BIOGEOCHEMICAL CHARACTERIZATION OF A CONSTRUCTED WETLAND FOR ACID MINE DRAINAGE TREATMENT

DISSE RTATION

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By

Wendy Buell Gagliano, M.S.

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Dissertation Committee:

Professor Jerry M. Bigham, Advisor

Professor Samuel J. Traina

Professor Olli H. Tuovinen

Professor Frederick C. Michel

Approved by

Advisor

Soil Science
ABSTRACT

Drainage from abandoned coal mines has resulted in severe water quality problems. The oxidation of sulfide minerals in coal and associated rocks releases iron-rich, acidic solutions that damage vegetation and aquatic ecosystems. The objective of this study was to characterize the sediment column of an established compost wetland constructed for the treatment of acid mine drainage to gain insight into biogeochemical processes that might impact treatment efficiency. To do this, mineralogy and geochemical stability of ochreous sediments were examined, spatial and seasonal trends in porewater chemistry were measured, and bacterial community composition profiled. The mineralogical composition of the ochre portion of the sediment column was a mixture of schwertmannite \([\text{Fe}_8\text{O}_6(\text{OH})_{4.8}\text{(SO}_4)_{1.6}]\) and goethite \((\alpha-\text{FeOOH})\). Initial drainage conditions favored the precipitation of schwertmannite, which transformed at a rate of 10-30 mol/m\(^3\)/yr to goethite. The sulfide minerals, pyrite \((\text{FeS}_2)\) and greigite \((\text{Fe}_3\text{S}_4)\), were identified along with magnetite \((\text{Fe}_3\text{O}_4)\) in the compost layer of the sediment. Vertical gradients in porewater chemistry were similar throughout the wetland system and, with the exception of dissolved sulfide concentration, no consistent seasonal trends were detected. Dissolved sulfide was elevated in the compost relative to the ochre and in June compared
to February. Porewater pH ranged from 3 to 7 and increased with depth; whereas, the Eh ranged from 110 to 750 mV and decreased with depth. Both pH and Eh changed abruptly near the interface between the ochre and compost layers. Dissolved Fe occurred primarily as Fe(II) and peaked within the interface region. Concentrations of other major elements (Al, Ca, K, Mg, Mn, and Na) in the pore waters showed some variation between cells and sampling dates, but vertical gradients generally reflected wetland stratigraphy. Terminal restriction fragment length polymorphism analysis (T-RFLP) of 16S rRNA genes was used to profile bacterial community composition. Bacterial diversity was found to be similar throughout the sediment profile; however, bacterial communities clustered together and could be correlated to sediment properties. Many terminal restriction fragment’s (TRF’s) consistent with bacteria relevant to wetland treatment efficiency were found. These included multiple TRF’s consistent with eight genera of sulfate-reducing bacteria as well as iron-reducers like *Shewanella* and *Peleobacter*. 
Dedicated to my family
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VITA

February 28, 1973 ................................................................. born, Buffalo, NY

1994 ................................................................. B.A., Biology SUNY Buffalo

1998 ................................................................. M.S., Soil Science, Ohio State University

2000-2003 ................................................................. NASA Graduate Student Research Fellow

PUBLICATIONS


FIELDS OF STUDY

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What is Acid Mine Drainage?

Acid mine drainage refers to metal-rich sulfuric acid solutions released from mine tunnels, open pits, and waste rock piles (Table 1.1). Similar solutions are produced by the drainage of some coastal wetlands, resulting in the formation of acid sulfate soils. Acid mine drainage typically yields pH values ranging from 2 to 4; however, extreme sites such as Iron Mountain, California have produced pH values as low as -3.6 (Nordstrom and Alpers, 1999). Neutral to alkaline mine drainage is also common in areas where the surrounding geologic units contain carbonate rocks to buffer acidity (Table 1.1).

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<td>2.7 - 7.3</td>
<td>5.2</td>
<td>3.6</td>
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<td>Fe (mg/L)</td>
<td>0.16 - 512.0</td>
<td>43.0</td>
<td>58.9</td>
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<tr>
<td>Al (mg/L)</td>
<td>0.01 - 108.0</td>
<td>1.3</td>
<td>9.8</td>
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<td>Mn (mg/L)</td>
<td>0.12 - 74.0</td>
<td>2.2</td>
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<td>SO₄ (mg/L)</td>
<td>120 - 2000</td>
<td>580.0</td>
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Table 1.1- Summary of mine drainage chemistry from 101 bituminous coal mine sites in Pennsylvania*.
Why is Acid Mine Drainage a Problem?

Soils and spoils exposed to acid mine drainage do not support vegetation and are susceptible to erosion. When acid mine drainage enters natural waterways, changes in pH and the formation of voluminous precipitates of metal hydroxides can devastate fish populations and other aquatic life (Fig. 1.1). The corrosion of engineered structures such as bridges is also greatly accelerated. There may be as many as 500,000 inactive or abandoned mines in the United States, with mine drainage severely impacting approximately 19,300 km of streams and more than 72,000 ha of lakes and reservoirs (Kleinmann, 1989; Lyon et al., 1993). Once initiated, mine drainage may persist for decades, making it a challenging problem to solve.

What Causes Acid Mine Drainage?

Mine drainage results from the oxidation of sulfide minerals such as pyrite (cubic FeS$_2$), marcasite (orthorhombic FeS$_2$) and chalcopyrite (CuFeS$_2$). These minerals are commonly found in coal and ore deposits and are stable until exposed to oxygen and water. Their oxidation causes the release of metals and the production of sulfuric acid. This process can occur as a form of natural mineral weathering, but is exacerbated by mining because of the sudden, large-scale exposure of unweathered rock to atmospheric conditions.
Figure 1.1- Mixing of acid mine drainage (right) with a natural stream resulting in the formation of voluminous precipitates.
The formation of mine drainage is commonly represented by a single chemical reaction (reaction [1]), which describes the oxidation of pyrite by oxygen in the presence of water to form Fe hydroxide [Fe(OH)$_3$] and sulfuric acid.

\[
\text{FeS}_2(s) + \frac{3}{4}O_2(g) + \frac{3}{2}H_2O(l) \rightarrow \text{Fe(OH)}_3(s) + 2H_2SO_4(aq) \quad [1]
\]

The actual process is considerably more complicated involving oxidation-reduction, hydrolysis, precipitation, and dissolution reactions, as well as microbial catalysis (Nordstrom and Alpers, 1999).

Pyrite and related sulfide minerals contain both Fe and S in reduced oxidation states. When exposed to oxygen and water the sulfur moiety is oxidized first, releasing Fe$^{2+}$ and sulfuric acid to solution (reaction [2]). The rate of oxidation is dependent on environmental factors like temperature, pH, Eh, and relative humidity, as well as mineral surface area and microbial catalysis.

\[
\text{FeS}_2(s) + \frac{3}{2}O_2(g) + H_2O(l) \rightarrow \text{Fe}^{2+}(aq) + 2\text{SO}_4^{2-}(aq) + 2H^+(aq) \quad [2]
\]

Reaction [2] is most important in the initial stages of mine drainage generation and can be either strictly abiotic or mediated by contact with sulfur-oxidizing bacteria (Rojas et al., 1995). The Fe$^{2+}$ released by pyrite decomposition is rapidly oxidized by oxygen at pH$>3$ (reaction [3]).
If acidity generated by reaction [2] exceeds the buffering capacity of the system, the pH eventually decreases. Below pH 3, Fe$^{3+}$ solubility increases and a second mechanism of pyrite oxidation becomes important (Nordstrom, 1982) (reaction [4]).

\[
\text{FeS}_2(s) + 14\text{Fe}^{2+}(aq) + 8\text{H}_2\text{O}(l) \rightarrow 15\text{Fe}^{2+}(aq) + 2\text{SO}_4^{2-}(aq) + 16\text{H}^+(aq) \]  

[4]

In this case, pyrite is oxidized by Fe$^{3+}$ resulting in the generation of more acidity than when oxygen is the primary oxidant. Pyrite decomposition is thus controlled by the rate at which Fe$^{2+}$ is converted to Fe$^{3+}$ at low pH (Singer and Stumm, 1970). At pH < 3, Fe$^{2+}$ oxidation is very slow, unless it is catalyzed by populations of iron oxidizing bacteria like *Acidithiobacillus ferrooxidans* or *Leptospirillum ferrooxidans*. These acidophilic bacteria oxidize Fe$^{2+}$ as a means of generating energy to fix carbon. In doing so, they supply soluble Fe$^{3+}$ at a rate equal to or slightly greater than the rate of pyrite oxidation by Fe$^{3+}$ (Nordstrom, 1982). Pyrite oxidation then regenerates Fe$^{2+}$ (reaction [4]), creating a cyclic situation that leads to vigorous acidification of mine drainage water.
Mine Drainage Mineralogy

The hydrolysis of Fe$^{3+}$ causes the precipitation of various Fe minerals, generally represented as [Fe(OH)$_3$], that are often the most obvious indicators of mine drainage contamination (reaction [5]).

$$\text{Fe}^{3+}_{(aq)} + 3\text{H}_2\text{O}_{(l)} \rightarrow \text{Fe(OH)}_3_{(s)} + 3\text{H}^+_{(aq)} \quad [5]$$

These precipitates are yellow-to-red-to-brown in color and have long been referred to by North American miners as “yellow boy.” The actual mineralogy of the precipitates is determined by solution parameters like pH, SO$_4$, and metal concentration and can vary both spatially and temporally. Some of the most common mine drainage minerals are goethite ($\alpha$-FeOOH), ferrihydrite (Fe$_5$HO$_8$·4H$_2$O), schwertmannite [Fe$_8$O$_8$(OH)$_6$SO$_4$], and jarosite [(H,K,Na)Fe$_3$(OH)$_6$(SO$_4$)$_2$] (Bigham and Nordstrom, 2000).

Goethite is a crystalline oxyhydroxide that occurs over a wide pH range, is relatively stable, and may represent a final transformation product of other mine drainage minerals. Ferrihydrite is a poorly crystalline ferric oxide that forms in higher pH (>6.5) environments. Schwertmannite is commonly found in drainage waters with pH ranging from 2.8 – 4.5, and with moderate-to-high SO$_4$ contents. It may be the dominant phase controlling major and minor element activities in most acid mine drainage. Jarosite forms in more extreme environments with pH
< 3, very high SO\textsubscript{4} concentrations, and in the presence of appropriate cations like Na and K.

Mine Drainage Microbiology

The most studied bacterial species in mine drainage systems belong to the genus *Acidithiobacillus* (formerly *Thiobacillus*) (Kelly and Wood, 2000). Species like *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* are important in S and Fe oxidation in acid drainage; however, many other microorganisms may also be involved (Gould et al., 1994). Bacteria have been found in close association with pyrite grains and may play a direct role in mineral oxidation, but they most likely function indirectly through oxidation of dissolved Fe\textsuperscript{2+} as described previously. In low pH systems (<3), *A. ferrooxidans* can increase the rate of Fe oxidation as much as five orders of magnitude relative to strictly abiotic rates (Singer and Stumm, 1970).

Iron oxidizing bacteria are chemolithotrophic, meaning they oxidize inorganic compounds, like Fe\textsuperscript{2+}, to generate energy and use CO\textsubscript{2} as a source of carbon. Iron oxidation, however, is a very low energy yielding process. It has been estimated that the oxidation of 90.1 moles of Fe\textsuperscript{2+} is required to assimilate one mole of carbon into biomass (Ehrlich, 1996). Thus, large amounts of Fe\textsuperscript{2+} must be oxidized to achieve even modest growth.

In addition to mediating Fe oxidation, bacteria may play an additional role in mineral formation. Bacteria in mine drainage systems have been shown to be
partially encrusted with mineral precipitates (Clarke, 1997). Bacterial cell walls provide reactive sites for the sorption of metal cations, which can accumulate and subsequently develop into precipitates, using the bacterial surface (living or dead) as a template (Schultze-Lam et al., 1996; Konhouser, 1998).

Environmental Impacts of Mine Drainage

Mine drainage is primarily released from open mine shafts or from mine spoil left exposed to the atmosphere. The drainage can have devastating effects on the surrounding ecosystem. Chemical precipitates can obstruct water flow, dramatically increase turbidity, and ruin stream aesthetics. Dissolved metals and acidity can also affect plant and aquatic animal populations.

Besides Fe, Al is the most common dissolved metal in acid mine drainage. The primary source of Al is the acid dissolution of aluminosilicates found in soil, spoils, tailings deposits and gangue material (Bigham and Nordstom, 2000). At high concentrations, Al can be toxic to plants and colloidal Al precipitates can irritate the gills of fish, causing suffocation. Aluminum occurs as a dissolved species at low pH, but rapidly hydrolyzes at about pH 5 to form “amorphous” basaluminite \( \text{[Al}_4\text{(SO}_4\text{)(OH)}_{10} \cdot \text{4H}_2\text{O]} \) or gibbsite \( \text{[Al(OH)_3]} \) (Bigham and Nordstom, 2000). Aluminum precipitates are white in color, but are readily masked by associated Fe compounds.

Elevated levels of trace elements like As, Cu, Ni, Pb, and Zn may be released during the oxidation of sulfide minerals. These elements can play a role
in mineralization processes by forming coprecipitates but occur primarily as sorbed species (Winland et al., 1991). Mine drainage precipitates can sorb both anions and cations, depending on pH. While coprecipitation and sorption function to immobilize trace elements by removing them from solution, this effect may not be permanent. Dissolution of precipitates and shifts in pH can result in the release of sorbed species, providing a latent source of pollution.

Dealing with Mine Drainage

Successful control of mine drainage usually involves elements of both prevention and treatment.

Prevention

Prevention techniques include sealing mine shafts, burying or submerging spoil piles, and adding bactericides to limit the function of iron oxidizing bacteria. These techniques often have limited success. Sealing of mines is extremely difficult due to fractures and the permeability of surrounding rocks. Covering spoil with soil material can decrease the degree of sulfide oxidation by limiting exposure to oxygen, but establishment of a vegetative cover is necessary to prevent erosion from re-exposing the spoil. Inhibition of iron oxidizing bacteria with bactericides can decrease sulfide oxidation and reduce metal mobility. However, re-application is often necessary and adequate distribution to all
affected areas is difficult. In addition, target bacteria may develop resistance, and beneficial bacteria may be harmed (Ledin and Pedersen, 1996).

