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# REMOVAL OF MANGANESE FROM AN ALKALINE MINE DRAINAGE USING A BIOREACTOR WITH DIFFERENT ORGANIC CARBON SOURCES

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# ABSTRACT OF THESIS

# REMOVAL OF MANGANESE FROM AN ALKALINE MINE DRAINAGE USING A BIOREACTOR WITH DIFFERENT ORGANIC CARBON SOURCES

The treatment of Mn and $SO_4^{2-}$ contaminated mine drainage via a sulfate reducing bioreactor is expected to result in near-permanent immobilization of significant amounts of Mn and a portion of the sulfates within the matrix. This study tested several different combinations of organic amendments and inorganic substrates in an attempt to optimize sulfate reducing conditions and Mn removal capacity. Five different organic carbon sources, including corn mash, wood mulch, biosolids, soybean oil, and sorghum syrup in combination with five different inorganic substrates, including creek sediment, marble and limestone chips, polished gravel, and sand were tested in batch experiments. Results indicate a widely Mn variant removal potential among the treatments, ranging from 35% for soybean oil to 97% for the mulch mixture, with respective Eh ranges of +60 mV and -320 mV. Sulfate removal ranged from less than 10% to 85%. The most favorable combinations were tested in small scale bioreactors under dynamic conditions. Greater than 90% of Mn and 70% of sulfate was removed over a 65 day test period. Results indicate Mn removal mechanisms include sulfide, oxide, and carbonate formation and simple sorption and $SO_4^{2-}$ removal mechanisms of sulfide gas evolution, gypsum and MnS precipitation, and anion sorption/cation bridging.

KEYWORDS: Alkaline Mine Drainage, Bioreactor, Sulfate Reduction, Manganese, Surface Mining

Jared D. Edwards

April 7, 2008

# REMOVAL OF MANGANESE FROM AN ALKALINE MINE DRAINAGE USING A BIOREACTOR WITH DIFFERENT ORGANIC CARBON SOURCES

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THESIS

Jared Daniel Edwards

The Graduate School

University of Kentucky

2008

# REMOVAL OF MANGANESE FROM AN ALKALINE MINE DRAINAGE USING A BIOREACTOR WITH DIFFERENT ORGANIC CARBON SOURCES

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THESIS

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

Jared Daniel Edwards

Lexington, Kentucky

Co-Directors: Dr. Anastasios Karathanasis, Professor of Soil Science
Dr. Christopher Barton, Professor of Forestry

2008

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# Table of Contents

# *List of Tables*

## *List of Figures*

# *List of Files*

# CHAPTER 1

# Manganese and Sulfate Removal from an Alkaline Mine Drainage in Eastern Kentucky

## *1.1 General Background*

In an environmental impact statement assessing the effects of mountaintop mining and valley fills in Appalachia, the U.S. Environmental Protection Agency determined there are approximately 1,200 miles of headwater streams (2% of the total stream length in the area) “directly impacted” by the mining practices (2005). While 2% does not seem significant, headwater streams generally encompass the majority of the surface to water interactions in any drainage basin, comprising approximately 86% of the total U.S. stream length (Leopold et al., 1964). Consequently, small alterations to these streams result in large cumulative impacts affecting water quality and biotic integrity downstream (Webster et al., 1992).

In 2005, Kentucky had 594 active coal mines, producing 124.4 million tons of coal. Of those mines, approximately 95% of them are located in Eastern Kentucky, in the Appalachian region (KYOMSL, 2006). One of the problems typically associated with mining, especially with abandoned mines, is acid mine drainage (AMD). As previously undisturbed material is exposed to oxygen and water, the sulfide-bearing materials are oxidized, releasing metals and sulfides and forming sulfuric acid. The sulfuric acid then further reacts with the material releasing metals into solution, including high concentrations of copper, lead, arsenic, iron, manganese, and zinc, forming soluble sulfate salts. As these effluents reach undisturbed areas, there is a rapid decrease in ecological functionality and health. In some areas, the limestone geology buffers the AMD to near-neutral conditions, causing the majority of the metals to precipitate. However, Mn has a high solubility, thus the primary contaminants in near-alkaline mine drainage are sulfates and Mn.

A significant portion of the surface mining in Eastern Kentucky utilizes head-of-hollow fills to dispose of the overburden material, which covers the existing headwater streams, resulting in more than 730 miles of permanently buried streams in Kentucky (USEPA, 2002). Any disturbance in a watershed alters the chemical and physical attributes of the drainage water. The most common changes associated with mining include an increase in dissolved ions, including metals and sulfate, and an increase in sediment loading in the stream (USEPA, 2002; Pond, 2004). Historically, the most common solution to acid mine drainage focused on increasing the pH of the water to remove toxic contaminants (Skousen, 1995; Younger & Robins, 2002). More recently, research has focused on sulfate reduction (McIntire & Edenborn, 1990; Webb et al., 1998; Benner et al., 1999; Cocos et al., 2002).

### *1.2 Sulfate Reduction Chemistry*

Sulfate reduction as a means of metal removal from the environment was first documented by Huntsman et al. in 1978 by utilizing a natural Sphagnum bog to treat coal mine drainage. In the last 30 years, much research has focused on the potential for treatment and the design characteristics necessary to maximize efficiency and efficacy in natural and constructed wetlands. During that time, several innovations have been developed and explored, including alternating aerobic and anaerobic treatments, subsurface and surface flow wetlands, limestone drains and many more.

One of the potentially most useful treatment strategies for many mine drainage systems involves the use of naturally occurring sulfate reducing bacteria (SRB). Carbon, typically in the form of waste materials such as municipal compost, biosolids, spent mushroom compost, etc., is added to a reducing environment. The carbon and other nutrients provide fuel for SRB to multiply and reduce the sulfate in the mine drainage to sulfide. Sulfide then precipitates with the metals in solution to form insoluble metal-sulfides, as shown below. (M here indicates a divalent metal cation).

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^-$$
$$M^{2+} + S^{2-} \rightarrow MS$$

A system designed to enhance the natural processes in an expedited fashion is generally called a bioreactor. The focus of this research is to determine the optimum mixture of organic matter for remediation of the Guy Cove mine drainage in Breathitt County, Kentucky using a bioreactor system.

### *1.3 The Bioreactor System*

A sulfate bioreactor requires four conditions to be appropriate to successfully function (Boudreau & Westrich, 1984; Ludwig et al., 2002; Neculita et al., 2007). First, there must be a consistent source of sulfate to feed the SRB and metals to complex the generated sulfide. Secondly, the bacteria need carbon and nutrients. Thirdly, the bacteria need a substrate for physical support. The precipitation of metal-sulfides will also be greatly enhanced if an adhesion surface is available. Lastly, chemical conditions must be appropriate. For example, SRB function best at near-neutral pH and in the absence of non-sulfate electron acceptors, including nitrate and oxygen. The second and third criteria are typically combined; thus, in essence, every bioreactor requires sulfate and contaminant metals, a substrate, and appropriate chemical conditions.

The source of the bacteria is another major concern in a bioreactor design. SRB are fairly ubiquitous in the environment, especially in the waste organic matter typically used in bioreactors. Some researchers have used animal manure (Machemer & Wildman, 1992; URS, 2003), sediments from anaerobic areas of streams (Cocos et al., 2002; Gibert et al., 2003), or various organic materials (Dvorak et al., 1992; Waybrant et al., 1998) as SRB sources. Others have cultured specific bacteria strains for their research (Christensen et al., 1996). While pure cultures effectively work in a bioreactor system, natural specimens tend to be much more effective (Skousen et al., 2000). Some SRB species are only capable of utilizing a specific carbon compound, such as acetate or methanol. Thus, a pure culture will function adequately given appropriate conditions, but natural samples contain a variety of microorganisms, including those capable of degrading large molecular weight organic compounds. Consequently, systems with a diverse population of organisms will much more readily adapt to the existing environment (Tuttle et al., 1969; URS, 2003).

Sulfate reduction research has expanded from batch and column experiments in laboratories to full-scale bioreactors, wetlands, and permeable reactive barriers. Permeable reactive barriers (PRB) are the in-ground equivalent of bioreactors. Typically, a trench is dug in the flow path of the contaminated water to be treated. The trench is then filled with relatively high permeability materials very similar in nature to the bioreactor substrate. The trench is then capped and monitoring wells are installed above- and below-gradient of and within the PRB for continuous monitoring. Due to the similarities between PRBs and bioreactors, research from both types of projects is evaluated herein.

Research utilizing sulfate reduction to encourage metal and sulfate removal has found widely varying efficiencies. Waybrant et al. (1998) attained greater than 99% $SO_4^{2-}$ removal, while Champagne (2005) showed 73% removal of sulfates. Chockalingam and Subramanian (2006) compared the differences between real mine drainage and synthetic drainage and found $SO_4^{2-}$ removal rates of 40% and 73%, respectively. Several field based experiments in either permeable reactive barriers or constructed wetlands reached sulfate removal efficiencies approximating 70% (McIntire & Edenborn, 1990; Sass, et al., 2001; Benner et al., 2002). As evidenced by the variance in removal efficiencies, there are several variables impacting the efficacy of sulfate-reducing treatments including the initial $SO_4^{2-}$ concentration, temperature, residence time (Waybrant et al., 2002) and the carbon source (Gibert, 2002; Waybrant et al., 1998).

### *1.3.1 Environmental Factors*

As with other biota, temperature plays an important role in the life of a bacterium. Sikora et al. (1996) found an increase in sulfide production in warmer waters, which they attributed to increased microbial activity. Similar temperature-induced fluxes were observed by Sobolewski (1999) and Gammons and Fraudsen (2001). However, Neculita et al. (2007) and Gusek (2004) did not observe seasonal changes, indicating a negligible impact on bacteria due to lower temperatures. Gusek (2004) documented a functioning bioreactor in Wyoming where temperatures were typically less than 5° C and as low as 0.5° C during the winter. While the winter treatment rate was approximately 80% of the summer rate, the bioreactor was still effectively treating the mine effluent. Low temperatures affect microbial activity, including decomposers and SRB; however, research indicates SRB are resilient to cold and freezing temperatures once they have become established in the reactor cell (Tsukamoto et al., 2004; Kuyucak et al., 2006).

