<b>Total Moles Metals:</b>		0.054	0.018	0.043	0.020
<b>Total Moles Sulfate:</b>		0.027	0.018	0.016	0.018
<b>Total Moles Metals: (less moles Mn<sup>2+</sup>)</b>		0.045	0.012	0.042	0.014
<b>Total Moles Sulfate:</b>		0.027	0.018	0.016	0.018

**APPENDIX E:**  
**Experiment #4 Data**

## INFLUENT SAMPLE DATA

Experiment: **DO-2**  
Date: **12/9/94**

Sample Time: 1400

<u>Variables</u>	<u>Units</u>	<u>Tank</u>
Eh (observed)	mV	403.2
Eh (corrected)	mV	612.2
DO	mg/L	4.8
pH	su	2.8
SpC	umhos	2070
Chloride	ppm	263.810
Nitrite	ppm	0.0
Nitrate	ppm	0.245
Phosphate	ppm	0.0
Sulfate	ppm	921.458

<b>Zn<sup>2+</sup></b>	<i>ppm</i>	<b>118.4</b>
Std. Dev.		0.012
RSD		0.99
<b>Mn<sup>2+</sup></b>	<i>ppm</i>	<b>51.9</b>
Std. Dev.		0.046
RSD		0.89
<b>Cd<sup>2+</sup></b>	<i>ppm</i>	<b>0.59</b>
Std. Dev.		0.01
RSD		1.49
<b>Al<sup>3+</sup></b>	<i>ppm</i>	<b>5.7</b>
ICP DL = 0.5		
<b>Cu<sup>2+</sup></b>	<i>ppm</i>	<b>8.14</b>
Std. Dev.		0.09
RSD		1.09

## SAMPLE ANALYSIS DATA SHEET

Experiment: DO-2

Date: 12/9/94

Column: A1

Flow:	1	2	3	Average	
	14.4	14.8	14.4	14.5	ml/min

Volume:	29364	ml
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Residence Time:	33.7	hrs
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## PORT SAMPLES

Sample Time:	A1-1	A1-2	A1-3	A1-4	A1-5	A1-6
	1355	1330	1245	1230	1208	1103

<u>Variables</u>	<u>Units</u>					
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Eh (observed)	mV	255.7	214.9	-39.7	-57.0	132.9	-67.1
Eh (corrected)	mV	464.7	423.9	169.3	152.0	341.9	141.9
DO	mg/L	1.83	0.98	0.65	0.74	0.80	0.80
pH	su	5.0	5.7	5.8	6.1	5.8	6.3
SpC	umhos	1697	1718	1742	1741	1733	1784
Chloride	ppm	281.451	263.700	310.606	256.436	241.567	10.149
Nitrite	ppm	0.0	10.8	0.0	9.7	10.0	0.0
Nitrate	ppm	0.4	2.1	0.0	0.1	0.0	0.0
Phosphate	ppm	0.0	0.0	0.0	0.407	0.0	0.0
Sulfate	ppm	955.416	849.006	747.586	718.507	770.933	36.139

<b>Zn2+</b>	<i>ppm</i>	<b>105.6</b>	<b>90.5</b>	<b>39.2</b>	<b>22.9</b>	<b>62.7</b>	<b>7.1</b>
Std. Dev.		0.009	0.012	0.005	0.004	0.006	0.002
RSD		0.88	1.38	1.38	1.84	1.0	3.17
<b>Mn2+</b>	<i>ppm</i>	<b>58.64</b>	<b>53.6</b>	<b>54.93</b>	<b>43.0</b>	<b>48.14</b>	<b>44.67</b>
Std. Dev.		0.038	0.028	0.037	0.024	0.031	0.019
RSD		0.65	0.51	0.67	0.56	0.64	0.42
<b>Cd2+</b>	<i>ppm</i>	<b>0.31</b>	<b>0.31</b>	<b>0.01</b>	<b>0.02</b>	<b>0.23</b>	<b>0.01</b>
Std. Dev.		0.01	0.01	0.0	0.0	0.0	0.0
RSD		2.05	1.95	18.86	10.37	1.09	33.28
<b>Al3+</b>	<i>ppm</i>	<b>&lt; 0.5</b>					
ICP DL = 0.5							
<b>Cu2+</b>	<i>ppm</i>	<b>2.28</b>	<b>0.51</b>	<b>0.0</b>	<b>0.01</b>	<b>0.09</b>	<b>0.01</b>
Std. Dev.		0.03	0.01	0.0	0.0	0.0	0.01
RSD		1.20	1.01	error	44.68	4.14	61.58