Treatment

Solution pH usually underestimates the total acid producing capacity of mine drainage which is the sum of “proton acidity” and “mineral acidity” generated upon oxidation and hydrolysis of metals like Fe$^{2+}$, Fe$^{3+}$, Mn and Al$^{3+}$. The traditional approach to treatment of acid mine drainage involves neutralization of total acidity by the addition of alkaline agents like caustic soda (NaOH) or hydrated lime [Ca(OH)$_2$]. This method is effective in neutralizing acidity and precipitating dissolved metals. However, it requires continuous oversight and produces large amounts of waste sludge that require disposal. Newer remediation strategies focus on low-cost, sustainable methods for treatment of drainage waters. For example, limestone drains coupled with compost wetlands have shown some promise as passive remediation technologies. In these systems, drainage is channeled through either oxic or anoxic limestone substrates to neutralize active acidity. Dissolved metals are then allowed to hydrolyze and precipitate in wetland cells. A major difficulty is the loss of reactive surface by armoring of limestone particles with precipitates of Fe and Al that eventually obstruct flow.

Compost wetlands are designed to stimulate the development of anaerobic microbial populations, particularly sulfate-reducing bacteria. The bacteria use the compost as an organic substrate and remove SO$_4$ from solution,
either by converting it to H$_2$S, which is lost to the atmosphere, or by forming insoluble Fe sulfides (reactions [6] and [7]).

$$2\text{CH}_3\text{CHOHCOO}^-_{(aq)} + \text{SO}_4^{2-}_{(aq)} \rightarrow 2\text{CH}_3\text{COO}^-_{(aq)} + 2\text{HCO}_3^-_{(aq)} + \text{H}_2\text{S}_{(g)} \quad [6]$$

$$\text{H}_2\text{S}_{(s)} + \text{Fe}^{2+}_{(aq)} \rightarrow \text{FeS}_{(s)} + 2\text{H}^+_{(aq)} \quad [7]$$

Bicarbonate is formed as a by-product of SO$_4$ reduction, and functions to buffer acidity. These systems have also shown limited success in the field. The SO$_4$ removal rates are usually low (<10%), and pH often remains unchanged or decreases within the wetland (Mitsch and Wise, 1998).
CHAPTER 2

TREATMENT OF ACID MINE DRAINAGE USING CONSTRUCTED WETLANDS

Coal mining has produced one of the most pervasive water quality problems in the eastern United States. Iron sulfide minerals, most commonly pyrite ($\text{FeS}_2$), in coal and associated rocks are exposed to water and oxygen through the mining process. This exposure results in accelerated weathering and the formation of acid solutions that have high concentrations of dissolved metals, Fe and $\text{SO}_4^-$ (Rose and Cravotta, 1998; Nordstrom and Alpers, 1999). The environmental effects of acid mine drainage (AMD) can be devastating. Acidity destroys vegetation, accelerates erosion, and increases the susceptibility of fish to disease while dissolved and precipitated metals contaminate soils and render water supplies unsuitable for human consumption (Hyman and Watzlaf, 1997). In the state of Ohio there are 25 watersheds and over 1300 miles of streams severely impacted by mine drainage (BUSML, 1974; ODNR, 1999).

Treatment of mine drainage is a challenge because of its persistent nature. Once initiated, mine drainage can continue for decades and often long after active mining operations have ceased. Traditional treatment methods based on neutralization with alkaline reagents are not ideal because they require
continual oversight and a substantial investment of resources. Variations in local conditions also mean that treatments must be tailored to individual sites. More recently, constructed wetlands have been proposed and implemented as low-cost, long-term treatment options (Watzlaf and Hyman, 1995). The development of this technology stemmed from observations of native sphagnum bogs near mine drainage sites that were found to improve mine drainage quality (Hedin et al., 1994; Weider and Lang, 1982). The utilization of constructed wetlands has evolved to enhance natural remediation processes and make use of locally available materials. Over 1000 wetlands have been constructed in the eastern United States for the treatment of coal mine drainage (Skousen et al., 1998).

The design and technology incorporated into constructed wetlands varies greatly, as does treatment efficiency (Weider, 1989; Hedin and Nairn, 1993). Peat-containing wetlands have been successfully utilized for heavy metal removal (Frostman 1993; Soboleski 1996), while systems composed of simple aerobic cells or ponds function to adequately remove Fe when surrounding soils and rocks have sufficient buffering capacity to neutralize acidity. At near neutral pH, Fe precipitates spontaneously through abiotic oxidation and hydrolysis. Under low pH (< 4.5) conditions, acidophilic iron-oxidizing bacteria mediate Fe oxidation but Al, Mn and other metals remain in solution; therefore, more elaborate systems are required to improve drainage quality. At a minimum, constructed wetlands receiving AMD consist of a series of clay-lined cells layered with limestone and an organic substrate (compost wetland). These systems rely strictly on the diffusion of alkalinity from underlying layers and often are not
adequate to fully neutralize acidity. In many cases, pH may actually decrease throughout the wetland system as Fe hydrolysis reactions produce additional acidity (Brodie et al., 1993; Manyin et al., 1997; Stark et al., 1991; Sritharan et al., 1992). More elaborate but sustainable alkalinity producing systems (SAPS) have since been developed that incorporate anoxic limestone drains and subsurface flow to combat decreases in pH and enhance SO$_4$ reduction (Kepler and McCleary, 1994; Ziemkiewicz et al., 1997).

A variety of organic substrates, based on local availability, such as spent mushroom compost, composted manures, hay mixtures and decomposed wood products have been incorporated into wetlands constructed for AMD treatment to stimulate beneficial anaerobic microbial populations, particularly sulfate-reducing bacteria (SRB) (Hedin et al., 1989; Gross et al., 1993; Karathanasis and Thompson, 1993; Stark and Williams, 1994). SRB reduce SO$_4$ to HS$^-$, which is either lost to the atmosphere as H$_2$S under acidic conditions or is combined with a divalent metal and precipitated (Machemer et al., 1993). In addition, SRB metabolic activities can consume protons, thereby neutralizing acidity. SRB populations have been characterized in mine tailings (Fortin et al., 1995) and acidic sediments (Blodau et al., 1998; Koschorreck et al., 2003) and have demonstrated the potential to efficiently remove SO$_4$ from acid-sulfate waters in several laboratory studies (Christensen et al., 1996; Webb et al., 1999; Waybrant et al., 2002). Results from field studies have been less promising, with SO$_4$ removal in wetland treatment systems often measuring less than 10% (Mitsch and Wise, 1988; Sritharan et al., 1992; Shimala, 2000).
In addition to SRB, bacteria capable of Fe reduction may also play an important role in wetland sediments. Bacterial reduction of Fe is a widely distributed trait in the domain bacteria and has been shown to be the dominant mechanism for Fe reduction in soils and sediments (Lovely et al., 1991; Lonergan, 1996). Release of Fe and Mn under reducing conditions was described in microcosms designed to simulate mine drainage wetlands (Tarutis and Unz, 1995), and remobilization of Fe and trace metals was attributed to reduction of iron precipitates by a *Clostridium* sp. in contaminated soils (Francis and Dodge, 1990). In addition, Fe reduction by an *Acidiphilium* sp. has been characterized in the acidic sediments of a lake impacted by mine drainage (Küsel et al., 1999; Küsel and Dorsch, 2000).

Much of the research on constructed wetlands has focused on design criteria or measurements of treatment efficiency through influent and effluent monitoring. Other studies have focused on a particular aspect of the wetland system such as characterization of precipitates (Karathanasis and Thompson, 1995) or geochemical modeling of drainage waters (Foos, 1997). Although these studies contribute to our knowledge of wetland systems, an integrated study of chemical, mineralogical, and microbiological relationships is essential to identify important biogeochemical processes that may affect long-term treatment efficiency. Thus, the goal of this study was to characterize the sediment column of an established wetland constructed for the treatment of AMD. The field site examined was near Carbondale in Athens Co., Ohio, and consisted of two compost wetlands constructed by the Ohio Department of Natural Resources in
1990 to improve the quality of drainage associated with two seeps from a drift mine abandoned in 1923 (Fig. 2.1). This research focused on the larger of the two wetlands, which received drainage at an average rate of 8.4 L/s. Long-term monitoring in two separate studies indicated the influent drainage had an average pH of 3.5, and average Fe and SO$_4$ concentrations of 110 and 1400 mg/L, respectively (Sritharan, 1992; Shimala, 2000).

The first objective of this study was to determine the mineralogy of ochreous precipitates and quantify rates of mineral transformation in the sediment column of the Carbondale wetland (Fig. 2.2). Mineralogy of Fe precipitates formed in drainage waters is dependent on solution chemistry parameters like pH and Fe and SO$_4$ activities. Previous studies have identified poorly ordered oxides and hydroxysulfates like ferrihydrite (HFe$_5$O$_8$·4H$_2$O) and schwertmannite [Fe$_8$O$_6$(OH)$_6$SO$_4$] in mine drainage sediments (Bigham et al., 1996). These minerals are efficient sorbents of contaminant trace metals and oxyanions and can effectively remove them from solution. Both minerals have been found to be unstable in laboratory experiments (Bigham et al., 1996) and can transform with time to more stable minerals like goethite (α-FeOOH) that may be less efficient sorbents. The transformation of schwertmannite to goethite can potentially result in the release of SO$_4$ and contaminant species to solution (Bigham et al., 1996; Rose and Ghazi, 1997; Desborough et al., 2000). Previous studies (Brill, 1999; Piene et al., 2000) have also shown that pH and redox gradients can develop within the sediment column, thereby accelerating mineral transformations. Thus, the mineralogy and geochemical stability of Fe
precipitates within the wetland sediment is of vital importance to long-term treatment efficiency. Characterization of mineral precipitates and quantification of transformation rates within the Carbondale wetland is the focus of chapter 3.

Figure 2.1- Cell 1 of the Carbondale wetland system in June 2000.
Figure 2.2- Sediment core collected from the Carbondale wetland.
A second objective was to evaluate the effects of seasonal changes in pore water chemistry and mineral formation/ transformation processes on the distribution of metals with depth in the sediment column. Several studies have found that the treatment efficiency of mine drainage impacted sediments varies seasonally. Herlihy and Mills (1985) found that SO₄ reduction rates in freshwater sediments receiving acid mine drainage were elevated in summer compared to winter, and a similar trend was described by Hsu and Maynard (2000) in a study that used stable S isotopes to monitor SO₄ reduction in a constructed wetland. August et al. (2002) found a natural wetland receiving mine drainage acted as a net sink for metals during the summer months, only to serve as a net source during the winter months. Chapter 4 includes seasonal analyses of surface and pore water chemistry for the first three cells in the Carbondale wetland as well as the chemical composition of associated solid phases.

A third objective was to profile bacterial community composition in the Carbondale sediment column in relation to basic pore water chemistry. Compost wetlands are designed to serve as a reservoir for Fe precipitates as well as provide an environment for the development of beneficial microbial communities. Several reviews have described microbial diversity in acid environments (Johnson, 1998; Baker and Banfield, 2003), and microbial community composition in acid-sulfate systems has been characterized using traditional culturing techniques (Batal et al., 1990; Webb et al., 1998; Wielinga et al., 1999). In addition, Edwards et al., (1999) used molecular techniques to characterize
seasonal changes in bacterial, archeal and eukaryal populations in an extreme acid mine drainage site. However, little is known about bacterial communities in the diverse range of environments that may exist within a wetland.

Because culture-based techniques can only describe a small portion of the bacterial diversity in environmental samples, often less than 1% (Amann et al., 1995), 16S rRNA based technology, specifically, terminal restriction fragment length polymorphism analysis of 16S rRNA genes (T-RFLP) (Liu et al., 1997) was used for this work. T-RFLP technology has been used to evaluate microbial communities in a range of environmental samples including marine sediments (Urakawa et al., 2000), soils (Dunbar et al., 2000) and compost materials (Michel et al., 2002); and other 16S rRNA based techniques have been used to characterize natural SRB communities (Devereux et al., 1996). Bacterial community profiles as well as the identification of putative species relevant to treatment processes within the sediment column are the focus of chapter 5.
3.1 Abstract

The objective of this study was to examine the mineralogy and geochemical stability of ochreous sediments accumulated in a compost wetland constructed in 1990 for acid mine drainage treatment. Intact sediment cores were collected in 1996 and 2000 from an area that had accumulated 33 cm of ochre. Solids and pore waters were subsequently separated by centrifugation and analyzed using conventional methods, including x-ray diffraction, infrared spectroscopy, scanning electron microscopy, and wet chemical techniques. The solid phase had an average Fe content of 585 g/kg and was predominantly schwertmannite $[\text{Fe}_8\text{O}_8(\text{OH})_{4.8}(\text{SO}_4)_{1.6}]$ in the upper portion of the sediment column, but transformed to goethite ($\alpha$-FeOOH) with depth. The rate of transformation was calculated to be 30 mol/m$^3$/yr in the initial 6 yr of sedimentation as compared to 10 mol/m$^3$/yr for the 4 yr period from 1996 to 2000. Pore water composition was affected by this mineral transformation through production of acidity and the release of Fe and SO$_4$. These results demonstrate that the sediment column was not a static environment. In addition, the transformation of schwertmannite
to goethite, which has been observed under laboratory conditions, also occurs in natural systems.

3.2 Introduction

Drainage from coal mines has resulted in a severe water quality problem for the eastern United States. The oxidation of sulfide minerals in coals and associated rocks releases Fe-rich, acidic solutions that may contain elevated levels of trace metals (Winland et al., 1991; Hyman and Watzlaf, 1997; Rose and Cravotta, 1998; Nordstrom and Alpers, 1999). When mine drainage enters natural waterways, changes in pH and the formation of ochreous precipitates can have devastating effects on aquatic ecosystems.