As with all other life forms and chemical reactions, pH is extremely important to sulfate reduction. It has been reported that SRB require a near neutral pH range (approximately 5-8) to flourish (Dvorak et al., 1992; Willow & Cohen, 2003). However, sulfate reduction in water with pH below 3 has been documented (Bolis et al., 1991; Kolmert & Johnson, 2001). The ability to sustain sulfate reduction may be linked to the carbon source and bacterial consortium involved in the reaction. Tuttle et al. (1969) were able to attain sulfate reduction utilizing a mixed bacterial culture at pH 3.0, but pure SRB cultures were unable to function below pH 5.5. SRB may survive in pockets of the substrate with a neutral pH, while the bulk solution is much different (Skousen et al., 2000; Zagury et al., 2005). Some research also indicates SRB are capable of controlling their micro-environment, thus explaining their ability to survive in both cold and in acidic environments (Zagury et al., 2005).

### *1.3.2 Carbon Source*

In addition to temperature, other environmental conditions may have a significant impact on the efficacy of bacterial sulfate reduction (BSR). The quantity and quality of the organic matter appears to be a determining factor in the speed and efficacy of metal remediation. The source and characteristics of the organic matter have a significant impact on the removal efficiency of a sulfate-reducing system. Factors such as the particle size of the organic matter, the labile: recalcitrant C ratio, and the availability of N and P are perhaps the most important (Gibert et al., 2002; Waybrant et al., 2002). In addition, two moles of labile C are required to reduce one mole of $SO_4^{2-}$, thus the concentration of labile carbon is a determining factor in the effectiveness and lifespan of a BSR bioreactor (Dvorak et al., 1992). Currently, most bioreactors have had a lifespan of approximately 3-4 years (URS, 2003). The rate limiting step in BSR in marine sediments was determined to be the lack of appropriate organic substrates (Boudreau & Westrich, 1984). Similarly, Eastman & Ferguson (1981) determined the solubility of the solid organic substrate limited the SRB activity in anaerobic digestion. Thus, the problem becomes an issue of balancing labile carbon with more recalcitrant organic matter for longer operating life spans.

In a statistical analysis of eight different combinations of wood chips and leaf compost with three different amounts of poultry manure as the carbon source, sulfate reduction rates varied from 45.9 mg $L^{-1}$ $d^{-1}$ to 156.3 mg $L^{-1}$ $d^{-1}$ (Cocos et al., 2002). Waybrant et al. found similar results through several different experiments, with sulfate reduction rates ranging from 22.5-154.4 mg $L^{-1}$ $d^{-1}$ (1995), 37.0-194.2 mg $L^{-1}$ $d^{-1}$ (1998), and 41.2-116.1 mg $L^{-1}$ $d^{-1}$ (2002). The large variation in reduction rates indicates minor differences in the substrate will have a significant long-term impact on bioreactor efficiency. Several studies have shown the highest sulfate reduction rates in the mixtures containing the widest variety of organic C sources (Waybrant et al., 1995; Waybrant et al., 1998; Cocos et al., 2002). Presumably, this is due to the widely variant types of carbon and the differences in lability between the carbon sources. Sulfur reducing bacteria utilize low molecular weight compounds such as acetate and methanol (Dvorak et al., 1992) however, use of these compounds as the primary C source requires specific SRB species (Nagpal et al., 2000). If a multitude of C sources are combined, the decomposition products allow for a greater variability of SRB species.

In addition, bioreactors using easily degraded carbon substances will generally require more frequent replenishment, and thus a higher operating cost. If more complex carbon compounds are used, such as manure or compost, the lifespan of the substrate will increase. However, SRB are not capable of decomposing large or recalcitrant carbon compounds into the optimum compounds, thus a consortium of bacteria are required (Drury, 2000; Neculita et al., 2007). In general, the substrates will have all the requisite bacteria to create a bioreactor, but an acclimation period is often necessary, ranging from a period of several days to several weeks (Waybrant et al., 1995; Cocos et al., 2002; URS, 2003).

### *1.3.3 Initial Sulfate Concentration*

In the majority of acid mine drainage situations sulfate concentrations are extremely high, typically greater than 1000 mg $L^{-1}$ (Neculita et al., 2007). Consequently, sufficient sulfate concentration to support SRB is typically not a concern. Above a concentration of approximately 10 mM (300 mg $L^{-1}$) the sulfate reduction rate is independent of concentration (Boudreau & Westrich, 1984; Wellsbury et al., 1996). The sulfate reduction rate increased rapidly from 0-5 mM and then completely plateaus before concentrations reached 15 mM (Boudreau & Westrich, 1984). When sulfate concentration is not the rate limiting step, the lack of oxidizable organic carbon generally becomes the determining factor (Westrich, 1983; Boudreau & Westrich, 1984; Drury, 2000).

## 1.4 Manganese Removal

Chemically, Mn is very similar to iron and is found in oxidation states from +1 to +7, although +2, +3, and +4 are the most common oxidation states. Manganese (II) is the most prevalent and most stable oxidation state. The most widespread

Mn compounds are pyrolusite ($MnO_2$) and rhodochrosite ($MnCO_3$). Unlike some other metals, Mn is not extremely toxic, though it has been found to cause nervous system damage in high concentrations. The most common exposure pathway is occupational inhalation, though Mn in bath water may be a significant problem among the general population. While Mn is toxic in extremely high concentrations over a period of time, it is also an important micronutrient in many enzymes and mitochondria for all living organisms (USEPA, 2004; WHO, 2004a). Similarly to Fe, Mn may also oxidize and form a flocculent or coating in stream channels, covering important locations for macroinvertebrate survival. In addition, Mn will coat plumbing fixtures and piping and causes taste and odor problems in high concentrations.

Manganese pollution in water has been widely studied. Manganese is a common earth mineral associated with acid mine drainage and other disturbed environments. Like most other minerals, Mn is rarely found in pure form. Rather, Mn is typically a trace mineral found in association with iron, copper, nickel, and zinc. In theory, a treatment system designed to remediate high concentrations of any of these other trace metals would be effective for Mn remediation. However, Mn has a much higher solubility product than most other metal complexes. Another confounding factor is the relationship between Fe and Mn. In the presence of oxidized Mn, ferrous iron ($Fe^{2+}$) will oxidize to ferric iron ($Fe^{3+}$), with Mn as the electron acceptor ($Mn^{4+} + 2Fe^{2+} \rightarrow Mn^{2+} + 2Fe^{3+}$). Thus, any Mn complexes in contact with the solution will re-solubilize and re-introduce the Mn into solution. As a result of the interaction between iron and manganese, virtually all iron ($< 5$ mg $L^{-1}$) must be removed from solution prior to Mn removal (Karathanasis & Barton, 1997; Sikora et al., 2000). In addition, Mn precipitates will re-solubilize if $Fe^{2+}$ concentrations spike (Stumm & Morgan, 1996; Karathanasis & Barton, 1997).

In many of the hollow fill sites in Eastern Kentucky, the drainage flowing through the fill exits the fill at the toe and is rapidly oxidized. Consequently, the ferrous iron oxidizes and forms yellow-boy in the stream channel. While aesthetically unattractive and ecologically harmful, yellow-boy formation removes many of the trace metals from solution through precipitation and the subsequent increase in pH. At the Guy Cove hollow fill, Mn and sulfate are the primary contaminants once the iron is removed from solution. A promising method for Mn removal from coal mine effluents is the biochemical reduction of soluble sulfates to sulfides, with a subsequent removal of manganese by MnS precipitation.

Metal sulfides are fairly insoluble and stable (Stumm & Morgan, 1996). However, there are several coinciding mechanisms occurring in a fully functioning treatment system, which are difficult to separate, including metal sulfide precipitation, hydroxide and carbonate formation, simple sorption and physical filtration (Wildeman and Updegraff, 1997; Neculita et al., 2007). In addition, removal mechanisms are not consistently the same. At the beginning, sorption seems to predominate until sorption sites are filled (Machemer & Wildeman, 1992; Gibert et al., 2005). As the system matures and microbial populations flourish in the system, metal-sulfide precipitation ideally begins to predominate.

Several researchers have shown an acclimation period in bioreactors, ranging from zero to 21 days (Waybrant et al., 1995; Cocos et al., 2002). Most experiences have shown stabilization periods of less than 10 days. However, even after the bacteria have stabilized and bacterial sulfate reduction (BSR) begins to occur, the primary removal mechanism may not be metal sulfide precipitation. Sikora et al. (1996) saw a decreasing Mn removal rate over a period of several months. Within the first 100 days, anaerobic wetland cells removed approximately 75% of the influent Mn; however, by day 250, the removal rate had decreased to approximately 35%.

Wildeman et al. (1993) observed a similar trend, with 50% removal efficiency during the first three months in a treatment wetland. However, from 6-24 months the overall Mn removal rate decreased to 10%. Stark et al. (1995) detected similar results with Mn removal slowly decreasing over the life of the experiment. One plausible explanation for the decreasing trend in removal efficiency is the filling of the available sorption sites. Manganese may complex with organic matter or react with carbonates in solution to form rhodochrosite ($MnCO_3$). Once the available ligands are depleted, Mn removal may be controlled by sulfide precipitation, which may be a much slower reaction than sorption if BSR conditions are not satisfactory.