## SAMPLE ANALYSIS DATA SHEET

Experiment: DO-2

Date: 12/9/94

Column: A2

Flow:	1	2	3	Average	
	5	5.2	4.9	5.0	ml/min

Volume: 29364 ml

Residence Time: 97.2 hrs

## PORT SAMPLES

A2-6

Sample Time: 1110

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-316.4
Eh (corrected)	mV	-107.4
DO	mg/L	0
pH	su	6.8
SpC	umhos	1829
Chloride	ppm	231.889
Nitrite	ppm	11.007
Nitrate	ppm	0.116
Phosphate	ppm	17.098
Sulfate	ppm	572.133

<b>Zn2+</b>	<i>ppm</i>	<b>1.60</b>
Std. Dev.		0.003
RSD		17.95
<b>Mn2+</b>	<i>ppm</i>	<b>15.51</b>
Std. Dev.		0.013
RSD		0.83
<b>Cd2+</b>	<i>ppm</i>	<b>0.01</b>
Std. Dev.		0.0
RSD		28.27
<b>Al3+</b>	<i>ppm</i>	<b>&lt; 0.5</b>
ICP DL = 0.5		
<b>Cu2+</b>	<i>ppm</i>	<b>0.0</b>
Std. Dev.		0.01
RSD		error

## SAMPLE ANALYSIS DATA SHEET

Experiment: **DO-2**  
Date: **12/9/94**

Column: **B1**

Flow: 1 2 3 *Average*  
14.8 14.8 15.2 14.9 ml/min  
Volume: 24904 ml  
Residence Time: 27.8 hrs

PORT SAMPLES		B1-1	B1-2	B1-3	B1-4	B1-5	B1-6
Sample Time:		1400	1340	1255	1240	1135	1120
<u>Variables</u>	<u>Units</u>						
Eh (observed)	mV	292.0	169.0	20.0	10.1	-48.2	-115.7
Eh (corrected)	mV	501.0	378.0	229.0	219.1	160.8	93.3
DO	mg/L	2.3	1.5	0.5	1.0	0.9	0.6
pH	su	4.2	5.6	5.8	5.8	5.9	6.0
SpC	umhos	1703	1706	1725	1728	1741	1741
Chloride	ppm	229.611	x	228.721	228.582	223.787	242.031
Nitrite	ppm	0.0	x	0.0	7.545	8.01	0.0
Nitrate	ppm	0.0	x	0.0	0.0	0.0	0.0
Phosphate	ppm	0.0	x	0.0	0.0	0.0	0.0
Sulfate	ppm	779.406	x	757.748	753.679	732.883	794.424

<b>Zn2+ **</b>	ppm	<b>122</b>	<b>115.9</b>	<b>92.4</b>	<b>81.5</b>	<b>53.4</b>	<b>36.5</b>
Std. Dev.		0.01	0.02	0.03	0.02	0.03	0.02
RSD		1.09	1.87	0.67	0.61	1.26	1.03
<b>Mn2+</b>	ppm	<b>57.86</b>	<b>58.11</b>	<b>57.4</b>	<b>56.59</b>	<b>57.0</b>	<b>55.47</b>
Std. Dev.		0.022	0.039	0.042	0.037	0.042	0.018
RSD		0.38	0.68	0.73	0.66	0.73	0.33
<b>Cd2+</b>	ppm	<b>0.48</b>	<b>0.35</b>	<b>0.02</b>	<b>0.01</b>	<b>0.01</b>	<b>0.0</b>
Std. Dev.		0.01	0.01	0.0	0.0	0.0	0.01
RSD		1.97	1.56	18.46	10.32	19.24	<i>error</i>
<b>Al3+</b>	ppm	<b>1.8</b>	<b>&lt; 0.5</b>				
ICP DL = 0.5							
<b>Cu2+</b>	ppm	<b>3.15</b>	<b>0.0</b>	<b>0.01</b>	<b>0.02</b>	<b>0.01</b>	<b>0.0</b>
Std. Dev.		0.06	0.0	0.0	0.0	0.0	0.0
RSD		1.88	<i>error</i>	36.74	20.46	17.24	<i>error</i>