Mine drainage may persist for decades, making it a challenging problem to address. Treatment methods often involve the addition of alkaline reagents that neutralize acidity and precipitate dissolved metals (Skousen, et al., 1998). These methods are effective, but require continuous oversight and a large investment of resources. More recently, constructed wetlands have been implemented as a low-maintenance, cost-effective means of achieving long-term treatment of mine drainage (Hedin et al., 1994). The specific design parameters and technology implemented in these wetlands varies with drainage quality and local site conditions, but a common feature is their function as a reservoir for secondary Fe precipitates (Hedin et al., 1994; Skousen, et al., 1998; Barton and Karathanasis, 1999).
The Fe precipitates from mine drainage include a variety of poorly ordered oxides and hydroxysulfates that are effective sorbents of trace metals and oxyanions (Karathanasis and Thompson, 1995; Webster et al., 1998; Bigham and Nordstrom, 2000). The geochemical stability of these Fe precipitates and their associated contaminant loads are an issue because poorly ordered minerals like ferrihydrite (HFe$_5$O$_8$·4H$_2$O) and schwertmannite [Fe$_8$O$_6$(OH)$_6$SO$_4$] spontaneously transform with time into more stable minerals, such as goethite (α-FeOOH), that may be less efficient sorbents of contaminants (Bigham et al., 1996; Rose and Ghazi, 1997; Desborough et al., 2000). This transformation process may be accelerated by the development of pH or redox gradients within the sediment column of lakes (Peine et al., 2000), reservoirs, or wetlands receiving mine drainage. Such conditions could facilitate the dissolution of Fe precipitates and/or desorption of metals resulting in a latent source of pollution. The objective of this study was to determine the mineralogy of ochreous precipitates and quantify rates of mineral transformation in the sediment column of a wetland receiving acid mine drainage (AMD).

3.3 Materials and methods

Field Site Description and Sampling

The field site used in this study was a wetland constructed for mine drainage treatment near Carbondale in Athens Co., Ohio. This wetland was
established in 1990 and consists of a series of six, rectangular sedimentation cells, each approximately 1020 m$^2$, followed by a deeper pond covering a total area of over 6000 m$^2$ (Fig. 3.1). The sedimentation cells were clay lined, layered with 30 cm of limestone substrate and 38 cm of compost material (either spent mushroom or manure compost), and planted initially with cattails (Typha spp.) at a density of 17 plants per m$^2$. Each cell was divided into thirds by two retaining boards to distribute the drainage water evenly, prevent short-circuiting, and maintain a water depth of about 10 cm.

Multiple sediment cores were collected during 1996-1997 (Brill, 1999) and 2000-2001 using clear plastic cylinders that were 7 cm in diam. and approximately 70 cm in length. Each cylinder was pushed into the sediment column, a stopper was placed on top to create a vacuum, the core was gently withdrawn, and a second stopper was placed in the bottom to retain the sediment. The sediment cores were transported on ice to the laboratory where they were extruded from the tubes under flowing Ar and divided into segments of 3-4 cm length or corresponding to visual banding patterns. The segments of sediment were then transferred to 250-ml centrifuge bottles under Ar and centrifuged for 15 min at 9100 RCF to separate pore waters from the solids for prompt geochemical and mineralogical analyses, respectively. The data presented in this paper are from cores collected on September 17, 1996 and October 21, 2000 near the inlet of the first sedimentation cell, which had accumulated the greatest thickness of ochre (Fig. 3.1). Analyses were limited to the ochre portion of the sediment column.
Fig. 3.1 – Schematic diagram of the Carbondale Wetland.
Pore Water Samples

An aliquot of the pore water was allowed to warm to room temperature for pH and Eh measurements using an Orion pH electrode and a Corning combination redox electrode with a Pt-sensing element and a Ag/AgCl reference element. The remainder of the pore water was filtered using a 0.2 \( \mu \)m polypropylene syringe filter and split into two subsamples, one of which was acidified with approximately 1 ml of ultra-pure 6 M HCl per 100 ml of sample for measurement of dissolved metals. The other subsample was not acidified and was used for SO\(_4\) analysis with a Dionex ion chromatograph. Total concentrations of Al, Ca, Mg, Mn, Na, K in the pore waters sampled in 2000 were measured using a Perkin-Elmer Optima 3000, inductively coupled plasma optical emission spectrometer (ICP-OES). Ferrous and total dissolved Fe (Fe\(_t\)) (following reduction with hydroxylamine hydrochloride) were measured colorimetrically by the ferrozine technique (To et al., 1999). A similar procedure was followed for the 1996 samples except that the Fe\(_t\) was measured by ICP-OES and other metals were not analyzed (Brill, 1999).

Solid Samples

Solid samples were freeze dried, and total C and S were determined by combustion using a CE instruments NC 2100 soil analyzer and a LECO Model 521 induction furnace, respectively. Total Fe, Al, Ca, Mg, Mn, Na, and K in the
solids were determined by selective dissolution of a 100 mg sample in 20 ml of ultra-pure 6 M HCl for a period of 48 h (Winland et al., 1991). The acid solutions were then centrifuged for 10 min at 1010 RCF, the supernatants were removed and analyzed by ICP-OES, and the acid insoluble residues were quantified gravimetrically. The oxalate-extractable Fe fraction was measured by reacting 50 mg samples with 40 ml of 0.2 M ammonium oxalate (pH 3) (McKeague and Day, 1966). The samples were shaken in the dark for 4 h, centrifuged for 10 min at 1010 RCF, and Fe in the supernatant was quantified by atomic absorption (AA) spectroscopy.

The mineralogical composition of the precipitates was evaluated by X-ray diffraction (XRD) analysis of randomly oriented powder samples using CuKα radiation and a Phillips PW 1316/90 diffractometer equipped with a theta-compensating slit, a 0.2-mm receiving slit, and a diffracted-beam monochromator (Brady et al., 1986). Freeze-dried samples were scanned from 3 to 80° 2θ using a step interval of 0.01° 2θ and a counting time of 4 s. Infrared (FTIR) absorption spectra were recorded with a Mattson Instruments spectrophotometer using powdered samples diluted to 2.5 wt.% in KBr (Carlson et al., 2002). Diffuse reflectance spectra were collected from 150-1500 cm⁻¹ as the average of 300 sample scans at 1 cm⁻¹ resolution. Selected sediment samples were gold coated to improve conductivity and examined with a Phillips XL30 scanning electron microscope (SEM). Uncoated samples were analyzed using an energy-dispersive x-ray spectrophotometer (EDS) for chemical analysis.
Specific surface area was determined with a Micromeritics Flowsorb II 2300 surface area analyzer (Carlson et al., 2002). Approximately 75-100 mg of sediment was placed in a sample holder, dried over P_2O_5 for 48 h, and analyzed by the single-point BET method using N_2 as the absorbate.

Color determinations were performed on dry, homogeneously ground samples using a Minolta CR-300 Chroma Meter with standard illuminant C (Post et al., 1993). The measuring probe was rested in a vertical position on the sample and a xenon arc lamp was used to measure light reflected from the sample in the visible portion of the spectrum. Data were reported as an average of three readings using Munsell color notation.

3.4 Results and discussion

Wetland Properties and Sedimentation Rates

The Carbondale wetland was commissioned in 1990, and both discharge and influent drainage quality were monitored in two, year-long studies beginning in April, 1991 (Sritharan et al., 1992) and February, 1999 (Shimala, 2000). Drainage entering the wetland had pH values ranging from 3.5 to 4.5 and average Fe and SO_4 concentrations of 110 and 1400 mg/L, respectively, and did not change appreciably over time. Fe removal efficiency decreased slightly from 1991 to 2000, but was consistently greater than 60%. Removal rates for SO_4, Al and Mn were much lower than Fe, and pH decreased from inlet to outlet.
Flow rate into the main wetland system ranged from 6 to 9 L/s, with significant losses due to seepage into the groundwater. Residence time was calculated to be 4 d using the reservoir volume and inflow rate (Sritharan et al., 1992), but was measured at 7.4 h using a tracer technique in April, 2000 (Shimala, 2000). This deviation between the calculated and actual residence time was likely due to the general failure of the plant cover, increased sediment level, preferential flow, and channeling through collapsed tunnels burrowed by animals (Shimala, 2000).

Maximum deposition of ochre during the first decade of operation occurred in the first cell. Cores collected in September, 1996, showed an accumulation of approximately 33 cm of sediment, which essentially filled the first basin. Dry densities measured in 2000 ranged from 0.29 to 0.50 g/cm$^3$ and averaged 0.41 g/cm$^3$ (Table 3.1). If similar densities were applied to the 1996 core, a deposition rate of 64 g/m$^2$/day over the first 6 yr can be calculated. Assuming an average Fe content of 585 g/kg (Table 3.2), this rate would correspond to 37 g Fe/m$^2$/day. Lower densities would yield rates more similar to the 20 g Fe/m$^2$/day reported by Hedin et al., (1994). Minimal sedimentation occurred after 1996 due to channeling of drainage away from the sampling site (Fig. 3.1).

The sediment cores in both years were collected from a stable area (uneroded) with a crusty, aggregated surface layer (0 – 6 cm) that became more fine-grained and loose with depth. The color graded from reddish brown at the surface to yellowish brown at the base, with no distinct stratification (Table 3.1). The 1996 core tended to be darker red in the upper 18 cm (average hue of 8.5
<table>
<thead>
<tr>
<th>Depth from surface (cm)</th>
<th>Color</th>
<th>Depth from surface (cm)</th>
<th>Color</th>
<th>Carbon % solid</th>
<th>Acid insoluble residue % solid</th>
<th>Density (D_b)</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>8.4YR 3.7/5.0</td>
<td>8.1YR 3.7/5.8</td>
<td>0.7 0.6</td>
<td>4 0</td>
<td>0.29</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>7.8YR 3.8/5.7</td>
<td>8.3YR 4.2/6.4</td>
<td>0.4 0.7</td>
<td>1 0</td>
<td>0.50</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>8.4YR 4.5/6.8</td>
<td>9.0YR 5.0/7.8</td>
<td>0.3 0.6</td>
<td>2 0</td>
<td>0.44</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>10.5</td>
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<td>9.5YR 5.5/8.5</td>
<td>0.3 0.6</td>
<td>3 4</td>
<td>0.41</td>
<td>127</td>
<td></td>
</tr>
<tr>
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<td>9.4YR 5.4/8.7</td>
<td>0.3 0.4</td>
<td>2 1</td>
<td>0.43</td>
<td>170</td>
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</tr>
<tr>
<td>16.5</td>
<td>8.8YR 5.1/8.1</td>
<td>9.5YR 5.4/8.6</td>
<td>0.3 0.5</td>
<td>4 0</td>
<td>0.40</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>19.5</td>
<td>9.1YR 5.2/8.2</td>
<td>0.1Y 5.8/7.9</td>
<td>0.3 0.7</td>
<td>4 1</td>
<td>0.40</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>22.5</td>
<td>9.1YR 5.4/7.4</td>
<td>9.3YR 5.2/8.4</td>
<td>0.6 0.6</td>
<td>1 1</td>
<td>0.46</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>25.5</td>
<td>9.3YR 5.4/7.8</td>
<td>9.3YR 5.3/8.6</td>
<td>0.6 0.5</td>
<td>10 3</td>
<td>0.47</td>
<td>177</td>
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<tr>
<td>28.5</td>
<td>9.1YR 5.3/7.5</td>
<td>0.1Y 5.8/8.5</td>
<td>2.4 0.6</td>
<td>15 9</td>
<td>0.40</td>
<td>140</td>
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<td>15 4</td>
<td>0.29</td>
<td>120</td>
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</tr>
</tbody>
</table>

D_b - Dry bulk density

Table 3.1- Wetland sediment properties
Table 3.2- Major and minor element composition\(^a\) of the solid phase for samples collected in 1996 and 2000.

<table>
<thead>
<tr>
<th>Depth from surface (cm)</th>
<th>Fe</th>
<th>S</th>
<th>Al</th>
<th>Ca</th>
<th>Mg mg/kg</th>
<th>Mn</th>
<th>Na</th>
<th>K</th>
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<td>519</td>
</tr>
<tr>
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<td>551</td>
<td>553</td>
<td>65</td>
<td>55</td>
<td>951</td>
<td>1788</td>
<td>767</td>
<td>635</td>
</tr>
<tr>
<td>7.5</td>
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<td>577</td>
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<td>606</td>
<td>50</td>
<td>25</td>
<td>668</td>
<td>969</td>
<td>713</td>
<td>654</td>
</tr>
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<td>613</td>
<td>591</td>
<td>45</td>
<td>24</td>
<td>720</td>
<td>1166</td>
<td>933</td>
<td>697</td>
</tr>
<tr>
<td>19.5</td>
<td>617</td>
<td>484</td>
<td>38</td>
<td>20</td>
<td>627</td>
<td>1869</td>
<td>828</td>
<td>853</td>
</tr>
<tr>
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<td>601</td>
<td>31</td>
<td>22</td>
<td>2140</td>
<td>1293</td>
<td>893</td>
<td>820</td>
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<td>630</td>
<td>595</td>
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<td>22</td>
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<td>1600</td>
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<td>906</td>
</tr>
<tr>
<td>28.5</td>
<td>558</td>
<td>628</td>
<td>14</td>
<td>19</td>
<td>13091</td>
<td>3634</td>
<td>13511</td>
<td>1053</td>
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<tr>
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<td>18</td>
<td>11</td>
<td>6928</td>
<td>6201</td>
<td>22893</td>
<td>1023</td>
</tr>
</tbody>
</table>

\(^a\) Concentrations corrected for water content (110°C) and acid insoluble residue.

\* Concentration in acid extract was below detection limit.
YR) than that collected in 2000 (average hue of 9.0 YR). Total C contents were greater at most depths in the 2000 core but were generally small (<0.75%) in both cores (Table 3.1). The solid phase was composed almost entirely of ochreous precipitates with minor acid insoluble residue (Table 3.1).

The upper 6 - 9 cm of the sediment column in both collection years was composed primarily of schwertmannite, which is a poorly ordered oxyhydroxysulfate that commonly forms in mine drainage waters with pH in the range of 2.8 – 4.5 and SO$_4$ concentrations ranging from 1000-3000 mg/L (Bigham et al., 1992). X-ray patterns displayed the eight broad peaks typical for schwertmannite with additional weak reflections indicating trace amounts of goethite and/or quartz (Fig. 3.2 and 3.3). Chemical analyses indicated an Fe/S mole ratio of 5.0 that corresponded to the formula Fe$_8$O$_6$(OH)$_{4.8}$(SO$_4$)$_{1.6}$, which is within the range considered typical for schwertmannite (Bigham et al., 1996).