Once sulfide generation becomes the controlling factor, Mn may readily be removed from solution if concentrations are high enough. However, the majority of Mn will pass through most treatment systems due to the high solubility of MnS (Costello, 2003; URS, 2003). As a result, Mn treated through an anaerobic wetland or bioreactor will generally exceed regulatory limits (Sikora, 1996). Machemer and Wildman (1992) utilized sulfate reduction to treat mine drainage with high concentrations of Mn, Fe, Cu, Zn, and $SO_4^{2-}$. There was a clear relationship between metal and $SO_4^{2-}$ concentrations over time, suggesting sulfide precipitation can be a significant metal removal mechanism. However, of the four metals tested, Mn-sulfides were the least likely precipitates, as indicated by the $K_{sp}$ values.

A potential problem with long term passive treatments is the possibility of the treatment becoming a source of contamination, rather than a sink (Watzlaf, 1988) as with the Jones Branch wetland discussed in Barton and Karathanasis (1999). In order to determine the likelihood of leaching from a wetland substrate, Knox et al. (2006) used sequential extraction to determine the propensity of Mn to desorb from the substrate. Of the five metals analyzed (Cu, Pb, Zn, Fe, and Mn), Mn was the most easily desorbed ion in the floc, organic, and inorganic layers of the wetland.

### *1.5 Manganese Precipitation Chemistry*

Manganese chemistry is often difficult to experimentally understand. Due to the interactions with iron and other metals commonly found in solution, a pure Mn system will react differently than a more typical solution. There are several dominant Mn precipitates in nature. Under typical atmospheric conditions, Mn predominates as an oxide/hydroxide or a carbonate (Morgan, 2000). Of the many metals generally found in mine drainage environments, Mn is among the most soluble of the precipitates, as shown in Table 1.1 (McBride, 1994). In addition, manganese and iron are closely related. If Fe concentrations are above approximately 2 mg $L^{-1}$, Mn will not precipitate and Mn-precipitates are re-solubilized if iron is introduced into the solution; consequently, Mn is often found co-precipitated with or on Fe-hydroxides (Evangelou, 1998).

The most common Mn minerals are the manganese oxides such as $MnO_2$ (pyrolusite), $Mn_2O_3$, and MnOOH. In addition, Mn is contained in several mineral structures such as rhodochrosite ($MnCO_3$), todorokite ($(Mn,Mg,Ca,Ba,K,Na)_2Mn_3O_{12}\cdot 3H_2O$), and birnessite ($(Na,Ca,K)(Mg,Mn)Mn_6O_{14}.5H_2O$). Each of the above minerals is extremely stable and generally has a Mn oxidation state of 3-4. One of the Mn minerals is capable of precipitating in virtually every environment on Earth, either auto catalyzing if pH and redox conditions are appropriate, or through the interaction of microbial species (Schwertmann & Fitzpatrick, 1992; Morgan, 2000). Contrary to most metals, including Fe, Mn requires a pH above 8.0 to precipitate, which is one reason historic methods of treating AMD is difficult (Ghiorse & Ehrlich, 1992)

Fairly little is understood about Mn sulfides, as they require very specific redox and chemical characteristics to exist. Mn-sulfides (hereafter, MnS and $MnS_2$ will not be differentiated) typically require strongly reducing conditions of Eh less than -400 mV and a pH between approximately 9 and 12. The majority of the stability diagrams available do not show MnS because of this relatively small region of stability. However, the stability of these species is not well understood because of poor crystallinity and the potential for multiple interactions between in-situ chemical species and even microorganisms which catalyze Mn reduction and oxidation (Fox, 1988; Ghiorse & Ehrlich, 1992). MnS are most commonly found in deep oceanic regions. A profile of Mn species in the Black Sea by Landing and Lewis determined MnS did not begin to occur until a depth of approximately 150 meters (Morgan, 2000). At that depth, the water is extremely anoxic and sulfate concentrations are sufficiently high to predominate as ligands. As total sulfur concentrations increase from less than 10 μM to greater than 250 μM, sulfides are able to complex with dissolved Mn, which concentrations also increase with depth (Morgan, 2000).

### *1.6 Health Effects*

While Mn is not as toxic as many of the other metals commonly found in mine drainage, there is still several chronic health risks associated with Mn. The national median Mn concentration in drinking water is 10 μg $L^{-1}$ and in river water, the range is 11-51 μg $L^{-1}$ (USEPA, 2004). The exact effects and the required dosage for noticeable impacts vary based on the method of intake, the form of the Mn, and the age and nutritional status of the person; however, effects are primarily neurological (USEPA, 2004). The primary route of Mn ingestion is typically inhalation from occupational exposure; consequently, the majority of the research available focuses on inhalation effects and there is relatively little research connecting Mn oral exposure to health effects in humans (USEPA, 2004).

One of the sources of the toxicity appears to be the inhibition of Fe absorption (Keen et al., 2000). Several studies cited by the EPA resulted in no fatalities of mice and rats at very high ingestion levels of up to 2,250 mg $kg^{-1}$ $d^{-1}$ (2004). The most common effects determined in the studies cited by EPA on mice, rats, cattle, pigs, and rabbits are generally muscular weakness, loss of weight, and alterations in the gastro-intestinal organs, such as the liver and stomach (2004). In addition, a decrease in hemoglobin levels and alterations in brain neurochemistry were reported by some researchers (USEPA, 2004). Mn has also been shown to be toxic to some plant species (Suresh et al., 1987) and the most important consideration may be the potential to oxidize more toxic metals such as arsenic (Dixon & Skinner, 1992)

There is a wide range of natural sulfate concentrations in surface and ground waters used for public consumption. In an analysis of water supply systems by the EPA (2002), 98% of all samples contained $SO_4^{2-}$, with concentrations ranging from less than one to 770 mg $L^{-1}$. However, only 3% of the supplies exceeded the recommended maximum of 250 mg $L^{-1}$. Sulfate ingestion has been found to cause temporary illnesses, including diarrhea and catharsis (WHO, 2004b). Dehydration is also possible as a side-effect of ingestion of excessively high concentrations of sulfate. However, at this time, sulfate is not considered a carcinogen or to have other adverse long-term health impacts (WHO, 2004b; USEPA, 2006). The U.S. drinking water standard of 250 mg $L^{-1}$ is a drinking water advisory only and is not health-based. It has been determined that 250 mg $L^{-1}$ is the approximate threshold of taste and smell detection in the average population (USEPA, 2006).

### *1.7 Site History*

The E.O. Robinson Forest in Breathitt, Knott, and Perry counties in Eastern Kentucky is the University of Kentucky’s research forest site. It is comprised of approximately 15,000 acres, primarily in one large tract with several outlying parcels (Fig. 1.1). The forest is a prime example of the mixed mesophytic forest native to much of eastern North America. Mixed mesophytic forests are one of the most biologically diverse ecosystems in the world. During the past 200 years, over 95% of this ecosystem has been heavily degraded or destroyed by development. The main block of the forest, comprising approximately 10,000 acres, is one of the largest contiguous blocks of property in Eastern Kentucky undisturbed by mining.

In 1912, E.O. Robinson and F.W. Mowbray purchased the land with the intent of logging and potentially mining the property (Blanton et al., 2003). By 1922, the property had been completely logged and in October 1923, Robinson deeded the surface rights to the property to the University with the intent the area be used to improve the quality of life of the Appalachian people and for research and educational purposes. One of the University's major concerns with taking ownership of the property was the lack of control over the mineral rights. In 1925, Robinson and Mowbray sold the coal rights on credit. The buyer proceeded to default on the loan, returning the rights to Robinson and Mowbray. In 1928, Robinson became the sole owner of the mineral rights to the property. By late 1930, Robinson conceded the attempt to sell the coal rights and donated the rights to the University. Though Robinson was unable to find an interested party for the coal rights to the property, he was more fortunate with the oil and gas rights. To this day, the University does not own the oil and gas rights to any of the 15,000 acres.

Since the University first acquired Robinson Forest in 1923, millions of dollars of improvements and research funding has been applied to the property. According to a report to the University Board of Trustees in 2003, approximately 700 forestry and natural resource personnel are trained annually, impacting more than 660,000 acres of forestland in Kentucky every year. This results in more $26 million of benefits to landowners and industry within the state. In addition, the main block of the forest contains two of the most pristine watersheds in the state. The forest was allowed to regenerate following the acquisition of the property and has been monitored closely over the past 80 years. Several forest inventories have been completed in that time and hydrology has been monitored on eight watersheds in the forest since 1972, which represents one of the longest continuous monitoring projects in the country.

The University Board of Trustees has consistently faced pressure to use mining as a source of revenue. Until approximately 1985, the Board had declined to engage in mining. However, in the late 1980s a coal company began pursuing mining on two parcels adjacent to Robinson Forest. In an attempt to block mining in the area, a large group of activists and organizations applied to the state of Kentucky for a “land unsuitable to mine” designation for all of the Forest. In 1991, the State ruled the main block of the Forest was unsuitable; however, declined to apply the same designation to adjacent areas and the outlying parcels. At the same time, the Board of Trustees initiated mining on some of the outlying parcels, specifically, Fishtrap Branch, Little Caney Creek, Hurricane Fork, Beaver Dam Creek, Rose Branch, and Laurel Fork. The mining company still maintains the leases for Beaver Dam Creek and Rose Branch as these two areas have not yet been mined. Reclamation of the mined sections is proceeding.

This research is based on the effluent from a valley in the headwaters of the Laurel Fork known as Guy Cove (Fig. 1.1). Prior to mining, the valley was approximately 105 acres in size and was the location of a perennial stream with several thousand feet of ephemeral and intermittent stream channels. As with the rest of Robinson Forest, the valley was logged in the early 20th century. Between the logging and the mining approximately 80 years later, the valley was undisturbed and left to regenerate. As part of the mining process, approximately 2.4 million cubic yards of material were placed in the valley. The drainage currently is funneled off the site through side drains and an underdrain. As part of the reclamation process, approximately one mile of stream channels will be re-created, and the bioreactor will be used to treat drainage seeping through the material and draining through the toe.