## SAMPLE ANALYSIS DATA SHEET

Experiment: DO-2

Date: 12/9/94

Column: B2

Flow:	1	2	3	Average	
	5	5	4.9	5.0	ml/min
Volume:				24904	ml
Residence Time:				83.6	hrs

## PORT SAMPLES

B2-6

Sample Time:

1100

<u>Variables</u>	<u>Units</u>	
Eh (observed)	mV	-299.5
Eh (corrected)	mV	-90.5
DO	mg/L	0.0
pH	su	6.7
SpC	umhos	1775
Chloride	ppm	223.204
Nitrite	ppm	7.227
Nitrate	ppm	0.0
Phosphate	ppm	8.598
Sulfate	ppm	510.817

<b>Zn2+</b>	<i>ppm</i>	<b>8.4</b>
Std. Dev.		0.003
RSD		3.87
<b>Mn2+</b>	<i>ppm</i>	<b>26.95</b>
Std. Dev.		0.023
RSD		0.85
<b>Cd2+</b>	<i>ppm</i>	<b>0.01</b>
Std. Dev.		0.0
RSD		23.4
<b>Al3+</b>	<i>ppm</i>	<b>&lt; 0.5</b>
ICP DL = 0.5		
<b>Cu2+</b>	<i>ppm</i>	<b>0.0</b>
Std. Dev.		0.0
RSD		31.0

### SULFATE UTILIZATION AND METAL REMOVAL

Experiment: **DO-2**  
Date: **12/9/94**

Column:		<b>A1</b>	<b>A2</b>	<b>B1</b>	<b>B2</b>
Flow Rate:	ml/min	14.5	5	14.9	5
Residence Time:	hrs	33.7	97.2	27.8	83.6
<b>Influent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		118.4	118.4	118.4	118.4
Mn <sup>2+</sup>		51.9	51.9	51.9	51.9
Cd <sup>2+</sup>		0.59	0.59	0.59	0.59
Al <sup>3+</sup>		6	6	6	6
Cu <sup>2+</sup>		8.14	8.14	8.14	8.14
SO <sub>4</sub> <sup>2-</sup>					
<b>Effluent</b>					
Concentration:	mg/L				
Zn <sup>2+</sup>		7.1	1.6	36.5	8.4
Mn <sup>2+</sup>		44.67	15.51	55.47	26.95
Cd <sup>2+</sup>		0.01	0.01	0	0.01
Al <sup>3+</sup>		0	0	0	0
Cu <sup>2+</sup>		0.01	0	0	0
SO <sub>4</sub> <sup>2-</sup>					
<b>Net Loss</b>					
	moles/day				
Zn <sup>2+</sup>		0.036	0.013	0.027	0.012
Mn <sup>2+</sup>		0.003	0.005	-0.001	0.003
Cd <sup>2+</sup>		0.000	0.000	0.000	0.000
Al <sup>3+</sup>		0.005	0.002	0.005	0.002
Cu <sup>2+</sup>		0.003	0.001	0.003	0.001
SO <sub>4</sub> <sup>2-</sup>		0.000	0.000	0.000	0.000
<b>Total Moles Metals:</b>		0.046	0.020	0.033	0.018
<b>Total Moles Sulfate:</b>					
<b>Total Moles Metals: (less moles Mn<sup>2+</sup>)</b>		0.043	0.015	0.035	0.015
<b>Total Moles Sulfate:</b>					