Infrared spectra from the surface samples were dominated by absorption bands due to $\nu_1$ (SO$_4$) at 970 cm$^{-1}$, $\nu_4$ (SO$_4$) at 610 cm$^{-1}$, and a splitting of the $\nu_3$ fundamental of SO$_4$ to yield features at 1210, 1130, and 1040 cm$^{-1}$ (Fig. 3.4 and 3.5). These bands, along with a band at 700 cm$^{-1}$ result from OH-stretching and have been shown to be typical of schwertmannite (Bishop and Murad, 1996). Scanning electron microscope examinations and EDS analyses of the surface samples revealed Fe- and S-rich spheres ranging in size from 1 - 2 $\mu$m (Fig. 3.6a and 3.7a). The “pincushion” morphology was similar to that previously described for synthetic schwertmannite with smaller spheres ranging from 0.1 – 0.2 $\mu$m in diameter (Bigham et al., 1990). Schwertmannite from the 0 – 3 cm depth was
Fig. 3.2 – X-ray powder diffraction patterns for samples at various depths below the sediment surface in 1996. Gp=gypsum, Gt=goethite, Jt=jarosite, Qz=quartz. d-spacings are in Å.
Fig. 3.3 – X-ray powder diffraction patterns for samples at various depths below the sediment surface in 2000. Gp=gypsum, Gt=goethite, Jt=jarosite, Qz=quartz. d-spacings are in Å.
Fig. 3.4 – Infrared (IR) spectra for samples at various depths below the sediment surface in 1996. Gp=gypsum, Gt=goethite Sh=schwertmannite.
Fig. 3.5 – Infrared (IR) spectra for samples at various depths below the sediment surface in 2000. Gp=gypsum, Gt=goethite Sh=schwertmannite.
Fig. 3.6 – Scanning electron microscope images of a) schwertmannite (0-3 cm), b) goethite (30-33 cm), c) gypsum (30-33 cm) and d) jarosite (25-30 cm) from the Carbondale wetland.
Fig. 3.7 – Energy dispersive x-ray spectra (EDS) of a) schwertmannite (0-3 cm), b) goethite (30-33 cm), c) gypsum (30-33 cm) and d) jarosite (25-30 cm) from the Carbondale wetland.
densely aggregated, probably due to periodic drying. Aggregation may have accounted for the reddish-brown color and small specific surface area (Table 3.1) as compared to synthetic specimens with surface areas that usually exceed 200 m$^2$/g. The actual surface area of the Carbondale schwertmannite was presumably underestimated because internal surfaces were not accessible to N$_2$ (Carlson and Schwertmann, 1981).

The dominant mineral phase at the bottom of both sediment columns (33cm) was goethite. SEM observations indicated that the average diameter of the goethite particles was about 0.15 µm (Fig. 3.6b), and this small particle size was reflected in specific surface areas exceeding 100 m$^2$/g (Table 3.1). IR-spectra also had characteristic absorption bands at 892 and 795 cm$^{-1}$, corresponding to the δ-OH and γ-OH bending vibrations in goethite (Cornell and Schwertmann, 1996). Samples collected from the bottom of the sediment column in 1996 were shown by XRD, FTIR and EDS analysis to contain gypsum (CaSO$_4$·2H$_2$O) (Fig. 3.2, 3.4, and 3.7c) in addition to goethite. Presumably, dissolution of carbonates in the compost material or limestone at the base of the wetland cell released sufficient Ca into the porewater to induce gypsum precipitation in the form of large, lath-like crystals (Fig. 3.6c). Chemical analysis of the solids for Ca indicated gypsum contents ranging from 6 – 10 wt % in the lower 6 cm (Table 3.2). The lack of detectable gypsum in XRD patterns from samples collected at similar depths in 2000 was likely due to a depletion of carbonates over time, thus lowering Ca concentrations in solution, and resulting in conditions favorable for gypsum dissolution.
Although little gypsum was present in the 2000 core, total S content of the solid phase was slightly elevated in the 25.0 - 28.5 cm zone compared to the 1996 samples (Table 3.2). This increase coincided with a region where the sediment was most yellow (Table 3.1) and where nodules of jarosite [(K,Na)Fe₃(SO₄)₂(OH)₆] were found (Fig. 3.8a). The nodules were approximately 5 mm in diameter, and SEM observations showed a hexagonal, plate-like morphology for the jarosite crystals (Fig. 3.6d). EDS spectra (Fig. 3.7d) and analyses following dissolution of the jarosite in 6 M hydrochloric acid (data not given) showed the jarosite contained mostly K (4.0 wt%) with some Na (0.8 wt %) as the monovalent cation. The nodules also yielded 26 wt% acid insoluble residue. X-ray diffraction analysis of the residue revealed mostly clay minerals, including illite, which likely provided a source of K for jarosite formation (Ross et al., 1982) (Fig. 3.8b).

The middle portions of both sediment columns (6-24 cm) represented a transition zone where the proportion of schwertmannite decreased as goethite increased with depth. XRD patterns contained peaks for both schwertmannite and goethite, and the goethite exhibited line broadening indicative of poor ordering compared to that occurring at the base of the ochre column (Fig. 3.2). The two OH bending vibrations of goethite became more intense with depth as bands characteristic of SO₄ diminished (Fig. 3.3). Likewise, total solid phase S decreased with depth (Table 3.2). These data support previous laboratory (Bigham et al., 1996; Desborough et al., 2000) and field studies (Peine et al., 2000) that indicated schwertmannite was a metastable precursor to goethite.
Fig. 3.8 – X-ray diffraction patterns for a) jarosite and the associated b) acid insoluble residue in nodules isolated from a depth of 25-30 cm in the 2000 sediment core. d-spacings are in Å.
The depth distribution of schwertmannite and goethite was quantified and conversion rates estimated using samples extracted in the dark with acid (pH 3) ammonium oxalate to selectively dissolve schwertmannite and leave goethite as a mostly insoluble residue. The acid ammonium oxalate procedure was developed for the selective removal of ferrihydrite from soils with recommended extraction times ranging from 2 to 4 h (Schwertmann, 1964; McKeague and Day, 1966). Brady et al. (1986) and Dold (2003) observed that schwertmannite can be dissolved in as little as 15 min, but schwertmannite in the Carbondale sediments was found to be more recalcitrant, perhaps due to its highly aggregated nature. Therefore, an extraction time of 4 h was defined in preliminary experiments by incrementally increasing the extraction time until XRD confirmed the residues were free of schwertmannite. The oxalate-extractable Fe was then converted to % schwertmannite by using the formula weight defined previously, and goethite contents were obtained by difference. Estimates obtained by this procedure were also confirmed by XRD analysis of binary mixtures of schwertmannite and goethite (Klug and Alexander, 1954). The mixtures were prepared from samples taken at 0-3 cm and 30-33 cm depths in the 2000 core.

In the 1996 samples, schwertmannite decreased from almost 100 wt% at the sediment surface to 10 wt% at the base of the sediment column, whereas goethite increased from 5 - 80 wt% (Fig. 3.9). Assuming that 1) all goethite was produced by transformation of schwertmannite and 2) active deposition of schwertmannite was still occurring, the minimum conversion rate for schwertmannite to goethite during the period 1990-1996 was calculated as 30
Fig. 3.9 – Depth distributions of schwertmannite, goethite and gypsum from the 1996 sediment core and schwertmannite and goethite from the 2000 sediment core.
mol/m$^3$/yr. Except for the surface 6 cm, schwertmannite was more abundant at all depths in 1996 as compared to 2000, which indicated that conversion continued after active sedimentation had ceased (Fig. 3.9). As a whole, the sediment column collected in 1996 contained 7% more schwertmannite than samples analyzed in 2000, suggesting a slower conversion rate of approximately 10 mol/m$^3$/yr. The persistence of schwertmannite at the surface of the sediment could have reflected active Fe cycling between dissolved and precipitated phases with constant replenishment of schwertmannite in the capillary fringe of the sediment column. Alternatively, the dense aggregation, low exposed surface area (Table 3.1) and oxic conditions could have prevented dissolution of schwertmannite.

Porewater pH, Eh, Fe and SO$_4$

Porewater samples from 1996 (Brill, 1999) and 2000 showed striking spatial and temporal differences in pH (Fig. 3.10a). In 1996, pH decreased in the upper half of the sediment column from 2.6 to 2.0 and then increased to 6.2 at the bottom. In 2000, the surface pH was greater and increased less with depth from 3.4 to 4.4. Increased pH toward the bottom of the sediment column was expected in both years due to influence of the limestone layer. In addition, the activity of sulfate-reducing bacteria (SRB) in compost underlying the ochre, as indicated by the odor of H$_2$S, may have contributed to increases in pH through the production of bicarbonate. Over time, dissolution or armoring of the
Fig. 3.10 – Porewater a) pH, b) sulfate, c) Eh, d) Fe(II), e) Fe₄ for samples collected in 1996 and 2000.
limestone decreased the neutralizing capacity and resulted in less dramatic increases in pH with depth for the 2000 sampling. Lower porewater pH at the sediment surface in 1996 compared to the influent drainage (pH 3.6) was likely due to active Fe precipitation through oxidation and hydrolysis. By the 2000 sampling, flow of drainage water was channeled, which decreased the rates of Fe precipitation in most areas of the first cell. Thus, the pH of porewater at the top of the sediment was similar to the influent drainage. Decreased pH in the upper portion of the sediment column in 1996 corresponded with the observed mineralogical transformation of schwertmannite to goethite, which releases protons (reaction 1)(Bigham et al., 1996).

\[
\text{Fe}_8\text{O}_8(\text{OH})_4(\text{SO}_4)_{1.6(s)} + 3.2 \text{ H}_2\text{O}(l) \rightarrow 8 \text{ FeOOH}(s) + 3.2 \text{ H}^+(aq) + 1.6 \text{ SO}_4^{2-}(aq) \quad (1)
\]

Sulfate is also released when schwertmannite is converted to goethite, which was clearly demonstrated in the samples collected in 1996. Sulfate concentrations in the porewater increased from 1010 mg/L near the sediment surface to 4630 mg/L at 25.5 cm depth. Assuming a bulk density \(D_b\) of 0.4 g/cm\(^3\), a particle density \(D_p\) of 4.4 g/cm\(^3\) and 90% porosity \([1-(D_b/D_p)]\), the decay of only 1.5 g of schwertmannite/100cm\(^3\) of sediment would increase the \(\text{SO}_4\) concentration by over 3000 mg/L above background. Below 26 cm, the \(\text{SO}_4\) concentration decreased to 3070 mg/L (Fig. 3.10b). In 1996 this decreased \(\text{SO}_4\) was mostly due to the precipitation of gypsum, but may also have involved SRB activity and loss of sulfide to the atmosphere as \(\text{H}_2\text{S}\) or to solids through
precipitation as FeS (sulfides were detected in compost beneath the ochre).

Sulfate concentrations in porewater samples collected in 2000 showed no distinct gradients with depth, but varied from 1420 mg/L to 2030 mg/L (Fig. 3.10b and Table 3.3). These results indicated a much less dynamic system.

The Eh of porewater samples collected from the upper half of the sediment column in 1996 ranged from 605 mV to 642 mV and then decreased sharply with depth to 115 mV (Fig. 3.10c). In 2000, Eh gradually decreased with depth from 776 to 429 mV. Lower Eh with depth was expected due to the consumption of O\textsubscript{2} diffused from the surface; however, the lower Eh near the bottom of the sediment column in 1996 may have also resulted from greater microbial activity. The compost material in 1996 presumably had more biodegradable electron donors for SO\textsubscript{4} reduction as compared to 2000.

Total Fe and Fe(II) concentrations in 1996 showed depth trends similar to SO\textsubscript{4}; both parameters increased with depth to 25.5 cm and then decreased (Fig. 3.10d – e). Total Fe ranged from 30 mg/L to 900 mg/L, with Fe(II) comprising 40-100% of the total. Ratios of Fe(II)/ Fe\textsubscript{t} that exceeded unity were observed with some samples and have been reported in previous analyses of pore waters using the ferrozine method (e.g. Luther et al., 1996). The generally high dissolved Fe contents could be related to the fact that Fe in poorly ordered schwertmannite was more susceptible to reduction than in relatively well ordered goethite (Münch and Ottow, 1980; Bigham et al., 1996). Moreover, high rates of Fe(III) reduction have recently been reported in acidic coal mine lakes (Blodau et al., 1998; Peine et al., 2000) where Küsel et al. (1999) demonstrated that the reduction of
<table>
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<th>Depth from surface (cm)</th>
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<th>K</th>
<th>Mg</th>
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Table 3.3- Porewater composition for samples collected in 2000.
Fe(III)-hydroxides could be mediated by *Acidophilium* species. Thus, the observed distribution of dissolved Fe(II) in the sediment column may be related to the distribution of iron-reducing bacteria as well as the susceptibility of the Fe precipitates to reduction.

In 2000, both Fe(II) and Fe$_i$ increased with depth; however, the concentrations were much lower than in 1996 even though redox profiles were similar to 25 cm depth (Fig. 3.10c and Table 3.3). Total Fe ranged from 12 mg/L near the surface to 290 mg/L near the bottom of the core, with the percentage of Fe(II) increasing with depth from 13 to 100%. Lower concentrations of Fe in solution during 2000 could perhaps be attributed to the greater proportion of goethite relative to schwertmannite in these sediments and to the fact that goethite was less soluble than schwertmannite at comparable Eh values.

**Al, Ca, Mg, Mn, Na and K in the Solid and Pore Water Samples**

Aluminum was the most abundant cation in the solid phase after Fe (Table 3.2). Precipitation of Al as Al(OH)$_3$ or a hydroxysulfate is pH dependent, with Al solubility decreasing above pH 4.5 (Nordstrom and Alpers, 1999). Thus, Al concentrations in the solid phase were at a maximum and those in the pore waters at a minimum near the bottom of the sediment column where pH was in the range of 4.5-6.0 (Tables 3.2 and 3.3). No Al minerals were detected in the solids due to low concentration and/or the poor structural order of any Al precipitates. Calcium content of the solids also increased with depth due to the
precipitation of gypsum in the 1996 samples (Table 3.2), and was presumably coupled to elevated solution concentrations of Ca arising from the dissolution of carbonates in the wetland cell liner. Calcium concentrations in the solids were less in the 2000 samples (Table 3.2) due to the dissolution of gypsum, but the distribution of dissolved Ca still increased with depth (Table 3.3).