### *1.8 Research Objectives*

The overall objective of this research was to optimize removal of Mn through a sulfate bioreactor. Several matrices were tested in batch experiments (Phase I) to determine the combination most likely to enhance sulfate reduction and Mn removal. Each combination consisted of an inorganic substrate and an organic amendment.  Subsequently, the most effective treatment combinations were tested in a more dynamic system representative of what would be found in a field environment using small scale bioreactors (Phase II).  Both Phase I and Phase II used a synthetic mine drainage to minimize interferences and develop an understanding of the maximum potential Mn and $SO_4^{2-}$ removal capacity. Recommendations based on the results of Phases I and II will be implemented in a full scale bioreactor in Guy Cove to test the capacity of the most effective matrix in a field environment.

Table 1.1 Solubility products of common metal compounds. All values are the negative log of the solubility product (-log $K_{so}$), thus higher values indicate lower solubility. Data derived from McBride, 1994.

**Carbonates**

| Pb | Cd | Fe | Mn | Zn | Ca |
|---|---|---|---|---|---|
| 13.1 | 11.7 | 10.7 | 10.4 | 10.2 | 8.42 |

$K_{so} = (M^{2+})(CO_3^{2-})$

**Oxides and Hydroxides**

| $Fe^{3+}$ | $Al^{3+}$ | $Hg^{2+}$ | $Cu^{2+}$ | $Zn^{2+}$ | $Pb^{2+}$ | $Fe^{2+}$ | $Cd^{2+}$ | $Mn^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|---|---|
| 39 | 31.2 | 25.4 | 20.3 | 16.9 | 15.3 | 15.2 | 14.4 | 12.8 | 11.2 |

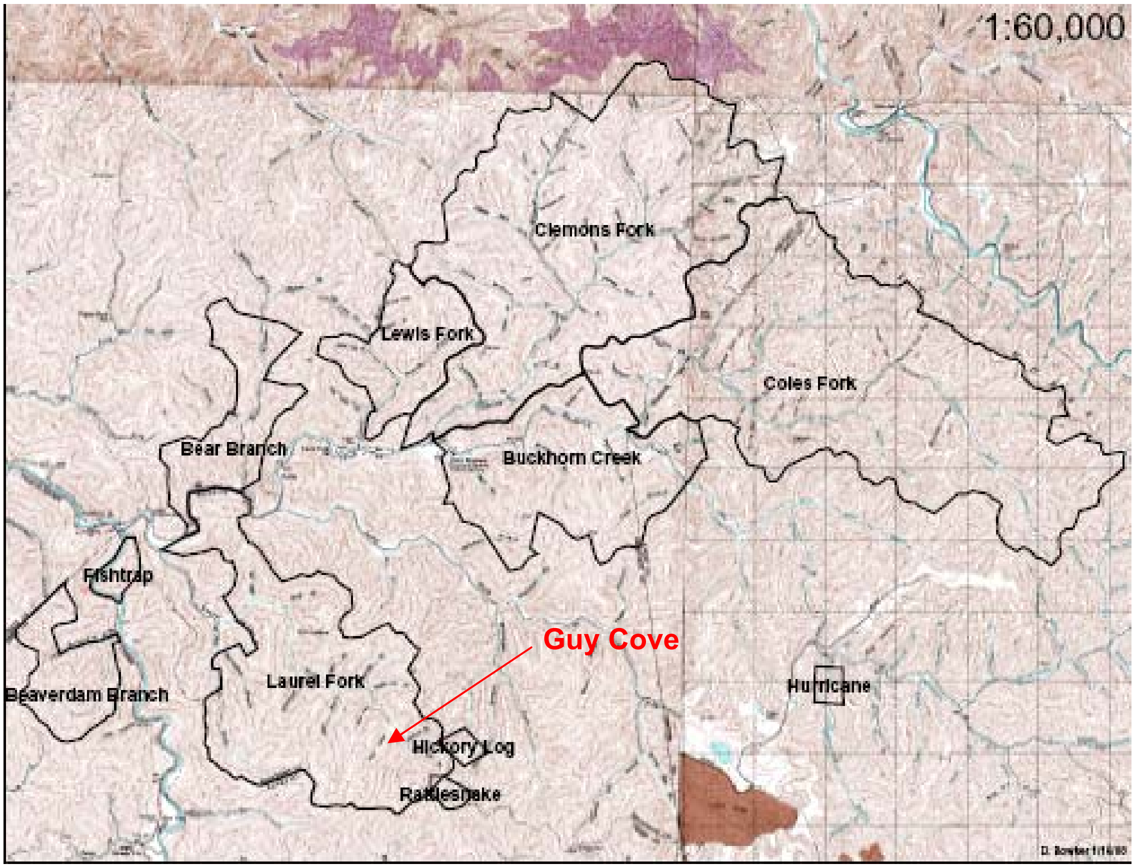
$K_{so} = (Mn^{+})(OH^{-})_n$

**Sulfides**

| Hg | Cu | Pb | Cd | Zn | Fe | Mn |
|---|---|---|---|---|---|---|
| 52.1 | 36.1 | 27.5 | 27 | 24.7 | 18.1 | 13.5 |

$K_{so} = (M^{2+})(S^{2-})$

Figure 1.1 A map showing the location and extent of Robinson Forest. Outlined areas are forest property and are labeled by the watershed name. The arrow indicates the location of Guy Cove.



United States Geological Survey 1977 Topographic Map, Noble Quadrangle

# CHAPTER 2

# Pilot Scale Batch Experiments for Removal of Manganese and Sulfate from a Near-Alkaline Mine Drainage in Eastern Kentucky

## *2.1 Background*

Coal has been a part of Kentucky's economy for more than 200 years; however, until the twentieth century, Western Kentucky was the source of the majority of the coal mined in Kentucky due to topographic limitations in the Appalachian areas of Eastern Kentucky (Carey et al., 2001). Beginning around 1970, the surface mining method known as mountaintop removal and valley fill rapidly expanded in eastern Kentucky. Eastern Kentucky now accounts for 62% of the coal produced in the state (KYOMSL, 2005).

Using the mountaintop removal and valley fill method, the soil and rock above the coal seams (overburden) is removed and placed in adjacent valleys. Generally, a significant portion of the overburden consists of sulfide bearing minerals, such as iron sulfides and manganese sulfides. When the overburden is exposed to surface weathering a rapid solubilization of the materials result in acidic, highly contaminated water, called acid mine drainage (USEPA, 2002; Pond, 2004). In some places of the world, including much of Eastern Kentucky, the native geology has substantial buffering capacity, typically derived from carbonates, capable of moderating the impacts of acidic drainage. Buffering of the acidity results in a natural removal of the majority of the soluble metals through precipitation due to a neutral to alkaline solution. However, Mn has a much higher solubility than other metals resulting in solutions with high concentrations of Mn and sulfate, which is not impacted by pH. Unless sulfate concentrations in the mine drainage are reduced to sulfide, Mn removal is very difficult. Therefore, sulfate reduction is a viable first step approach to treat mine drainage high in Mn. Research utilizing sulfate reduction to encourage metal and sulfate removal has found widely varying efficiencies. Waybrant et al. (1998) attained greater than 99% $SO_4^{2-}$ removal, while Champagne et al. (2005) showed 73% removal of sulfates. Chockalingam and Subramanian (2006) compared the differences between real mine drainage and synthetic drainage and found $SO_4^{2-}$ removal rates of 40% and 73%, respectively. Several field based experiments in either permeable reactive barriers or constructed wetlands reached sulfate removal efficiencies approximating 70% (McIntire & Edenborn, 1990; Benner et al., 1999; Sass et al., 2001).

As evidenced by the variance in removal efficiencies, there are several variables impacting the efficacy of sulfate-reducing treatments including the initial $SO_4^{2-}$ concentration, temperature, residence time (Waybrant et al., 2002) and the carbon source (Gibert et al., 2002). The source and characteristics of the organic matter have a significant impact on the removal efficiency of a sulfate-reducing system. Factors such as the particle size of the organic matter, the labile: recalcitrant C ratio, and the availability of N and P are perhaps the most important (Waybrant et al., 2002; Gibert et al., 2002). The theoretical metal removal mechanism in a bioreactor is metal sulfide precipitation. Metal sulfides are fairly insoluble and stable (Stumm & Morgan, 1996). However, there are several coinciding mechanisms occurring in a fully functioning treatment system, which are difficult to separate, including metal sulfide precipitation, hydroxide and carbonate formation, simple sorption and physical filtration (Wildeman and Updegraff, 1997; Neculita et al., 2007). In addition, removal mechanisms may change over time. At the beginning, sorption seems to predominate until sorption sites are saturated (Machemer & Wildeman, 1992; Gibert et al., 2005). As the system matures and microbial populations flourish in the system, metal sulfide precipitation begins to predominate.

Of the many metals and metalloids commonly found in AMD, Mn is one of the more difficult to remove due to the complexity of the interactions governing Mn solubility. Manganese precipitation is inhibited if the Fe: Mn ratio is too high

(Karathanasis & Barton, 1997) and Mn precipitates will dissolve if $Fe^{2+}$ concentrations are too high (Stumm & Morgan, 1996; Evangelou, 1998). In addition, most other metals preferentially complex with sulfides before Mn (Stumm & Morgan, 1996). Research has shown widely variant success in Mn removal. Tabak et al. (2003) successfully removed 95% of the Mn load from AMD, while Machemer and Wildeman (1992) were only able to see a temporary decrease in Mn concentrations. Machemer and Wildman also observed a relationship between metal and $SO_4^{2-}$ concentrations over time, suggesting that following the saturation threshold stage, sulfide precipitation can be a significant metal removal mechanism. However, of the four metals tested (Mn, Zn, Cu, Fe), Mn-sulfides were the least likely precipitates, as indicated by the $K_{sp}$ values.