**APPENDIX F:**  
**Experiment #5 Data**

**BENCHSCALE MANURE SORPTION BEAKER TEST #2****Calibration Curve for Copper on AA**

Standard (ppm)	Absorbance	Final Reading (ppm)
0.8	0.109	0.793
2.0	0.269	1.952
4.0	0.504	3.856
6.0	0.704	5.56
8.0	0.876	7.78

**Results**

	<b>Beaker A</b>	<b>Beaker B</b>
<b>T=0 minutes</b>	<b>69.22</b>	<b>71.01</b>
StD	0.048	0.049
RSD	0.69	0.69
<b>T=10 minutes</b>	<b>40.43</b>	<b>46.67</b>
StD	0.024	0.019
RSD	0.6	0.4
<b>T=20 minutes</b>	<b>38.12</b>	<b>43.31</b>
StD	0.024	0.046
RSD	0.64	1.06
<b>T=35 minutes</b>	<b>32.97</b>	<b>39.45</b>
StD	0.013	0.027
RSD	0.4	0.69
<b>T=60 minutes</b>	<b>28.78</b>	<b>34.25</b>
StD	0.016	0.02
RSD	0.54	0.58
<b>T=180 minutes</b>	<b>21.22</b>	<b>26.9</b>
StD	0.02	0.009
RSD	0.93	0.34
<b>T=1440 minutes</b>	<b>6.47</b>	<b>6.38</b>
StD	0.007	0.005
RSD	1.05	0.79
<b>T=2880 minutes</b>	<b>5.18</b>	<b>4.71</b>
StD	0.007	0.003
RSD	1.32	0.56
<b>T=4320 minutes</b>	<b>4.65</b>	<b>4.64</b>
StD	0.003	0.005
RSD	0.65	1.1
<b>T=5760 minutes</b>	<b>5.42</b>	<b>4.86</b>
StD	0.006	0.007
RSD	1.07	1.38

**APPENDIX G:**  
**Experiment #6 Data**

## ADSORPTION COLUMN EFFLUENT ANALYSIS

### AA/AE Spectrophotometer Calibration Curve (Cadmium)

Standard (ppm)	Absorbance	Final Reading (ppm)
0.8	0.140	0.867
2.0	0.286	2.087
4.0	0.444	4.160
6.0	0.529	5.930
8.0	0.593	7.890

<u>Sample Date</u>	<u>Volume Passed (L)</u>	<u>Cd<sup>2+</sup> (ppm)</u>	<u>StD.</u>	<u>RSD</u>
Influent-11/28	0	<b>0.368</b>	0.004	1.010
11/28/94	?	<b>0.010</b>	0.004	40.870
11/30/94	?	<b>0.003</b>	0.003	96.080
12/2/94	?	<b>0.010</b>	0.003	33.520
12/5/94	29.7	<b>0.000</b>	0.004	<i>error</i>
12/7/94	33.6	<b>0.001</b>	0.004	<i>error</i>
12/9/94	37.7	<b>0.005</b>	0.003	49.500
12/12/94	42.4	<b>0.009</b>	0.002	18.610
12/14/94	45.3	<b>0.009</b>	0.003	34.080
12/16/94	50.7	<b>0.002</b>	0.003	<i>error</i>
12/19/94	55.2	<b>0.006</b>	0.002	27.360
12/27/94	69.1	<b>0.000</b>	0.002	<i>error</i>
1/9/95	74.3	<b>0.000</b>	0.003	<i>error</i>
1/11/95	77.8	<b>0.010</b>	0.003	26.630
1/13/95	81.0	<b>0.010</b>	0.002	17.380
1/16/95	86.6	<b>0.005</b>	0.004	81.740
1/18/95	89.4	<b>0.007</b>	0.002	30.100
1/20/95	92.3	<b>0.007</b>	0.003	40.310
1/23/95	95.7	<b>0.005</b>	0.003	62.250
1/25/95	98.9	<b>0.014</b>	0.003	17.780