Solid phase Mg and Mn in the 1996 samples increased with depth and were generally greater than in 2000 when both porewater and solid concentrations were relatively constant with depth. The Mg distribution probably reflected contributions from the compost or limestone. Sodium and K showed no distinct trends with depth in the solid phase for either sampling period except that K contents were elevated in the samples from 2000 over the depth increment where jarosite was found (Table 3.2). Solution K also showed some increase with depth in the 2000 samples (Table 3.3).

3.5 Conclusions

The Carbondale wetland examined in this study has served as a reservoir for Fe precipitates from influent mine drainage. Drainage chemistry favored an initial accumulation of schwertmannite, which over time has partially transformed to goethite. The calculated rate of transformation has varied from 10-30 mol/m$^3$/yr. These rates demonstrate that the sediment column has not been a static system, and the instability of schwertmannite has had a significant impact on pore water chemistry through production of acidity and the release of Fe and
SO$_4$. Trace metal cycling could also become an issue in systems with greater contaminant loads. High Fe(II)/Fe$_t$ ratios in the pore waters probably reflect the activity of acidophilic, Fe-reducing bacteria. Microbially mediated reduction of Fe oxides under acidic conditions has rarely been reported and deserves further investigation because of the potential significance to many acid sulfate waters.

The compost and limestone added to the base of the wetland cells have functioned to partially neutralize the low pH drainage, and the compost has modified the redox potential through stimulation of anaerobic microbial decomposition and SO$_4$ reduction. Both effects, however, have been spatially limited by diffusion and have mostly impacted the compost layer and sediment immediately adjacent to the compost. In addition, both effects diminished with time as reactive components were consumed.

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CHAPTER 4

SPATIAL AND SEASONAL VARIATIONS IN PORE WATER CHEMISTRY WITHIN THE SEDIMENT COLUMN OF AN ACID MINE DRAINAGE WETLAND

4.1 Abstract

Constructed wetlands offer promise as a low maintenance, cost-effective means of achieving long-term treatment of mine drainage. Most studies evaluating the treatment efficiency of such wetlands have focused on short-term influent/effluent monitoring. Few have investigated geochemical processes occurring within the accumulating sediment column. The objective of this study was to measure both spatial and seasonal trends in the pore water chemistry of a compost wetland constructed in 1991 for treatment of acid mine drainage near Carbondale, in Athens Co., OH. Sediment cores were collected from the first three wetland cells in October 2000, February 2001 and June 2001 and the solids and pore waters were analyzed. Mineralogical analyses showed that goethite (\(\alpha\)-FeOOH) and schwertmannite \([\text{Fe}_8\text{O}_6(\text{OH})_5(\text{SO}_4)_{1.5}]\) were primary components of the accumulated ochre, and spatial trends indicated a gradual transformation of schwertmannite to goethite. Analyses of pore waters from all three cells gave similar results for Fe, pH and Eh with little variation within sites based on
The pH ranged from 3 to 7 and increased with depth; whereas the Eh ranged from 100 to 750 mV and decreased with depth. Both pH and Eh changed abruptly near the interface between the ochre and compost layers indicating that the ochre was an effective barrier to the diffusion of both alkalinity and oxygen. Dissolved Fe occurred primarily as Fe(II) and peaked within the interface region. Concentrations of other major elements (Al, Ca, K, Mg, Mn, and Na) showed some variation between cells and sampling dates, but vertical gradients generally reflected wetland stratigraphy. Dissolved sulfide concentrations varied with sampling date and were elevated in the compost compared to the ochre and in June compared to February. Accordingly, the sulfide minerals pyrite (FeS$_2$) and greigite (Fe$_3$S$_4$) were detected along with magnetite (Fe$_3$O$_4$) in the compost. Trace metal concentrations in the wetland system were generally low, but solid-phase concentrations were elevated in the compost materials relative to the overlying ochre.

4.2 Introduction

Constructed wetlands have been used with varied degrees of success to treat many different types of contaminated waters. These systems are meant to duplicate the purifying action that occurs in natural wetlands where numerous chemical and biological processes are integrated (Mays and Edwards, 2001). Constructed wetlands offer the potential for long-term treatment where release of contaminated drainage is persistent, and they have been widely implemented for
the mitigation of mine drainage. Abandoned mines and spoil piles often produce acidic, metal-rich drainage for decades after mining operations have ceased, and traditional methods of treatment such as the addition of alkaline reagents are not ideal because they require constant monitoring as well as a large investment of resources (Hyman and Watzlaf, 1997; Rose and Cravotta, 1998; Nordstrom and Alpers, 1999). The technology used in mine drainage wetlands ranges from simple aerobic ponds to more elaborate systems with anoxic limestone drains, organic substrates and subsurface flow (Hedin et al., 1994; Skousen, et al., 1998; Barton and Karathanasis, 1998).

Many of the wetlands initially constructed to treat acid mine drainage in the northeastern U.S. were compost wetlands that consisted of clay-lined cells layered with limestone and compost. The compost and limestone layers were included to provide an organic substrate and a source of alkalinity to promote the development of beneficial bacterial communities, particularly sulfate-reducing bacteria (SRB). Sulfate-reducing bacteria remove $\text{SO}_4^{2-}$ from the drainage through reduction to $\text{H}_2\text{S}$ which is either lost to the atmosphere or combined with a divalent metal to form a metal sulfide precipitate (Fortin et al., 1996; Fortin and Beveridge, 1997). Removal of Fe and other metals occurs either through oxidation and hydrolysis reactions in the surface waters or by precipitation as metal sulfides at depth.

Treatment efficiency in compost wetlands can be highly variable (Wieder, 1989; Hedin and Nairn, 1993). Rates of $\text{SO}_4^{2-}$ reduction are usually low compared to loading rates and also vary with seasonal activity of the SRB populations (Hsu
and Maynard, 1999). Oxidation and precipitation reactions result in the accumulation of an Fe-rich sediment layer within the wetland cell. These reactions also produce acidity and may result in a net decrease in pH from influent to effluent. In addition, the retention of Fe and other metals may only be temporary. Seasonal changes in porewater chemistry may cause the wetland to serve as a source rather than a sink for metals (August et al., 2002), and the mineralogy of Fe precipitates may affect their long-term stability (Tarutis and Unz, 1995; Bigham and Nordstrom, 2000; Piene et al., 2000; Gagliano et al., 2004).

Most evaluations of wetland efficiency have been short-term monitoring studies that compared influent and effluent surface water quality parameters (Mitsch and Wise, 1998). While these studies provide an overall view of wetland function, a detailed understanding of wetland processes is rarely achieved. Consequently, the objective of this study was to gain a better understanding of processes occurring within the sediment column of a compost wetland receiving acid mine drainage by examination of surface and pore water chemistry as well as the composition of the solid phase. We were specifically interested in evaluating the effects of seasonal changes in pore water chemistry and mineral formation/ transformation processes on the distribution of metals with depth.
4.3 Materials and methods

Site Description

The field site examined in this study was near Carbondale in Athens Co., Ohio. A duplex system was constructed by the Ohio Department of Natural Resources in 1990 to improve drainage quality from two seeps arising from a drift mine abandoned in 1923. The smaller system consisted of three cells and covered a total area of approximately \( 700 \text{ m}^2 \). A larger system, with five cells of \( 1020 \text{ m}^2 \) each and a total area of over \( 5000 \text{ m}^2 \), was the focus of this study (Fig. 4.1). Effluents from both systems were combined in a final wetland cell and polishing pond before entering nearby Hewitt Fork (Sritharan, 1992).

Initially, the cells were planted with 17 cattails (\( Typha \) spp.) per \( \text{m}^2 \) and divided into thirds with retaining boards to evenly distribute the drainage; however, by the year 2000 the plant cover had largely failed and the retaining boards were removed from the first cell due to the accumulation of large volumes of sediment. At the time of construction, drainage entered the large system at an average rate of 8.4 L/s, with an average pH of 3.5, and average Fe and SO\(_4\)
Fig. 4.1 – Schematic diagram of the Carbondale Wetland.
concentrations of 110 and 1400 mg/L, respectively (Sritharan, 1992). The influent drainage chemistry has changed little with time (Shimala, 2000).

Sample Collection and Processing

Sediment cores were collected using clear plastic cylinders approximately 7 cm in diameter and 70 cm in length. Each cylinder was pushed as deep as possible into the sediment column; a rubber stopper was placed in the top to create a vacuum; the core was gently withdrawn; and a second rubber stopper was placed in the bottom of the cylinder. The cores were transported on ice to the laboratory where the sediment was extruded under Ar(g). All cores had distinct ochre and compost layers with an interface approximately 5 cm in thickness. The sediment was divided under flowing Ar(g) into 1 to 5 cm increments according to the natural sediment stratification. The segments were transferred to 250 ml centrifuge bottles, purged with Ar(g) and centrifuged for 15 min at 9100 RCF to separate the pore waters from the solids for analysis. The data presented in this paper are from cores collected from the first three cells of the large wetland system in October 2000, February 2001 and June 2001 (Fig. 4.1). Subsequent cells (4 and 5) were not sampled because the ochre thickness was similar to cell 3 and because of mixing with drainage waters from the adjacent, smaller treatment system (Cell 6). The cores in each cell were taken from undisturbed areas as close as possible to each other over the three sampling dates.
Pore Water Analyses

A portion of the unfiltered porewater was used for pH, Eh and sulfide measurements. The pH and redox potential were measured with an Orion pH electrode and a Corning combination redox electrode with a Pt-sensing element and a Ag/AgCl reference element, respectively. Reported Eh values were calculated by correcting redox measurements using the standard reference potential for the electrode. Sulfide determinations were performed using a HACH™ kit, which reacts hydrogen sulfide and acid soluble metal sulfides with N,N-dimethyl-p-phenylenediamine oxalate to form methylene blue. The amount of sulfide was then quantified colorimetrically.

The remainder of the porewater was filtered using a 0.2 \( \mu \)m polypropylene syringe filter and split into two sub-samples; one was acidified with approximately 1 ml of ultra-pure 6M HCl per 100 ml of sample while the other was not. The acidified sample was used for measurements of dissolved metals. Total Fe and Fe\(^{2+}\) were determined colorimetrically using the ferrozine technique described in To et al. (1999). Other dissolved metals (Al, Ca, Mg, Mn, Na, K, Cd, Co, Ni and Zn) were measured by either atomic absorption (AA) or inductively coupled plasma optical emission spectrophotometry (ICP-OES). Total As was determined using a graphite furnace AA. The unacidified sub-sample was used for \( \text{SO}_4 \) analysis with an ion chromatograph.

Geochemical modeling of aqueous-phase chemical equilibria was conducted using Visual Minteq. Average porewater temperatures, pH and Eh
values were used as model inputs along with laboratory measurements of dissolved Al, Ca, Fe(II), Fe(III), Mg, Mn, Na, K, Be, Co, Ni, Zn and SO₄.

Chemistry and Mineralogy of Solids

Solid samples were fused with lithium metaborate/tetraborate, dissolved in weak nitric acid, and analyzed by ICP-MS for Fe, Al, Ca, Mg, Mn, Na, K, Cd, Co, Ni and Zn content. Analyses were conducted by Act Laboratories in Ontario, Canada. Total C and S were determined by combustion using a CE instruments NC 2100 soil analyzer and a LECO Model 521 induction furnace, respectively. Results were corrected for adsorbed water (110 °C). Arsenic content was determined by dissolution of the solids in 6M HCl followed by graphite furnace AA analysis. Uncoated samples were examined with a Philips XL-30 scanning electron microscope (SEM) equipped with an energy dispersive x-ray spectrophotometer (EDS) for chemical analysis.

The mineralogical composition of the precipitates was evaluated by X-ray diffraction (XRD) analysis of randomly oriented powder samples using CuKα radiation and a Philips PW 1316/90 diffractometer equipped with a theta-compensating slit, a 0.2-mm receiving slit, and a diffracted-beam monochromator (Brady et al., 1986). Random powder mounts of freeze-dried samples were scanned from 3 to 80° 2θ using a step interval of 0.05° 2θ and a counting time of 4 s.
Magnetic susceptibility of the solids was analyzed using a Bartington MS2 Susceptibility System. The corrected, volume-specific magnetic susceptibility ($\kappa_{\text{corr}}$) was determined using approximately 2.5 g of sample in a 10 cm$^3$ plastic pot with lid. The mass-specific magnetic susceptibility ($\chi$) in units of m$^3$kg$^{-1}$ on a scale of $10^{-8}$ was then calculated as: $\chi = \frac{\kappa_{\text{corr}}}{\text{sample weight(g)}}/100$. Samples with high $\chi$ were further characterized by XRD after separation of the magnetic fraction using a hand magnet.

4.4 Results and Discussion

Water Chemistry and Wetland Stratigraphy

Surface water pH was similar for the first three wetlands cells, ranging from 3-3.5 and was consistent with the long-term influent drainage pH (Sritharan, 1992). The precipitation of Fe oxyhydroxides and hydroxysulfates had resulted in an accumulation of 35, 15 and 5 cm of ochre in the first, second and third wetland cells, respectively, by the time the study was conducted. Active Fe precipitation did not result in a decrease in downstream pH (from cell 1 to cell 3) as noted in other studies (Brodie et al., 1993). Pore water pH for the ochre portion of the sediment columns was similar to the surface water pH, but increased to near neutral in the compost layers (Fig. 4.2). The transition in pH occurred over a few centimeters at the interface between the ochre and compost layers for all three cells. This increase in pH was due to proximity to the
Fig. 4.2 – Porewater pH in October (●), February (■) and June (▲) for cells 1, 2 and 3. Dashed lines indicate the interface between the ochre and compost layers for each cell.
dissolving limestone; however, the neutralizing effects were clearly limited to the compost and interface region, not reaching the upper portion of the ochre in cells 1 and 2 or the surface waters. These results indicated that the ochre layer served as an effective barrier to diffusion of alkalinity. They also confirmed that discharge into the wetland, at 8.4 L/s, was too fast to permit neutralization of the influent drainage by simple diffusion of alkalinity. The calculated residence time based on the flow rate and initial reservoir volume was 4 d; however, a tracer test found the actual residence time in 1999 to be approximately 7.4 h (Shimala, 2000).