Our first objective in this research was to develop a system that maximized long term Mn removal by utilizing the existing sulfate pool, triggered by the addition of an organic material to induce sulfate reduction. To do so, we tested various combinations of organic materials and mineral substrates to encourage sulfate reduction. The initial phase consisted of batch experiments conducted in the laboratory. A simulated mine drainage solution containing Mn and sulfate at levels similar to those observed at Guy Cove was added to each of the treatment combinations and allowed to mix and equilibrate for a period of 21 days.

## *2.2 Materials & Methods*

### *2.2.1 Research Materials*

Five organic amendments were collected from different sources including corn mash from a bourbon distillery, soybean oil, wood mulch, sorghum syrup, and biosolids. Similarly, five inorganic substrates were used in the experiment including limestone, marble, creek sediment from a reference stream near the project area, polished river gravel, and sand. One of the determinate factors for choosing the substrates and amendments was the availability and cost. Ideally, the treatment material is an inexpensive or free material available in large quantities on demand. Combining the substrates and the amendments together created the different treatments for use in the batch experiments. There were a total of 30 treatments and each treatment was duplicated. Each amendment was mixed with each substrate, each substrate was tested independently of any amendment, and a control treatment, consisting of no substrate or amendment was tested. For example, the following combinations were tested: creek sediment & biosolid, creek sediment & corn mash, creek sediment & mulch, creek sediment & soybean oil, creek sediment & sorghum syrup, and creek sediment without an amendment. The same pattern was replicated for each substrate.

The limestone was collected from a commercial quarry and had an average diameter of approximately one cm. The marble and the river gravel were purchased from a retail garden store and were intended for use as decorative stone. The gravel was highly polished and ranged in diameter from less than 5 mm to 3 cm in diameter. The marble was rough hewn material with an average diameter of approximately two centimeters. The sand was also purchased from a retail garden store and was intended for use in a child's sandbox. Each of the previous substrates was washed using a number 10 (1 mm) sieve to remove fine size particles. The creek sediment was collected from four stream beds in Robinson Forest, which are reference reach streams for the State of Kentucky due to their cleanliness and biotic and overall integrity. The sample collected comprised the top several centimeters of material from a section of each stream, and thus was a mixture of gravel (> 2 mm), sand and silt (2 – 0.002 mm), fine size particles (< 0.002 mm), and organic matter. Unlike the other substrates, the creek sediment was not sieved because of the desire to maintain the overall integrity of the matrix. In addition, the microbial population is generally attached to the smaller size fraction material and would thus be excluded from the analysis. After the samples were collected from each stream, they were combined and mixed to ensure homogeneity during the experiment and stored in sealed, zero head-space plastic bottles at 4° C until they were used.

Like several of the substrates, the sorghum syrup and the soybean oil are available from a retail grocery store. The biosolids were collected from the Winchester, Kentucky wastewater treatment plant. The biosolids were then dried, ground, and sieved through a Number 10 (1 mm) sieve to ensure homogeneity. The wood mulch was obtained from one of the University of Kentucky's research farms and consisted primarily of the woody waste material on the farm. The material was chopped and stored on-site until it was needed for farm use. After the mulch was collected, it was also dried, ground, and sieved through a Number 10 (1 mm) sieve. The corn mash was obtained from a local bourbon distillery and is waste material from the distilling process. The corn mash was dried and weighed to obtain moisture content and added to the flasks as a wet material on a dried mass basis. The mash was assumed to be homogenous due to the distilling process, which requires significant mixing, and thus was not dried as the other amendments were.

Rather than using the actual mine drainage solution, a synthetic solution was mixed to simplify the chemistry and minimize interferences. While the average Mn and sulfate concentrations in the mine effluent are approximately 30 and 1,300 mg $L^{-1}$, respectively, the maximum observed concentration has ranged significantly beyond that concentration (Tables 2.1-2.2). Consequently, the synthetic mine drainage was mixed to a final concentration of approximately 90 mg $L^{-1}$ Mn and 1,500 mg $L^{-1}$ $SO_4^{2-}$. Reagent grade manganese sulfate ($MnSO_4$) and calcium sulfate dihydrate ($CaSO_4\ 2H_2O$) were used as the $Mn^{2+}$ and $SO_4^{2-}$ sources for the synthetic drainage solution (Fisher Scientific). The mixture was tested in a batch experiment using an acid-washed glass 1 L suction flask (Fig. 2.1). The substrates and amendments were mixed in each flask on a 10:1 weight to weight ratio. The amendments were added on an oven-dried mass basis, except for the oils. The density ratio of the oils was used to determine the requisite masses of each. The mine drainage was added on an equivalent mass ratio to the substrate. For example, if 200 g of substrate were added to the batch, 200 mL of solution were also added.

In order to enforce and maintain an anaerobic environment in the flasks, purified nitrogen gas was flushed through the system continuously at a rate of approximately 0.7 cubic feet per hour. The nitrogen gas was supplied by a commercial supplier. Also, a gas trap was installed on each flask and filled with sodium thiosulfate to allow the nitrogen gas to leave the flask without allowing oxygen to enter the system.

### *2.2.2 Material Analysis and Preparation*

To gain an understanding of the materials used in the experiments, several common parameters were tested for each organic amendment and each inorganic substrate. Data collected included cation exchange capacity, water saturated pH, total nitrogen and phosphorus for the amendments, and particle size analysis for the creek sediment. To determine cation exchange capacity, 25 mL of ammonium acetate ($NH_3C_2H_30_2$) was added to 10 grams of sample and allowed to stabilize for a period of 24 hours. After that period, the sample was filtered through a Millipore filter system with a Whatman No. 42 filter fabric. As the solution was filtered through the sample, ammonium acetate was added in 20 mL increments until approximately 90 mL had been filtered. The effluent was poured into a 100 mL volumetric flask and filled to volume with ammonium acetate. The samples were discarded and the effluent was analyzed via atomic absorption analysis for magnesium, calcium, potassium, and sodium concentrations. The concentrations were then converted to millequivalents and summed to determine the cation exchange capacity. Total nitrogen and phosphorus were analyzed via the Kjeldahl digestion method using 0.1 grams of duplicate samples. For the particle size analysis of the creek sediment, a representative sample was dried in the oven at 55° C. The sample was then sieved through a series of sieves for a period of ten minutes. The mass of the material retained on each sieve was weighed and a cumulative percent was calculated. The pH for each substrate and amendment was measured in water. A sample was saturated with deionized water and allowed to equilibrate for one hour before each measurement.

### *2.2.3 Sample Collection & Analysis*

A sample was extracted from the flask for analysis each day for three weeks (21 days) or until the major constituents reached a stable concentration. For each sampling period, the redox potential (Eh), the pH, the Mn and sulfate concentrations were determined. Redox potential and pH were measured using a Fisher Scientific Accumet AP62 pH/mV meter immediately after sample collection. The pH was measured using an Accumet pH probe and the redox was measured using a silver/silver chloride Mettler Toledo InLab Redox Probe and values adjusted to reflect a hydrogen reference electrode. Several milliliters (2-4) were pipetted from each flask and filtered through a 0.45 μm membrane filter. Each sample was then preserved in an equivalent amount of two normal (2 N) hydrochloric acid to create several milliliters of one normal sample. Samples were stored for a maximum of seven days prior to analysis. Each sample was diluted as necessary and then analyzed. Each sample was analyzed for Mn and $SO_4$-S concentrations. Manganese concentrations were tested using a Solaar M5 ThermoElemental Atomic Absorption Spectrometer. Sulfate-sulfur concentrations were analyzed by a variation of APHA Method 4500-$SO_4^{2-}$ E (APHA, 1998). The method was adapted for use on a Molecular Devices Versamay Tunable Plate Reader at 450 nm. The samples for sulfate analysis were diluted as necessary using one normal (1 N) hydrochloric acid. A sample volume of 130 μL was placed in a sterile plate with an equivalent amount of a barium chloride and carboxy-methyl cellulose reagent. The samples were stirred for 45 minutes and then analyzed on the Plate Reader at 450 nm. The Mn samples were diluted and analyzed via atomic absorption.

Due to the highly acidic nature of the samples, they were each neutralized using sodium hydroxide prior to disposal. The treatment mixtures were collected and disposed of by approved University of Kentucky hazardous waste protocols. Everything used in the testing procedure was then washed and acid washed prior to reuse.

### *2.2.4 Mineralogical Analysis*

Following the completion of each batch experiment, the matrices were tested for mineralogical differences. Each treatment was dried in an oven at 55° C until the solution was completely evaporated. The matrices were then sieved through a number 100 sieve (150 µm) to maximize particle size homogeneity. The matrices were tested for mineralogy by thermogravimetric analysis and X-ray diffraction. X-ray diffraction was completed using a Philips PW 1729 X-ray generator and a Philips PW 1840 diffractometer. The x-ray source is cobalt, with a wavelength of 0.179 nm. Samples were analyzed from 0-60° 2θ. Thermogravimetric analysis was completed using a DuPont Instruments 951 Thermogravimetric Analyzer. Approximately 10 mg of soil was used in this analysis. The sample was scraped off of the Mg-saturated slide into a platinum dish and placed into the instrument and heated from 25° C to 900° C and the percent weight loss was recorded. Nitrogen gas was used to flush the sample container to prevent oxidation of mineral elements.

### *2.2.5 Statistical Analysis*

The statistical analysis of the data was completed using the SAS program, Version 9.1. The sulfate and Mn concentrations and the redox status were analyzed to determine differences. The majority of the treatments are not directly comparable because neither the substrates nor the amendments are the same. For example, the creek sediment-biosolid treatment is not comparable to the sand-mulch treatment. Consequently, the analysis was based on each substrate and each amendment. The “PROC MIXED” procedure with the LS-Means statement was used for the analysis based on the nature of the data and the correlation between each sampling point, or day, with those coming before and after it. The PROC MIXED procedure does not control Type-II error, thus due to the large number of comparisons for each data set, the maximum probability allowed to indicate significant differences is 0.001 ($\alpha$=0.001). The Satterthwaite approximation was utilized to calculate the degrees of freedom for the analysis.