\* 1:1 dilution for analysis

**ADSORPTION COLUMN EFFLUENT ANALYSIS**AA/AE Spectrophotometer Calibration Curve  
(Manganese)

Standard (ppm)	Absorbance	Final Reading (ppm)
0.8	0.118	0.792
2.0	0.277	1.969
4.0	0.517	4.002
6.0	0.706	5.997
8.0	0.857	8.035

<b>Sample Date</b>	<b>Volume Passed (L)</b>	<b>Mn<sup>2+</sup> (ppm)</b>	<b>Std.</b>	<b>RSD</b>
Influent-11/28	0	<b>63.85</b>	0.011	0.84
11/28/94	?	<b>0.35</b>	0.005	74.22
11/30/94	?	<b>0.15</b>	0.003	<i>error</i>
12/2/94	?	<b>0.30</b>	0.003	51.89
12/5/94	29.7	<b>0.20</b>	0.004	96.15
12/7/94	33.6	<b>0.10</b>	0.004	<i>error</i>
12/9/94	37.7	<b>0.35</b>	0.005	68.45
12/12/94	42.4	<b>0.40</b>	0.003	29.62
12/14/94	45.3	<b>0.00</b>	0.005	<i>error</i>
12/16/94	50.7	<b>0.15</b>	0.004	<i>error</i>
12/19/94	55.2	<b>0.75</b>	0.005	32.65
12/27/94	69.1	<b>9.35</b>	0.004	1.89
1/9/95	74.3	<b>24.45</b>	0.008	1.60
1/11/95	77.8	<b>43.20</b>	0.006	0.66
1/13/95	81.0	<b>44.50</b>	0.009	0.97
1/16/95	86.6	<b>47.25</b>	0.005	0.58
1/18/95	89.4	<b>48.30</b>	0.006	0.67
1/20/95	92.3	<b>49.25</b>	0.007	0.69
1/23/95	95.7	<b>50.00</b>	0.008	0.76
1/25/95	98.9	<b>55.00</b>	0.007	0.69

\* 1:50 dilution for analysis

**ADSORPTION COLUMN EFFLUENT ANALYSIS**AA/AE Spectrophotometer Calibration Curve  
(Zinc)

Standard (ppm)	Absorbance	Final Reading (ppm)
0.25	0.060	0.25
1.0	0.212	0.99
2.0	0.386	1.92
4.0	0.663	3.70
6.0	0.852	5.26

<u>Sample Date</u>	<u>Volume Passed (L)</u>	<u>Zn<sup>2+</sup> (ppm)</u>	<u>Std.</u>	<u>RSD</u>
Influent-11/28	0	106.5	0.020	0.96
11/28/94	?	5.5	0.01	8.38
11/30/94	?	7.5	0.01	3.41
12/2/94	?	7.0	0.01	8.07
12/5/94	29.7	8.0	0.01	4.86
12/7/94	33.6	4.0	0.01	9.04
12/9/94	37.7	2.5	0.01	19.61
12/12/94	42.4	5.0	0.01	7.96
12/14/94	45.3	3.5	0.01	10.60
12/16/94	50.7	2.5	0.01	10.57
12/19/94	55.2	4.0	0.01	7.53
12/27/94	69.1	4.0	0.01	8.28
1/9/95	74.3	4.0	0.01	8.67
1/11/95	77.8	8.0	0.01	6.88
1/13/95	81.0	12.5	0.01	3.09
1/16/95	86.6	11.5	0.01	2.70
1/18/95	89.4	15.0	0.01	2.58
1/20/95	92.3	15.0	0.01	2.58
1/23/95	95.7	18.0	0.01	2.59
1/25/95	98.9	23.5	0.01	2.36

\* 1:50 dilution for analysis

**APPENDIX H:**

**Composite Manure Mixture Physical Properties**

Using procedures set forth by Lemke (1989) for the analysis of the physical properties of constructed wetland substrates, the following information was determined:

<b>Physical Properties for the Reactor Substrate</b>	
Bulk density of composite substrate mixture	0.93 grams/cm <sup>3</sup>
Specific gravity of composted livestock manure	2.27