The interface region of all three cells was also characterized by a decrease in porewater Eh, which was likely due to restricted diffusion of oxygen from the surface waters through the ochre layer combined with increased microbial activity in the compost layer. Surface water Eh ranged from 500 to 700 mV, decreased approximately 400 mV in cell 2 and cell 3 with proximity to the interface region, and was then relatively constant with depth in the compost layer (Fig. 4.3). A similar trend with lower magnitude decreases in Eh was observed in cell 1 where only about 3 cm of compost were secured.

With the exception of the surface water samples, dissolved Fe occurred primarily as Fe(II) (Table 4.1) despite positive Eh conditions within the sediment column. Total dissolved Fe increased with depth in all three cells, including a sharp increase near the ochre-compost interface, before decreasing with depth in the compost of cells 2 and 3 (Fig. 4.4). Increases in dissolved Fe with depth in the ochre presumably resulted from the reductive dissolution of Fe precipitates,
Fig. 4.3 – Porewater Eh in October (●), February (■) and June (▲) for cells 1, 2 and 3. Dashed lines indicate the interface between the ochre and compost layers for each cell.
Table 4.1 - Surface and Pore Water Chemistry

<table>
<thead>
<tr>
<th>Cell 1</th>
<th>Surface water (n=3)</th>
<th>Ochre Pore Waters (n=28)</th>
<th>Compost Pore Waters (n=3)</th>
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<tbody>
<tr>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>28.9±2.8</td>
<td>25.8±2.0</td>
<td>*</td>
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<tr>
<td>Ca</td>
<td>141.8±14.3</td>
<td>186.7±11.2</td>
<td>299.3±33.9</td>
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<td>Fe(II)</td>
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<td>7.3±12.3</td>
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<tr>
<td>Fe₃</td>
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<td>193.6±50.1</td>
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<tr>
<td>Mg</td>
<td>76.8±3.7</td>
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<td>88.2±1.7</td>
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<td>8.2±0.4</td>
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<td>34±26</td>
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<td>Ni</td>
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<td>Ochre Pore Waters (n=15)</td>
<td>Compost Pore Waters (n=12)</td>
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<td>Mg</td>
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<td>Compost Pore Waters (n=6)</td>
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<tr>
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<td>59±14</td>
<td>*</td>
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<tr>
<td>Zn</td>
<td>1145±580</td>
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*below detection limit
**average for three sampling dates
Fig. 4.4 – Total dissolved Fe in October (●), February (■) and June (▲) for cells 1, 2 and 3. Dashed lines indicate the interface between the ochre and compost layers for each cell.
demonstrating their instability within the wetland system. Dissolution indicates potential mediation of Fe(III) reduction by iron-reducing bacteria, which has been shown to be the dominant mechanism for Fe reduction in most soils and sediments (Lovely et al., 1991) but which has rarely been reported under strongly acidic conditions. An exception is the recent work by Küsel et al., (1999) who investigated Fe-rich sediments from an acidic mine lake and found that Fe(III)-hydroxides could be reduced by a native Acidiphilium species. Biological activity would be expected to show seasonal trends, but such trends were not apparent in Fe(II), Eh or most other solution parameters. An exception was dissolved sulfide concentrations, which were elevated in the compost compared to the ochre and in June compared to February (Fig. 4.5). These differences presumably reflected changes in SRB abundance and activity. Despite the observed increases in dissolved sulfide with depth, the absolute concentrations were low compared to dissolved SO$_4$ (Table 4.1) and indicated poor removal efficiency.

The concentrations of other major elements (Al, Ca, K, Mg, Mn, and Na) in the surface water showed little change from cell 1 to cell 3 (Table 4.1). Pore water composition displayed some variation between cells, but vertical gradients generally reflected wetland stratigraphy. For example, dissolved Ca, K and Na increased with depth in the pore waters and were presumably influenced by the chemistry of the compost and underlying limestone. The limestone base may have caused increases in dissolved and solid phase Mg concentration with depth in cell 2; however, the dissolved concentrations in cells 1 and 3 remained
Fig. 4.5 – Dissolved sulfide in February (■) and June (▲) for cells 1, 2 and 3. Dashed lines indicate the interface between the ochre and compost layers for each cell.
relatively constant with depth (Table 4.1 and 4.2). Soluble Al concentrations were approximately 30mg/L in the surface waters and upper portions of the ochre where pH was <4.5, but decreased to below detection levels as Al precipitated with increases in pH near the compost (Table 4.2). Dissolved Mn concentrations decreased with depth in cells 2 and 3, but increased in the compost of cell 1.

Sediment Mineralogy and Chemistry

Ochre deposits in the Carbondale wetland had high total Fe concentrations ranging from 530-610 g/kg and low carbon contents ranging from 6-14 g/kg (Table 4.2). X-ray diffraction analyses showed that schwertmannite [Fe$_8$O$_8$(OH)$_5$(SO$_4$)$_{1.5}$] and goethite [α-FeOOH] were primary mineral components of the ochre and that goethite/schwertmannite ratios increased with depth and distance from the drainage inlet (Fig. 4.6). The pH and pe (pe = Eh (mv)/59.2) values for surface and pore waters of cells 1, 2 and 3 from the October sampling were plotted on a pe-pH stability diagram constructed using average activities for Fe$^{2+}$ and SO$_4^{2-}$ generated by Visual MINTEQ and log K$_{sp}$ values for schwertmannite and goethite of 18 and 1.40, respectively (Bigham et al., 1996)(Fig. 4.7). Most samples fell on or below the solubility line for schwertmannite. Only surface or near surface waters were superarated with respect to schwertmannite, whereas all solutions were supersaturated with respect to goethite. These results are consistent with previous observations (Gagliano et al., 2004) that schwertmannite precipitates and then transforms
### Table 4.2- Major element chemistry of solids

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<th>Compost</th>
</tr>
</thead>
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<td>(n=1)</td>
</tr>
<tr>
<td>g/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>6.5±1.5</td>
<td>39.4</td>
</tr>
<tr>
<td>Ca</td>
<td>0.6±0.1</td>
<td>21.2</td>
</tr>
<tr>
<td>Fe</td>
<td>611.8±8.8</td>
<td>35.1</td>
</tr>
<tr>
<td>K</td>
<td>0.9±0.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Mg</td>
<td>*</td>
<td>8.7</td>
</tr>
<tr>
<td>Na</td>
<td>0.2±0.1</td>
<td>2.9</td>
</tr>
<tr>
<td>C</td>
<td>6.1±0.4</td>
<td>99.6</td>
</tr>
<tr>
<td>S</td>
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<td>(n=3)</td>
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<td>g/kg</td>
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<tr>
<td>Al</td>
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<td>61.1±7.3</td>
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<td>9.2±4.1</td>
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<tr>
<td>Fe</td>
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<td>119.2±33.6</td>
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<tr>
<td>K</td>
<td>0.6±0.2</td>
<td>8.0±0.6</td>
</tr>
<tr>
<td>Mg</td>
<td>*</td>
<td>5.7±1.8</td>
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</tr>
<tr>
<td>g/kg</td>
<td></td>
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</tr>
<tr>
<td>Al</td>
<td>5.6±0.6</td>
<td>8.8±3.0</td>
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<tr>
<td>Ca</td>
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<tr>
<td>Fe</td>
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<td>K</td>
<td>1.5±0.1</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Mg</td>
<td>*</td>
<td>0.3±0.2</td>
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<td>Na</td>
<td>0.4±0.1</td>
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<td>C</td>
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<tr>
<td>S</td>
<td>35.4±5.0</td>
<td>5.5±4.3</td>
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</table>

*below detection limit
Fig. 4.6 – X-ray diffraction patterns for sediment samples collected from the second cell showing schwertmannite (sh) and goethite (gt) as the predominant mineral phases from 0-3 cm and 6-9 cm, respectively.
Fig. 4.7 – pE-pH diagram including surface (open symbols) and pore water (solid symbols) data for cells 1, 2 and 3 during the October sampling. Line equations for goethite (pE=17.65-3 pH) and schwertmannite (pE=18.97-2.5 pH) were calculated using average log activities for Fe$^{2+}$ and SO$_4^{2-}$ of –3.21 and –2.17, respectively.
to goethite resulting in a temporary release of Fe and SO$_4$ to solution (Gagliano et al., 2004) (Fig. 4.6). Increases in dissolved Fe near the interface of the ochre and compost (Fig. 4.4), and elevated SO$_4$ concentrations in the ochre pore waters compared to the surface waters for all three cells were likely due to this transformation (Table 4.1).

Dissolved sulfide measurements were elevated in the compost compared to the ochre, especially during the June sampling (Fig. 4.5). SRB reduce SO$_4$ in the presence of an organic substrate to HS$^-$, which can react spontaneously with Fe(II) and other metals to form sulfide minerals or with H$^+$ to yield H$_2$S (Fortin and Beveridge, 1997). Accordingly, pyrite (FeS$_2$) and the magnetic mineral, greigite (Fe$_3$S$_4$) were identified along with magnetite (Fe$_3$O$_4$), by XRD analysis of magnetic separates from the compost layers (Fig. 4.8 and 4.9). The size and morphology of the greigite and magnetite crystals were consistent with a biological origin (Fig. 4.10) (Delong et al., 1993). Magnetic minerals synthesized by magnetotactic bacteria within specialized intracellular compartments called magnetosomes are usually smaller in size and have more consistent morphology than their abiotic counterparts. Magnetic susceptibility measurements not only confirmed the presence of ferrimagnetic minerals in the raw compost materials, but also showed that the upper portion of the compost in cell 3 had a magnetic signature similar to the ochre (Fig. 4.11). The manure compost in cell 3 was much higher in Fe and lower in total C than the mushroom compost in cells 1 and 2, either because of mixing with the ochre or differences in starting composition. Despite the presence of sulfide minerals in the compost layers, total S contents
Fig. 4.8 – X-ray diffraction pattern from the composted manure showing pyrite (P), magnetite (M) and quartz (Q).
Fig. 4.9 – X-ray diffraction pattern from the magnetic portion of the composted manure in cell 3 showing greigite (G) and quartz (Q).
Fig. 4.10 – Scanning electron micrographs of pyrite (A) and magnetite (B) and a transmission electron micrograph of greigite (C).
Fig. 4.11– Magnetic susceptibility measurements with depth from cells 1(●), 2(■) and 3(▲).
were low compared to the ochre in all three cells (Table 4.2) and were another indicator of poor removal efficiency associated with SO$_4$ reduction. Total S in the compost layers also decreased downstream from cell 1 to cell 3.

Trace Element Composition of Water and Sediments

Trace metal concentrations in the influent drainage at Carbondale were low, with dissolved As, Be, Cd, Cu, Cr, and Pb falling below detection limits in the surface and pore waters. The conversion of schwertmannite to goethite did not have a detectable impact on the temporal or spatial distribution of trace metals within the ochre. Dissolved Co, Ni and Zn decreased with depth in the pore waters but analysis of the solids showed an opposite trend (Table 4.1 and 4.3). The trace metal content of the compost materials was substantially greater than in the ochre, indicating larger initial contents in the composts, preferential sorption of trace metals to organic materials, or precipitation of trace metals as sulfide minerals (Webb et al., 1998; Webster et al., 1998). The organic material used in cell 1 and cell 2 was spent mushroom compost, and it consistently showed higher trace metal contents, except for Cu, than the composted manure used in cell 3. Unfortunately, no data were available to characterize the initial composition of either material.
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*below detection limit

Table 4.3 -Minor element chemistry of solids
4.5 Summary

The objective of this study was to characterize spatial and seasonal trends in pore water chemistry within the sediment column of a wetland constructed for the treatment of acid mine drainage. Sediment cores were collected from the first three cells of a six-cell compost wetland system in October 2000, February 2001 and June 2001. All three cells had similar trends for Fe, pH and Eh with little variation based on sampling date. Values for pH ranged from 3 to 7 and increased with depth; whereas the Eh ranged from 100 to 750mV and decreased with depth. Both pH and Eh changed abruptly near the interface between the ochre and compost layers indicating that the ochre was an effective barrier to the diffusion of both alkalinity and oxygen. Dissolved Fe was predominantly present as Fe(II), and concentrations peaked at the interface of the compost and ochre in all three cells. Other major dissolved element concentrations had some variation between cells and with sampling date, but vertical trends generally reflected wetland stratigraphy. For example, Ca concentrations increased with proximity to the limestone layer. Trace metal concentrations in the porewaters were generally low, but those above detection levels decreased with depth and showed no consistent variations with sampling date. Solids showed the opposite trend where trace metal concentrations were elevated in the compost compared to the ochre. Dissolved sulfide was the only parameter measured that showed consistent variations with sampling date. Concentrations were elevated in June
compared to February and in the compost compared to the ochre, but were very low in relation to SO$_4$ loading rates.

Acknowledgements. We gratefully acknowledge Dr. D.C. Golden’s contribution of the transmission electron micrograph in figure 11 and the analytical assistance of Mr. D. Beak and Mr. F.S. Jones throughout the study. Funding for WBG was provided by the NASA graduate student research program, grant no. 9-51. Additional salary support was provided by OSU-OARDC.
CHAPTER 5

VARIATIONS IN BACTERIAL COMMUNITY STRUCTURE WITHIN THE SEDIMENT COLUMN OF A WETLAND CONSTRUCTED FOR ACID MINE DRAINAGE TREATMENT

5.1 Abstract

The objective of this study was to profile bacterial community composition in relation to porewater chemistry within the sediment column of an established wetland constructed for the treatment of acid mine drainage. Intact sediment cores were collected and divided into segments. Analyses of pore water chemistry showed that pH increased and Eh decreased with depth in the sediment column. To characterize bacterial communities, DNA was extracted from each segment and the 16S rRNA genes were amplified using the polymerase chain reaction with fluorescently labeled 3’ and 5’ primers. The amplified product was digested with three enzymes Hha I, Msp I and Rsa I, and the number and size of the terminal restriction fragments (TRF’s) were measured. Results showed that bacterial diversity (total number of TRF’s) was similar throughout the sediment profile; however, relatedness of bacterial communities correlated with sediment properties. Bacterial communities in samples from the base of the sediment column where pH was near neutral and Eh ranged from
100-150 mV showed a high degree of similarity to each other while samples from the top 3 cm of sediment where conditions were acidic and oxidizing were least related to other samples. Fragment sizes from the three digestions were also compared to those predicted by computer simulated digestion to identify putative bacterial species. Numerous TRF’s consistent with species of bacteria relevant to wetland function and treatment efficiency, including sulfate-reducing and iron-reducing bacteria, were found and showed population trends with depth.