## *2.3 Results & Discussion*

The final Mn and sulfate removal capacity of each treatment combination varied widely (Tables 2.3 and 2.4, respectively). The sulfate removal efficiency was influenced significantly by the redox potential in each system, as sulfate reducing bacteria generally need a strongly reducing environment (< -200 mV) to function well. The most effective treatments generally were subject to the most reducing conditions. Mn removal was impacted by the redox status of the systems due to the potential removal mechanism of Mn-sulfide precipitation; however, pH was also a determining factor in Mn removal. Above a pH of approximately 8, Mn will auto-oxidize and precipitate as a Mn-oxyhydroxide, which is one explanation for the efficacy of the biosolid treatments. The mean redox potential and the mean pH of each treatment are presented in Tables 2.5 and 2.6, respectively.

### *2.3.1 Material Characteristics*

Each of the substrates and amendments were tested for several basic characteristics, as summarized in Table 2.7. The biosolids are lime-stabilized and thus are strongly alkaline. The corn mash and the creek sediment were slightly acidic and the limestone was slightly alkaline, while the sand, marble, and mulch were moderately basic. River gravel was the single substrate or amendment which was strongly acidic, with a pH of 4.8. The three solid matrixes are similar in phosphorus content, ranging from a low of 0.76% to a maximum of 0.93%, and in nitrogen content, though the corn mash has more than twice the nitrogen concentration than either the wood mulch or the biosolids. Both the sorghum syrup and the soybean oil had very low or no nitrogen and phosphorus, as both elements are removed during processing to increase the longevity of the oils (Z. Christensen, personal communication, February 3, 2008). The marble, sediment, and limestone have nearly equivalent cation exchange capacity, while the exchange capacity of the river gravel was negligible.

### *2.3.2 Manganese Removal*

The majority of the treatments caused a significant decrease in Mn concentration over the trial period (Table 2.8). The biosolid and wood mulch amendments showed the most potential to remove Mn, as all of the combinations involving either of those amendments achieved a significant removal rate. The treatments without the addition of an amendment also caused a significant decrease in Mn concentration over time, but in contrast, the soybean oil, sorghum syrup, and corn mash only caused a significant change in two of the five treatments each. All of the substrates except the river gravel caused a significant decrease in Mn concentration in four or five of the six treatment combinations. Thus, river gravel appears to be the least effective substrate and the mulch and biosolid treatments the most effective amendments. The remaining amendments and substrates have shown varying Mn removal efficiencies and an order of effectiveness is not clearly definable. Table 2.9 defines the statistical relationships between the treatments. The differences and potential reasons are explored further below.

#### *2.3.2.1 Removal by Amendment*

Each of the organic amendments had a distinctive capacity to remove Mn from solution. The most effective amendments were the biosolids and the wood mulch, while the least effective treatments were the sorghum syrup and the soybean oil. Table 2.3 summarizes the effective removal capacities of each of the treatments.

##### *2.3.2.1.1 Biosolids*

The biosolid treatments removed 100% of the Mn from solution (Fig. 2.2); however, the biosolids were lime-stabilized, with a resultant treatment pH always greater than 10 and generally higher than 11. The Eh-pH equilibrium diagram (Fig. 2.3) indicates that Mn will form oxy-hydroxides at high pH under oxidized conditions and carbonates and/or sulfides under reducing conditions. As a result of the high pH, the Mn was likely precipitated as a Mn-oxyhydroxide or as Mn carbonate rather than as the anticipated MnS. Mineralogical analysis of the biosolid treatments consistently indicated increased amounts of rhodochrosite ($MnCO_3$) and manganite ($MnO(OH)$). This conclusion is further supported by Table 2.10, which shows the percentage of the Mn that was removed from solution within the first day. Previous research has found it generally takes a period of several days to develop sulfate-reducing conditions (Waybrant et al., 1995; Cocos et al., 2002; URS, 2003). Consequently, the removal of the Mn within the first 24 hours most likely occurred through precipitation of oxy-hydroxides due to the high pH or formation of rhodochrosite as a result of the substantial concentrations of carbonates in the biosolids.

#### *2.3.2.1.2 Wood Mulch*

The wood mulch treatments were almost as effective as the biosolid treatments, removing 94-97% of the Mn from solution as shown in Fig. 2.2. However, as with the biosolid treatments, the majority of the Mn was removed within the first 24 hours indicating a removal mechanism other than sulfate reduction. The pH of the mulch treatments generally stabilized slightly above 8 (Table 2.6). While this is considerably lower than the pH of the biosolid treatments, it may be high enough to allow Mn oxy-hydroxides to precipitate at the relevant Eh range (Fig. 2.3). The creek sediment and mulch combination caused a slight increase in MnS and a noticeable increase in rhodochrosite; however, it was the only mulch treatment to show changes in mineralogy after the treatment period. A sizeable proportion of the Mn may be sorbed to the organic matter by simple sorption and ion exchange. The mulch was found to have the highest CEC of the amendments by a sizable margin, as shown in Table 2.7. Given a CEC of approximately 50 meq/100 g of material, the mulch amendment would have more than sufficient sorption capacity to bind the Mn in the synthetic test solution. This hypothesis indicates there may be a time when Mn break-through could occur due to the sorption capacity of the mulch being exceeded. However, at this stage of the experiment, Mn was added in a single dose, rather than in a continuous feed. Therefore, the next phase of the experiment further explored this possibility.

#### *2.3.2.1.3 Corn Mash*

Our hypothesis presumed that the corn mash would have been a very effective amendment for Mn removal. The corn mash had a high CEC (Table 2.7), though not as high as the mulch. In addition, the corn mash contained the highest levels of nitrogen of any of the amendments, presumably making it an appropriate substrate to supplement sulfate reduction. However, only two of the treatment combinations attained a significant decrease in Mn concentration (Table 2.8). This was unexpected, given that the river gravel-corn and marble-corn treatments realized removal efficiencies of 45.2% and 31.8%, respectively. One replicate of the river gravel and corn treatment removed nearly 75% of the Mn from solution, while the second replicate removed only 17%, resulting in high variability, with a standard deviation of approximately 40%. Presuming that there were problems with the second replicate, which is plausible given only 0.6% of the sulfate was removed in that replicate, this treatment may have some potential benefits. However, the creek sediment and corn treatment only achieved a treatment efficiency of approximately 10%, while the marble and corn treatment produced a decrease of 32%, suggesting the corn mash may not be the optimal organic source for a successful treatment amendment.

###### *2.3.2.1.4 Soybean Oil*

Research at the Savannah River Site, both in laboratory scale experiments and field scale installations found that soybean oil was an effective amendment to encourage sulfate reduction (Phifer et al., 2001). However, our findings indicated neither the sorghum syrup nor the soybean oil was consistently effective in reducing Mn concentrations as the final Mn removal capacities in Fig. 2.2 indicate. The highest Mn removal rate for the sorghum and soybean oil amendments was 25% and 37.5%, respectively. Nitrogen and phosphorus analysis of the amendments indicated both were extremely low in total N and P, which are essential for bacterial growth (Gibert et al., 2002; Waybrant et al., 2002). The research at the Savannah River Site found it necessary to add rock phosphate and commercial fertilizer to the aquifer to provide the requisite nutrients for the SRB to flourish (Phifer et al., 2001). Without either of these key nutrients, sulfate-reducing bacteria were unable to convert the sulfate to sulfide in significant quantities to allow the majority of the Mn to precipitate. In addition, the syrup and the oil have very low sorption capacity to bind Mn (Table 2.7). In our experiment, the decrease observed in the various treatments may have been derived from minor amounts of MnS precipitation, the sorption capacity of the substrates, or oxidation of the Mn to Mn-hydroxides. Autooxidation of the Mn is unlikely given the equilibrium status as shown in Fig. 2.3 (see Tables 2.3-2.4 for Eh and pH status); however, the Mn concentration in the experiment was approximately twice the maximum concentration shown in the diagram. Analysis of the chemical speciation by MinEQL+ indicated the species most likely to be present are $MnSO_{4(aq)}$ and $Mn^{2+}$. The concentrations of the Mn-hydroxides were minute. Regardless of the cause for moderate Mn removal in these systems, neither sorghum syrup nor soybean oil was as effective as the biosolids nor the mulch amendments to remove Mn from an alkaline mine drainage.

#### *2.3.2.2 Removal by Substrate*

Each substrate was tested as a control without the addition of any organic amendment. The most effective substrate used in the study was the creek sediment, which removed 79% of the Mn from solution, while the least effective substrate was the polished river gravel, with only 2.5% removal efficiency. Of the five substrates used in the experiment, river gravel was the least effective for each of the added organic amendments. Four of the six river gravel treatments did not show a significantly different Mn concentration after the treatment period. As discussed previously, every biosolid treatment and wood mulch treatment was significantly different, thus indicating the controlling factor in the river gravel batches was the organic amendment rather than the substrate. The Mn removal by the treatments without an organic amendment was correlated to the total CEC for each treatment, indicating a moderate relationship between the two parameters (Fig. 2.4). The correlation coefficient for the relationship is 0.53, with a probability level of 0.16. The quality of the relationship decreased ($r^2$=0.27) when Mn removal is compared to the CEC for all of the treatments due to the increased complexity of the reactions; however, the significance increased greatly to less than 0.01 (Fig. 2.5). Thus, the results indicate simple sorption is a substantial source of Mn removal in these systems; however, it fails to account for the entirety of the Mn removed indicating other removal mechanisms are occurring. The differences between the substrates are explored in greater detail below.

#### *2.3.2.2.1 River Gravel*

Originally intended for use as decorative stone in aquariums, river gravel was the most processed of the substrates, as the stone had been cleaned and polished prior to packaging. Subsequently, many of the fissures and crevices commonly found on the other substrates were not evident on the river gravel, which reduced some of the inherent benefits of a mineral substrate by minimizing attachment points for bacteria. This is also supported by the low cation exchange capacity of the material (Table 2.7). The marble chips, although lower in CEC, showed a better Mn removal efficiency than the river gravel most likely due to the presence of carbonates. In addition, river gravel was the only substrate or amendment to have a strongly acidic pH. The corn mash and the creek sediment were both slightly acidic, but the acidity of the gravel may have inhibited formation of Mn-hydroxides, which was a dominant removal mechanism in other treatments. In the first day, the river gravel had a negative removal rate (Table 2.10), most likely due to disequilibrium conditions.

#### *2.3.2.2.2 Limestone/Marble Chips*

Both the limestone and marble chips were washed and sieved prior to use as described previously, thus, the grain size was uniform. Unlike the river gravel, however, both the limestone and marble chips were not polished. Consequently, there were rough faces and sharp angles, as well as fissures and crevices in the stones which would be ideal for microbial colonization and ion sorption. The limestone and marble treatments without an organic amendment provided remarkably high Mn removal rates of 54% and 32%, respectively, possibly due to carbonate or hydroxide precipitation. Both the limestone and the marble chips were chosen for evaluation in this portion of the experiment because of the likelihood for minor dissolution, which could then allow for the formation of $MnCO_3$, or rhodochrosite formation. Mineralogical analysis indicated there was a minor increase in both rhodochrosite and manganite in the limestone treatments.

Evaluation of the potential ions in solution by MinEQL+ indicated the solution may be saturated with $MnCO_3$. The autocatalytic formation of Mn-hydroxides generally does not occur until the pH exceeds 8.0. Neither the marble or limestone treatments exceeded that level, with pH generally below 8.0. However, it is possible that the extremely high concentrations of Mn (1.8 mM) may have allowed some Mn-hydroxides to form.

#### *2.3.2.2.3 Sand*

The sand was the second most effective substrate following the creek sediment (Fig. 2.6). As with the marble and limestone chips, sand was comprised of relatively uniform particles with many rough faces and sharp edges, which make ideal attachment points for bacteria. In addition, the smaller particle size of the sand particles provided a significantly higher specific surface area for increased microbial attachment and ion exchange. The broken mineral edges of quartz may sorb ions, which could also account for some of the Mn removal. Of the substrates used in this research, the sand had the highest pH value (9.26). This pH may have contributed to the formation of Mn-hydroxides or rhodochrosite if traces of carbonates were present. The sand substrate also had much smaller pores than either the marble or limestone substrates due to the smaller particle size. Several researchers have noted the capacity of bacteria to adapt the environment around themselves to suit their individual needs (Skousen et al., 2000; Zagury et al., 2005); consequently, even if the bulk solution chemistry and environment is unsuitable for sulfate-reducing bacteria and MnS production, the small pores within the sand substrate could allow bacteria to more readily control the microenvironment than in the marble and limestone substrates. The mineralogical analysis of the matrices following the treatment indicated an increase in rhodochrosite and manganite in both the corn and sand and biosolid and sand combinations. The remaining sand combinations did not cause any change in mineralogy.

#### *2.3.2.2.4 Creek Sediment*

The creek sediment provided the largest potential for Mn removal of the five substrates. The sediment consisted of a mixture of gravel (> 2 mm), sand and silt (2 – 0.002 mm.), fine size particles (< 0.002 mm.), and organic matter. The matrix of the inorganic portion of the sediment consisted of limestone, shale, and sandstone materials. As a result, the sediment possessed many of the properties of the limestone substrate, such as the abundance of carbonates and the inherently high buffering capacity. The combination of the autochthonous organic matter and the large specific surface area of the sediments also resulted in an ideal environment to support microbial communities. The other four substrates used in this research were all sieved to remove fine size particles; however, the purpose of using the creek sediment was to understand the potential for sulfate reduction in a natural environment, where it occurs naturally, if the conditions were ideal. Consequently, the sediment samples were not sieved to remove fine particles or organics. The sediment also had the highest cation exchange capacity of the five substrates (Table 2.7), most likely due to the combination of fine size particles and organic matter at all stages of decomposition. Hence, it is not surprising to see a significant difference between the creek sediment and the other substrates. According to our understanding of the optimum environment for microbial sulfate reduction, SRB require attachment points and a source of carbon and nutrients, generally found in a diverse organic source (Waybrant et al., 2002; Gibert et al., 2002; Boudreau & Westrich, 1984). Thus, the creek sediment matrix could serve as the ideal substrate due to diversity in particle sizes, organic material, microbial populations, mineralogy and chemistry.

### *2.3.3 Sulfate Removal*

Only the biosolid treatments were able to consistently attain a significant reduction in sulfate concentrations during the treatment period (Table 2.11). Surprisingly, the control treatment resulted in a reduction of 15% of the initial sulfate concentration. Each amendment and substrate produced at least one treatment with a statistically significant sulfate concentration reduction over the treatment period, except the corn mash treatments. Table 2.12 shows the statistical relationships between treatments for sulfate reduction. The differences between substrates and amendments are explored further below.

#### *2.3.3.1 Removal by Amendment*

Several of the treatments had high variations between replicates for sulfate removal, with standard deviations averaging around 10% and ranging from a low of 0.5% to a maximum of nearly 40%. Two of the five amendments did not show any statistically significant differences between the substrate combinations (Table 2.12). The lack of statistically significant differences between the corn treatments was caused by high variation between replicates. As seen in Table 2.4, the river gravel and creek sediment treatments had average sulfate removal efficiencies approximately three fold that for the other treatments. However, both the river gravel-corn and creek sediment-corn treatments had high standard deviations (39.3% and 18.1%, respectively), which accounted for the lack of significant differences between the treatments. Wood mulch, like the corn mash, was the other amendment not showing statistical differences between treatments, usually due to high standard deviations (10-20%). However, the mulch did consistently decrease $SO_4^{2-}$ concentrations during treatment. Similarly to the creek sediment, the wood mulch had gone through minimal processing prior to use, and thus was more likely to have sulfate-reducing bacterial communities, accounting for the consistent $SO_4^{2-}$ removal. Unfortunately, all of the treatments had a wide variability in sulfate removal between replicates, effectively limiting the value of statistical analysis.

The biosolids and wood mulch were the only amendments that produced a consistent contribution to sulfate removal (Fig. 2.7). The sorghum syrup, soybean oil, and treatments without an amendment each produced one combination with a significantly different sulfate concentration after the treatment period, while the wood mulch produced two successful treatment combinations.

The syrup and soybean oil were only effective in combination with the creek sediment, suggesting that the sediment, rather than the organic amendment, was the determining factor in sulfate reduction. Of all the organic amendments used, the biosolids were most likely to have native sulfate reducing bacteria, accounting for the substantial sulfate reduction in during the treatment period.

Soybean oil caused highly variant responses between the substrates. When added to the creek sediment, soybean oil contributed to a 76% reduction in sulfates, whereas in the sand, river gravel, and limestone treatments, there was an increase in sulfate during the trial period. Analysis of the nitrogen and phosphorous concentrations of the organic amendments indicated soybean oil has no N or P, which are both required for bacterial proliferation (Waybrant et al., 2002; Gibert et al., 2002). In accordance with research conducted at the Savannah River Laboratory Site (Phifer et al., 2001), soybean oil was expected to significantly reduce sulfate concentrations due to its carbon density. However, our results were highly erratic. Two soybean oil treatments resulted in an increase of sulfate concentrations, two others had moderate decreases in the sulfate concentration over the treatment period, and the final treatment had a significant reduction in sulfate concentration. This variability may have been caused by the diversity in microbial communities between the substrates. Some research has indicated sulfate reducing bacteria are incapable of utilizing large organic molecules and require other microbes to dissolve them into smaller, more soluble compounds (URS, 2003; Skousen et al., 2000). The creek sediment, which was the substrate producing the largest sulfate decrease in combination with the soybean oil, with its naturally diverse microbial population may have been the most efficient in breaking down the fatty acids in the oil to smaller compounds. As a result, the creek sediment treatment was capable of degrading the soybean oil and utilizing it as a source of carbon for the sulfate reduction process, whereas the other substrates were incapable of doing so.

#### *2.3.3.2 Removal by Substrate*

Comparison of the substrates was similar to the results of the analysis by amendment. Each substrate resulted in at least one combination with significantly reduced sulfate concentrations after the treatment period (Table 2.11). Two of the substrates, limestone and marble chips, were only effective in combination with the biosolid amendment. As shown previously, the biosolid amendment consistently resulted in decreased sulfate concentrations, thus we can exclude the limestone and marble chips from further analysis, as they did not have any positive impact on the sulfate concentration. The most beneficial substrate was the creek sediment, shown in Fig. 2.8. The primary sulfate removal mechanisms from these systems appeared to be sulfate reduction to sulfide and precipitation of Mn or evolution as sulfide gas. Most likely, a combination of the two resulted in the decreases observed, as the concentration of sulfate (7.5 mM) was four times the concentration of Mn (1.8 mM). Presuming the entire concentration of reduced $SO_4^{2-}$ precipitated with Mn to cause removal of 100% of the Mn, it could only account for a 20-30% decrease in the sulfate concentration. Consequently, we can assume a significant amount of $SO_4^{2-}$ was evolved as sulfide gas. The sediment contained a diverse population of natural microbial communities, including sulfate reducing bacteria, more so than any other substrate, thus accounting for the better sulfate removal efficiency.