5.2 Introduction

Drainage from abandoned coal mines can have devastating effects on terrestrial and aquatic ecosystems. The mining process exposes metal sulfide minerals to oxygen and water resulting in the generation of acid drainage with high concentrations of metals and \( \text{SO}_4 \) (Nordstrom and Alpers, 1999). Acidity kills plants and increases the susceptibility of fish to disease, while dissolved metals can pollute soils and natural waters. Once initiated Acid mine drainage (AMD) often flows from mining sites long after operations have ceased; thus, treatment options for AMD must be long-term. Traditional methods such as the addition of alkaline reagents are effective but require a large investment of resources and require continual oversight.

Constructed wetlands have been implemented for remediation of mine drainage and are considered a low-cost, long-term treatment option. The design and technology incorporated in these wetlands vary with site conditions. A
common system for AMD treatment consists of a series of clay-lined cells layered with limestone and compost (Hedin et al., 1994; Skousen et al., 1998). These compost wetlands serve as a reservoir for Fe precipitates and provide an environment for beneficial microbial communities to develop. Over time, the sediment column can become stratified. The upper portion of the sediment is usually composed of Fe precipitates, and pore waters are similar to the influent drainage with low pH and oxidizing conditions. The base of the sediment column is compost material, and pore waters have near neutral pH due to the influence of the underlying limestone layer. Conditions are also more reducing due to microbial activity and restricted diffusion of oxygen (Gagliano et al., 2004b).

Most research on the use of constructed wetlands for AMD remediation has focused on evaluating treatment efficiency through short-term influent and effluent monitoring or on optimizing design parameters (Stark et al., 1991; Sritharan et al., 1992; Manyin et al., 1997). Other studies have characterized the composition of chemical precipitates (Sobelewski, 1996; Webster et al., 1998; Gagliano et al., 2004) or porewater chemistry (Gagliano et al., 2004b), and a few have analyzed microbial communities using traditional culturing techniques (Batal et al., 1990; Webb et al., 1998). Fundamental biogeochemical processes occurring within the sediment column, however, remain poorly understood. An essential component to understanding these processes is the microbial community composition.

The objective of this study was to profile bacterial communities in the wetland sediment in relation to basic pore water chemistry. We were particularly
interested in the distribution of sulfate-reducing bacteria (SRB) and iron-reducing bacteria (IRB) within the sediment layers because of their relevance to wetland treatment efficiency. SRB use SO$_4$ as an electron acceptor, reducing it to H$_2$S, which is either lost to the atmosphere or combined with a divalent metal and precipitated as a metal sulfide. Metabolic activities of SRB can remove SO$_4$ and metals from drainage waters as well as neutralize acidity (Christensen et al., 1996). IRB can decrease Fe removal efficiency in wetland systems by dissolving Fe precipitates through reductive dissolution processes. In systems where drainage waters have high trace metal concentrations, Fe precipitates often have trace metals sorbed to particle surfaces. Reductive dissolution of these precipitates can result in the release of trace metals back into drainage waters (Tarutis and Unz, 1995).

Because culture-based techniques only describe a small portion of the bacterial populations in environmental samples, often less than 1% in soils and sediments (Amann et al., 1995), we employed terminal restriction fragment length polymorphism (T-RFLP) based on 16S rRNA genes to analyze community composition (Liu et al., 1997). T-RFLP has been used to evaluate microbial communities in a range of environments including marine sediments (Urakawa et al., 2000), soils (Dunbar et al., 2000) and compost materials (Michel et al., 2002). T-RFLP allowed us to compare bacterial community profiles as well as identify putative species within different segments of the sediment column.
5.3 Materials and Methods

Field Site and Sampling

The field site examined in this study was a wetland system constructed in 1990 by the Ohio Department of Natural Resources for the treatment of acid mine drainage. This system consisted of a series of six sedimentation cells each approximately 1000 m² in area, that were excavated, clay lined and layered with 30 cm of dolomitic limestone and 38 cm of an organic substrate. At the time of construction, cattails (*Typha spp.*) were planted at a density of 17 plants/m²; however, they failed to become established. The influent drainage entered the system at a rate of 8.4 L/s, with an average pH of 3.5. The average Fe and SO₄ concentrations were 100 and 1400 mg/L, respectively (Sritharan, 1992). The influent drainage chemistry has changed little with time (Shimala, 2000), and pore water chemistry and composition of solids within the sediment showed consistent trends throughout multiple samplings (Gagliano et al., 2004b). The data presented are from sediment cores collected from the third wetland cell where the organic substrate was composted manure. Samples analyzed for pore water chemistry and microbial community composition were collected on November 21, 2000 and October 19, 2001, respectively.

Sediment cores were collected using clear plastic cylinders approx. 7 cm in diam and 70 cm in length. Each cylinder was pushed into the sediment column; a rubber stopper was placed in the top to create a vacuum; the core was
gently withdrawn; and a second rubber stopper was placed in the bottom of the cylinder. Cores were transported to the laboratory on ice where they were extruded in approximately 3-cm segments corresponding to visual differences in the ochre and compost layers. Cores were segmented to analyze variations in biogeochemical properties with depth in the sediment.

Sediment Characterization

Extruded segments of cores used for pore water and solids analysis were transferred to 250 ml centrifuge bottles, purged with Ar and centrifuged for 15 min at 9100 RCF to separate the pore waters from the solids. The unfiltered porewater was used for pH and redox determinations with an Orion pH electrode and a Corning combination redox electrode with a Pt-sensing element and an Ag/AgCl reference element, respectively. Reported Eh values were calculated by correcting redox measurements using the standard reference potential for the electrode. Dissolved Fe measurements were performed on filtered pore waters using the ferrozine method described by To et al., (1999). Solids were freeze-dried and characterized by x-ray diffraction (XRD) analysis of randomly oriented powder samples using CuKα radiation and a Philips PW 1316/90 diffractometer equipped with a theta-compensating slit, a 0.2-mm receiving slit, and a diffracted-beam monochromator (Brady et al., 1986). Morphological studies were conducted with a Philips XL30 scanning electron microscope.
DNA Extraction and Amplification

The sediment samples used for microbial community composition were collected from two cores (A and B). Each segment from both cores was used for two separate extractions resulting in a total of four samples per sediment depth. Extraction of DNA directly from the sediments gave low yields and extracts could not be PCR amplified even after concentration and purification by low melting temperature agarose gel electrophoresis. This difficulty was likely due to the high Fe-oxide and humic substance content of the sediment samples, which have been found to inhibit the polymerase chain reaction (PCR) (Holben, 1994). Attempts to dissolve Fe precipitates with oxalate or EDTA to allow for concentration of cells were unsuccessful due to the crystallinity of the Fe minerals (Hao et al., 2002). Thus, an indirect extraction procedure was used. Approximately 5 g of sediment from the interior portion of the core was transferred to a 15 ml falcon tube and 10 ml of buffer (0.1% Na-pyrophosphate, 50mM NaCl and 100mM Tris) was added. The tubes were shaken on ice for 2 h to free bacteria from the solids and the sediment was allowed to settle for 4-6 h. The supernatant was then transferred to a separate tube and centrifuged at 10,000 RPM. The pellet was washed with TE (10 mM Tris base and 1mM EDTA) and resuspended in 200 µl sterile water. DNA was extracted from the suspensions using the QIAamp DNA mini extraction kit (Qiagen Inc.) with a cell lysis temperature of 95°C. DNA extractions yielded 0.2-0.8 µg/g of wet sediment.
Ribosomal 16S rRNA genes were PCR amplified from DNA extracts using universal bacterial primers 11F and 907R. This primer pair was selected because of its ability to anneal to multiple genera of SRB as well as several species of IRB; however, it would not anneal to bacteria such as *Acidithiobacillus ferrooxidans* or *Leptospirillum ferrooxidans* commonly found in mine drainage environments (Nicomart, 2001). The sequences of the labeled forward and reverse primers were: HEX-5’GTGGATCGTGGTCCAG-3’ and FAM-5’-CCGTCAATTTCTTTRATTATTT-3’, respectively (Lane, 1991; Giovannoni, 1991). PCR reactions contained 1 mM MgCl$_2$, 1X PCR buffer, 0.2 mM dNTP’s, 0.3 µg/µl BSA, 3% DMSO, 1µM each primer and 0.025 U/µl Hot Start *taq* (Qiagen, Valencia, CA). Reactions were heated at 95°C for 15 min then amplified by denaturing at 94°C for 45 sec, annealing at 56°C for 60 sec and extending at 72°C for 2 min for 30 cycles followed by a final extension at 72°C for 10 min. The size of PCR products was consistent with the amplification of a 900 bp rRNA gene fragment.

**Digestion and TRFLP Analysis**

PCR products were purified using DNA Clean and Concentrate-25 kits (Zymo Research). Each sample was digested for 3 h at 37°C followed by 20 min at 75°C. The digestions included 200 ng purified DNA, 0.5 µl of enzyme, (either *Hha I*, *Msp I* or *Rsa I*), and the appropriate buffer. The size (up to 500 base pairs) and intensity in fluorescence units of the labeled terminal restriction
fragments (TRF’s) were determined using capillary electrophoresis on an Applied Biosystems Instrument model 3700 DNA analyzer and Genescan software. Peak areas were adjusted to compensate for small peaks found in the negative control, and the relative fluorescence for each sample digest calculated by dividing individual TRF peak areas by the sum of all TRF peak areas. These data were imported into FRAGSORT version 4.0 (Michel and Sciarini, 2003), which identifies putative taxonomic units or species by comparison to a computer simulated digestion using defined primers and enzymes. The data presented matched the expected terminal fragment sizes for all three enzymes within a defined fragment length sizing error (eq. 1).

\[
\text{Sizing error} = 1.0 \times 10^{-6} F^3 + 1.0 \times 10^{-5} F^2 - 4.0 \times 10^{-4} F + 1.1201 \quad (1)
\]

In this equation, F is the observed terminal restriction size determined using capillary electrophoresis. We used this equation because the sizing error for polyacrylamide electrophoresis increases with fragment size. Discussion of the putative species includes only those identified with all three restriction enzyme profiles and found in all four replicates. The relatedness of bacterial communities or cluster analysis within the sediment layers was determined using digitized images of composite electropherograms generated from both the forward and reverse primers for all three enzyme digests. In this analysis, contributions from small peaks found in the negative control were considered insignificant and samples were analyzed using Bionumerics version 3.5 (Applied Maths, Kortrijk).
Similarity was determined using Pearson’s correlation, and the unweighted pair group method using arithmetic averages (UPGMA) was used to create dendograms (Liu et al., 1997; Michel et al., 2002).

5.4 Results and Discussion

Wetland Sediment Properties

All six cells in the Carbondale wetland system had a layer of ochre at the sediment surface (Fig. 5.1). The greatest accumulations were in the first and second cells where the ochre was 15-30 cm thick. Samples for this study were collected from the third wetland cell where 2-3 cm of ochre had accumulated. XRD patterns showed goethite ($\alpha$-FeOOH) was a primary component of the ochre; however, selective dissolution analysis (data not shown) indicated schwertmannite (Fe$_8$O$_6$(OH)$_6$SO$_4$) was also present (Fig. 5.2). Pore waters within the ochre were similar to the influent drainage with pH ranging from 3 to 4.5 and Eh ranging from 450 to 700 mV (Fig. 5.3). These conditions contrasted with the base of the sediment column where the solids were mostly composted manure and the pore waters had near neutral pH values due to the influence of the limestone layer and lower Eh’s of 100-200 mV. Between these layers was an interface region approximately 4-6 cm in thickness that was a mixture of highly degraded organic matter and Fe minerals. Porewater chemistry in this region displayed strong gradients, with pH increasing and Eh decreasing sharply.
Figure 5.1- Schematic diagram of the Carbondale Wetland.
Figure 5.2- X-ray diffraction pattern for Fe precipitates in the third wetland cell with goethite (gt) and quartz (qz) peaks labeled.
Figure 5.3- Porewater pH ● and Eh ■ in relation to sediment depth. Dashed lines represent boundaries of different sediment layers.
Dissolved Fe, which throughout the profile was predominantly in the reduced form, reached a peak in the interface region (Fig. 5.4).

**Bacterial Diversity and Community analysis**

Ecological theory suggests that bacterial diversity might decrease with lower organic substrate concentrations and greater acidity. Because each TRF represents at least one unique species, this data can be used to represent the relative diversity of samples. The average number of TRF’s was lower for all digestions of both the 0-3 and 3-6 cm segments as compared to the deeper sediments. However, these differences were only significant for the *Rsa I* 0-3 cm sample (Fig. 5.5).

In addition to changes in bacterial diversity within the sediment column, one would expect the composition of the bacterial communities to change with depth. Cluster analyses of the T-RFLP data for each of the six sediment depths show that the greatest degree of similarity (>80%) was in samples extracted from the same sediment core (Fig. 5.6). The two replicates from core A showed a high degree of similarity for each sediment depth, while the greatest degree of similarity in core B was between adjacent sediment depths within the same replicate.
Figure 5.4- Dissolved Fe(II) in relation to sediment depth.
Figure 5.5- Average number of terminal restriction fragments for *Hha I*, *Msp I* and *Rsa I* enzymes for 6 sediment depths from a wetland constructed for acid mine drainage treatment. Error bars represent standard error.
Figure 5.6- Dendogram depicting the relatedness of bacterial communities within the wetland sediment column.
Clusters with a lesser degree of similarity did, however, correlate with wetland sediment properties. All samples collected from the lower portion of the sediment column (6-18 cm) where pH was near neutral and Eh ranged from 100-150 mV (Fig. 5.3) formed two closely related clusters. One cluster included all samples from 6-12 cm with a similarity of 70% and another included all samples between 12-18 cm with 74% similarity. Samples from 0-6 cm in the top portion of the sediment column also formed two distinct clusters. One contained 3 of the 0-3 cm samples, and another included four samples from the 3-6 cm depth and the remaining 0-3 cm sample. The cluster of 0-3 cm samples was most distinct having only 48% similarity with other clusters, indicating that the acidic, oxidizing conditions of the ochre resulted in the development of a substantially different bacterial community. The samples from the 3-6 cm depth clustered more closely to the samples at the base of the sediment column than those at the top. This cluster also corresponded to the region in the sediment column where dissolved Fe concentrations reached a maximum (Fig. 5.4).

Identification of Relevant Species Corresponding to Observed TRF’s

TRF’s consistent with numerous SRB were identified by FRAGSORT (Table 5.1). SRB are important constituents in constructed wetlands because their metabolic processes can serve to remediate mine drainage waters. In the presence of an organic substrate, SRB use SO₄ as a terminal electron acceptor,
Table 5.1- TRF’s consistent with sulfate-reducing bacteria found within the segments of the wetland sediment. TRF’s for listed species were found in all three digestions and all four replicates.
reducing it to H$_2$S, which is either lost to the atmosphere or combined with a
divalent metal and precipitated as a metal sulfide. Multiple TRF’s consistent with
eight genera of SRB were identified in the wetland sediment including
*Desulfo bacter, Desulfo bacterium, Desulfo bulbus, Desulfo coccus, Desulfo halobium, Desulfor hapalus, Desulfo tomaculum* and *Desulfo vibrio* (Table 5.1). All these genera, with the exception of *Desulfo tomaculum*, which is a gram-positive spore former, are gram-negative, and all are mesophilic except for the
recently described *Desulfor hapalus* which is considered moderately psychrophilic (Castro et al., 2000). TRF’s consistent with SRB were found throughout the
sediment profile, even in the top segment where pore waters were oxygenated
and acidic. SRB prefer neutral to slightly alkaline conditions, but have been
described in acidic mine tailings (Fortin et al., 1995) and acidic sediments (Blodau et al., 1998; Koschorreck et al., 2003). In addition, SRB are considered
anaerobic but *Desulfo vibrio* spp. have been described in aerobic environments
and reduce oxygen as a means of environmental detoxification (Baumgarten et
al., 2001). Dissolved sulfide concentrations were consistently below detection
levels in the upper portion of the sediment, suggesting active SRB populations
were not present (Gagliano et al., 2004b). The number of TRF’s consistent with
SRB did, however, increase with depth, suggesting that diversity of SRB
increased as conditions within the sediment became more favorable (Table 5.1).
TRF’s from the SRB genera *Desulfo vibrio* and *Desulfo tomaculum* were identified
in the top segment while TRF’s from 5-6 genera were present in the bottom half
of the sediment column. In the case of *Desulfotomaculum* spp., spores may have been the source of nucleic acids rather than active bacterial populations.

Dissolved Fe in the wetland sediment was present predominantly as Fe(II), indicating the presence of IRB (Fig. 5.3 and 5.4). Reduction of Fe within the sediment column is important with regard to treatment efficiency because dissolution of Fe precipitates can decrease the efficiency of Fe removal from drainage waters. In addition, Fe precipitates often have trace metals sorbed to particle surfaces. Reductive dissolution of these precipitates can result in the release of trace metals back into the drainage waters. Release of Fe and Mn under reducing conditions was described in microcosms designed to simulate mine drainage wetlands (Tarutis and Unz, 1995), and remobilization of Fe and trace metals was attributed to the reduction of Fe precipitates by a *Clostridium* sp. in contaminated soils (Francis and Dodge, 1990). In addition, under acidic conditions *Acidophilium* spp. have been found to reduce Fe(III)-hydroxides in a mine drainage contaminated lake (Küsel et al., 1999). Iron-reduction is a widely distributed trait common in many species throughout the domain bacteria (Lonergan et al., 1996). Among the best described IRB are *Geobacter metallireducens* and *Shewanella putrefaciens*, but many sulfur reducing bacteria including members of the genera *Desulfuromusa* and *Desulfuromonas*, are also capable of using Fe(III) as an electron acceptor. Some SRB reduce Fe, but none have been identified that can use Fe(III) as the sole electron acceptor. TRF’s consistent with *Desulfuromusa bakii*, *Peleobacter acidigallica* and *Shewanella*
<table>
<thead>
<tr>
<th></th>
<th>Hha I (TRF size)</th>
<th>Msp I (TRF size)</th>
<th>Rsa I (TRF size)</th>
<th>Sediment depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron-reducing Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Peleobacter acidigallici</em></td>
<td>91</td>
<td>160</td>
<td>462</td>
<td>X</td>
</tr>
<tr>
<td><em>Shewanella sp.</em></td>
<td>372</td>
<td>495</td>
<td>223</td>
<td>X</td>
</tr>
<tr>
<td><em>Desulfuromusa bakii</em></td>
<td>91</td>
<td>122</td>
<td>208</td>
<td>X</td>
</tr>
<tr>
<td><strong>Sheathed Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptothrix species (L. cholodnii, L. mobilis and L. discophora sp-6)</em></td>
<td>199</td>
<td>131</td>
<td>465</td>
<td>X</td>
</tr>
<tr>
<td><em>L. discophora ss-1</em></td>
<td>199</td>
<td>482</td>
<td>465</td>
<td>X</td>
</tr>
<tr>
<td><em>Sphaerotilus sp.</em></td>
<td>197</td>
<td>480</td>
<td>463</td>
<td>X</td>
</tr>
<tr>
<td><strong>Magnetotactic Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Magnetobacterium bavaricum</em></td>
<td>90</td>
<td>128</td>
<td>447</td>
<td>X</td>
</tr>
<tr>
<td><strong>Cellulose degrading Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cytophaga sp.</em></td>
<td>92</td>
<td>87</td>
<td>308</td>
<td>X</td>
</tr>
<tr>
<td><em>Cellulomonas flavigena</em></td>
<td>366</td>
<td>159</td>
<td>452</td>
<td>X</td>
</tr>
<tr>
<td><strong>Ruminant and related Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Methanobrevibacter ruminantium</em></td>
<td>333</td>
<td>144</td>
<td>76</td>
<td>X</td>
</tr>
<tr>
<td><em>Fibrobacter succinogens</em></td>
<td>137</td>
<td>263</td>
<td>451</td>
<td>X</td>
</tr>
<tr>
<td><em>Campylobacter sp.</em></td>
<td>92</td>
<td>433</td>
<td>416</td>
<td>X</td>
</tr>
</tbody>
</table>

Table 5.2- TRF’s consistent with iron-reducing and other bacteria found within the segments of the wetland sediment. TRF’s for listed species were found in all three digestions and all four replicates.
spp. were identified throughout the sediment profile (Table 5.2) and may have contributed to the elevated concentrations of dissolved Fe(II) in the interface region (Fig. 5.3).

TRF’s consistent with several other bacteria were identified in multiple digestions that could have influenced the composition and morphology of mineral phases formed within the sediment column. TRF’s consistent with various species of sheathed bacteria including *Leptothrix discophora*, *L. cholodnii*, *L. mobilis* and two *Sphaerotilus* spp. were observed in the upper sediment layers (Table 5.2). *Leptothrix* species are known to mediate Fe and manganese oxidation at the oxic/anoxic interface of sediments under near-neutral pH conditions. *Sphaerotilus* spp. are common in fresh waters that are rich in organic substrates. Lattice-like structures in the upper layers of the wetland sediment (Fig. 5.7) may be Fe-oxide encrusted sheaths of these bacteria.

The magnetotactic bacterium, *Magnetobacterium bavaricum*, was identified throughout the sediment column. Magnetotactic bacteria synthesize magnetic minerals, most commonly magnetite (Fe$_3$O$_4$) and greigite (Fe$_3$S$_4$), in intracellular compartments called magnetosomes (Delong et al., 1993). Elevated magnetic susceptibility was measured and small crystals of magnetite and greigite separated from the compost in cell 3 (Fig. 5.8) (Gagliano et al., 2004b).

In addition to the bacterial species that were relevant to local geochemical conditions, several TRF’s consistent with species related to the composition and nature of the compost were found. Composted manure, which included straw bedding, was used as the organic substrate in cell 3. Associated bacterial
Figure 5.7- Scanning electron micrograph of Fe precipitates.
Figure 5.8-Scanning electron micrograph of magnetite crystals.

500 nm
species included common ruminant bacteria such as *Methanobrevibacter ruminantium* and *Fibrobacter succinogens* as well as a *Campylobacter* sp., a bacterium that can infect cattle. Also included were TFF’s consistent with two cellulose degrading bacteria, *Cellulomomas flavigena* and an unnamed *Cytophaga* sp.

5.5 Summary

Constructed wetlands have been widely implemented for treatment of acid mine drainage, but little is known about the bacterial communities that exist within the wetland sediment. We used 16S rRNA-based terminal restriction length polymorphism (T-RFLP) analysis to profile the vertical distribution of bacterial communities within the wetland sediment and underlying organic substrate. Relatedness of bacterial communities could be correlated to wetland sediment properties. The terminal fragments of bacteria isolated from the compost base of the sediment column, where pore water pH was near neutral and Eh ranged from 100-150 mV, formed two closely related clusters. Communities in the top portion of the sediment column, where pore waters were acidic and oxidizing were least related to other samples. Multiple TRF’s consistent with SRB were identified throughout the sediment profile; however, the diversity appeared to increase with depth. TRF’s from only two genera of SRB, *Desulfovibrio* and the spore-forming *Desulfotomaculum* were identified in the acidic, top portion of the sediment column while TRF’s consistent with 6-7
genera, including *Desulfbacter*, *Desulfobacterium*, *Desulfobulbus*,
*Desulfococcus*, *Desulfohalobium*, *Desulforhapalus* and *Desulfovibrio*, were
identified in the near-neutral, compost material lower in the profile. TRF’s
consistent with several species capable of Fe reduction were also identified,
including *Desulfuromusa bakii*, *Peleobacter acidigallica* and an unnamed *Shewanella sp.*

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CHAPTER 6
SUMMARY

Compost wetlands have been constructed and utilized throughout the northeastern United States for treatment of drainage from coal mines. Implementation of this technology has been widespread, based largely on positive evaluations of treatment efficiency in existing systems through short-term monitoring of influent and effluent drainage parameters. A more accurate assessment of the treatment potential, particularly with regard to long-term viability, requires a more complete understanding of both chemical and biological processes occurring within the wetland sediment. Thus, our objective was to perform an integrated study that characterized the mineralogy of chemical precipitates, the pore water chemistry, and the bacterial community composition of the sediment column in an established wetland to gain insight into biogeochemical processes relevant to treatment efficiency.

The field site used in this study was a 6-cell compost wetland constructed in 1991 near Carbondale, OH, for treatment of acid mine drainage. By 2003, treatment efficiency was determined to be inadequate, and the majority of the wetland system was buried in favor of an alkaline chemical doser combined with a sedimentation pond. While operational, the Carbondale wetland served as a
reservoir for iron precipitates removing, on average, 66% of the influent Fe and forming significant accumulations of ochre ranging in thickness from 30 cm in the first cell to 5 cm in the third and subsequent cells. The influent drainage conditions favored precipitation of schwertmannite \([\text{Fe}_8\text{O}_8(\text{OH})_{4.8}(\text{SO}_4)_{1.6}]\) which was unstable in the wetland system and transformed at a rate of 10-30 mol/m\(^3\)/yr to goethite \((\alpha-\text{FeOOH})\). Trace metal concentrations in the Carbondale system were low, but in systems with higher contaminant loads this mineral transformation may have implications for trace metal cycling and long-term treatment efficiency.

Compost and limestone layers incorporated into the wetland to stimulate the development of beneficial microbial populations resulted in distinct trends in pore water chemistry. The limestone functioned to neutralize acidity at the base of the sediment column, while the compost served to decrease redox potential through stimulation of microbial decomposition processes. Consequently, the pore water pH ranged from 3 to 7 and increased with depth, whereas the Eh ranged from 110 to 750 mV and decreased with depth. Both pH and Eh changed abruptly near the interface between the ochre and compost layers indicating that the ochre was an effective barrier to the diffusion of both alkalinity and oxygen. Dissolved Fe occurred primarily as Fe(II) and peaked within the interface region. Concentrations of other major elements showed some variation between cells and sampling dates, but vertical gradients reflected wetland stratigraphy.

Seasonal samplings were conducted to determine if variations in microbial activity affected pore water chemistry. There were no consistent trends based on
sampling date except for dissolved sulfide. Dissolved sulfide concentrations were elevated in the compost relative to the ochre and in June compared to February, but overall were low in relation to SO$_4$ loading. Sulfate concentrations remained unchanged from influent to effluent in the wetland system indicating the activity of SRB was inadequate for effective removal of SO$_4$ from the drainage waters.

Terminal restriction length polymorphism (T-RFLP) of 16S rRNA genes was used to profile bacterial diversity and community composition. DNA was extracted from the wetland sediment and the 16S rRNA genes amplified using polymerase chain reaction with fluorescently labeled 3’ and 5’ primers. The amplified product was digested, and the number and size of the terminal restriction fragments (TRF’s) were measured. Results showed bacterial diversity was similar throughout the sediment profile; however, relatedness of bacterial communities was correlated to wetland sediment properties. Terminal restriction fragments consistent with several genera of SRB, including *Desulfobacter*, *Desulfobacterium*, *Desulfobulbus*, *Desulfococcus*, *Desulfohalobium*, *Desulfurhapalus*, *Desulfotomaculum* and *Desulfovibrio*, were found throughout the sediment column. In addition, TRF’s consistent with other bacterial species were putatively identified that could have influenced wetland treatment efficiency and/or the composition and morphology of iron precipitates. For example TRF’s consistent with several species of bacteria capable of iron-reduction were identified. These included *Desulfuromusa bakii*, *Peleobacter acidigallica* and an unnamed *Shewanella* species. TRF’s consistent with a magnetotactic bacterium,
Magnetobacterium bavaricum, and several species of the iron-oxidizing, bacteria, Leptothrix, were also identified.
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