The sand resulted in significantly lower sulfate concentrations without an amendment and with the addition of wood mulch (Fig. 2.7). As discussed previously, the wood mulch may have supported native sulfate-reducing bacterial communities. The small particle size of the sand is an ideal matrix for bacteria as they are capable of maintaining a suitable micro-environment in the interstitial spaces. A similar occurrence may have developed in the biosolid treatment; however, the variability between replicates (13.5%) inhibits the determination of significance. Surprisingly, the river gravel exhibited high $SO_4^{2-}$ removal capacity, in contrast to the Mn removal capability. Four of the six treatment combinations removed greater than 30% of the initial sulfate concentration. However, only two treatments significantly reduced sulfate concentrations during the trial period. As with previous treatments, the lack of significance is primarily due to high variability between samples.

## *2.4 Conclusions*

The most effective treatment combinations for Mn removal were those utilizing biosolids or wood mulch as the amendment, especially in combination with the creek sediment substrate (Table 2.3). Similarly, sulfate removal was best in the creek sediment and biosolid combination, but without a clear second choice. The sorghum syrup and soybean oil were inconsistent in their capacity to induce sulfate reduction; however, based on the research of Phifer et al. (2001), the addition of fertilizer may have increased the $SO_4^{2-}$ removal by those amendments. Wood mulch and the treatments with no amendment were similar in most aspects to each other, showing moderate Mn removal capacities, which were clearly less than the sediment and biosolid treatment. Substrate particle size and chemistry appeared to be an important aspect in the success or failure of a treatment. Due to the carbonate chemistry of limestone, $MnCO_3$ formation was feasible from the dissolution elements, and the sand treatments allowed bacteria to form microclimates suitable for their needs. The creek sediment encompassed both of these aspects, as well as native microbial communities suitable for large organic molecule decomposition and sulfate reduction. Thermodynamics indicate no single removal mechanism (sorption, sulfide, oxide, or carbonate formation) was capable of removing all the Mn from solution in these treatments. Consequently, at least two, and most likely all, of the removal mechanisms were working in most treatments to varying degrees and similar results were expected for the second phase of the research. Based upon the results of the batch experiments, the most favorable choice for the second phase of the experiment was the creek sediment and biosolid treatment combination. However, due to the volume of biosolids required and the density of the material, which would inhibit the percolation of the mine drainage solution, a combination of the wood mulch and the biosolid amendments were used in the small scale bioreactor.

Table 2.1 Average Cation and Anion Concentrations in Water Samples from Restoration Sites.

| *Site** | EC | Cl | $SO_4^{2-}$ | Mg | Ca | K | Na |
|---|---|---|---|---|---|---|---|
| | µS | ----------------------------mg $L^{-1}$-------------------------------- | | | | | |
| LM (30yr)[†] | 46 | 0.6 | 10 | 1.8 | 2.3 | 1.3 | 1.1 |
| LMp[Δ] | 46 | 1.4 | 10 | 1.5 | 1.4 | 1.5 | 1.3 |
| LMi[Δ] | 24 | 1.7 | 10 | 1.7 | 1.4 | 1.5 | 1.4 |
| GC 1[‡] | 478 | 1.3 | 225 | 43 | 47 | 8.9 | 7.6 |
| GC 2 | 1732 | 2.2 | 1280 | 205 | 125 | 7.3 | 8.4 |
| GC 3 | 1440 | 2.1 | 1018 | 162 | 109 | 11.4 | 10.6 |
| GC 4 | 1723 | 2.3 | 1293 | 204 | 137 | 11.4 | 13.1 |
| LF up | 1692 | 2.5 | 1299 | 196 | 136 | 12.4 | 15.4 |
| LF down | 1685 | 2.4 | 1344 | 190 | 138 | 11.2 | 16.7 |

*LM =Little Millseat reference stream; GC = Guy Cove restoration stream; LF = Laurel Fork stream, upstream and downstream of the Guy Cove outlet.

[†] Average from weekly samples collected over a thirty year period.

[‡] Guy Cove samples collected monthly starting June, 2004.

[Δ] p = perennial weir, I = intermittent flume.

Table 2.2 Average Nutrient and Metal Concentrations in Water Samples from Restoration Sites.

| *Site** | pH | $NO_3$ | $NH^4$ | TOC | Alk | Fe | Mn |
|---|---|---|---|---|---|---|---|
| | S.U. | ----------------------------mg $L^{-1}$-------------------------------- | | | | | |
| LM (30yr)[†] | 6.46 | 0.13 | NA | 4.99 | 17 | -- | -- |
| LMp[Δ] | 6.53 | 0.14 | 0.04 | 4.83 | 25 | 0.12[Ψ] | 1.1[Ψ] |
| LMi[Δ] | 6.72 | 0.09 | 0.04 | 5.31 | 20 | 0.15[Ψ] | 0.9[Ψ] |
| GC 1[‡] | 7.94 | 0.09 | 0.07 | 30.1 | 324 | 0.28 | 4.0 |
| GC 2 | 6.35 | 0.02 | 0.47 | 12.1 | 88 | 2.96 | 26.0 |
| GC 3 | 6.46 | 0.02 | 0.10 | 15.2 | 115 | 3.49 | 23.5 |
| GC 4 | 7.04 | 0.01 | 0.09 | 9.6 | 79 | 0.69 | 21.4 |
| LF up | 7.03 | 0.02 | 0.17 | 9.5 | 79 | 2.48 | 19.5 |
| LF down | 6.87 | 0.03 | 0.21 | 9.4 | 72 | 3.56 | 17.6 |

*LM =Little Millseat reference stream; GC = Guy Cove restoration stream; LF = Laurel Fork stream, upstream and downstream of the Guy Cove outlet.

[†] Average from weekly samples collected over a thirty year period.

[‡] Guy Cove samples collected monthly starting June, 2004.

[Ψ] Samples collected monthly in 2006.

[Δ] p = perennial weir, I = intermittent flume.

Table 2.3 Percent of Mn (± 1 S.D.) removed from each replicated treatment combination after 21 days. The efficacy of each substrate and amendment is also averaged. (n=2)

| | Substrate | | | | | |
|---|---|---|---|---|---|---|
| Amendment | Creek Sediment | Limestone | Marble | River Gravel | Sand | Mean |
| Biosolid | 100.0 ± 0.0 | 99.7 ± 0.1 | 99.9 ± 0.1 | 99.8 ± 0.2 | 99.8 ± 0.0 | 99.8 ± 0.1 |
| Corn Mash | 10.2 ± 8.9 | 67.4 ± 9.6 | 31.8 ± 7.6 | 45.2 ± 40.0 | 39.8 ± 5.4 | 38.9 ± 20.8 |
| Sorghum Syrup | -119.5 ± 5.5 | | 10.0 ± 0.4 | 24.9 ± 9.1 | 7.5 ± 1.1 | -19.3 ± 67.3** |
| Soybean Oil | 33.2 ± 9.6 | 16.2 ± 0.1 | 37.5 ± 7.5 | 8.4 ± 8.4 | 34.0 ± 0.5 | 25.9 ± 12.8 |
| Wood Mulch | 94.1 ± 4.9 | 96.3 ± 0.9 | 96.7 ± 0.1 | 95.2 ± 0.5 | 97.0 ± 0.7 | 95.9 ± 1.2 |
| None | 78.9 ± 8.7 | 54.2 ± 7.3 | 32.1 ± 1.0 | 2.5 ± 0.8 | 60.6 ± 9.4 | 45.7 ± 29.4 |
| Mean | 32.8 ± 82.6* | 66.8 ± 34.2 | 51.3 ± 37.6 | 46.0 ± 42.6 | 56.5 ±36.7 | |

Control: 2.8 ± 0.3

* Removing the sorghum syrup treatment from the analysis increases the mean to 63.3 ± 14.1%.

** Removing the sorghum syrup treatment from the analysis increases the mean to 14.1 ± 9.4%.

Table 2.4 The percent of $SO_4^{2-}$ (± 1 S.D.) removed from each replicated treatment combination. Each substrate and amendment is also averaged. (n=2)

| | Substrate | | | | | |
|---|---|---|---|---|---|---|
| Amendment | Creek Sediment | Limestone | Marble | River Gravel | Sand | Mean |
| Biosolid | 84.7 ± 1.0 | 38.2 ± 6.0 | 62.8 ± 13.5 | 60.0 ± 16.8 | 32.3 ± 13.5 | 55.6 ± 21.0 |
| Corn Mash | 29.3 ± 18.1 | 13.4 ± 1.0 | 10.9 ± 7.5 | 28.4 ± 39.3 | 11.6 ± 10.9 | 18.7 ± 9.3 |
| Sorghum Syrup | 23.0 ± 0.5 | | -3.5 ± 8.6 | 29.0 ± 21.5 | 8.3 ± 3.2 | 14.2 ± 14.7 |
| Soybean Oil | 76.4 ± 8.5 | -7.1 ± 2.7 | 22.7 ± 24.3 | -2.9 ± 3.1 | 7.1 ± 1.2 | 19.2 ± 34.0 |
| Wood Mulch | 48.3 ± 18.6 | 34.3 ± 10.6 | 31.7 ± 20.7 | 58.3 ± 11.9 | 65.3 ± 13.3 | 47.6 ± 14.6 |
| None | 29.7 ± 34.1 | 33.9 ± 9.5 | 23.1 ± 3.2 | 14.1 ± 1.0 | 22.3 ± 3.2 | 24.6 ± 7.6 |
| Mean | 48.6 ± 26.3 | 22.5 ± 19.2 | 24.6 ± 22.3 | 31.2 ± 24.6 | 24.5 ± 22.2 | |

Control: 14.9 ± 6.0

Table 2.5 Mean redox potential (mV) of each treatment. Values are the means after redox stabilized and represents the mean of two treatments.

| | | Substrate | | | | | |
|---|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand | Mean |
| Amendment | Biosolid | -17 | 88 | -189 | -306 | -69 | -99 |
| | Corn Mash | -80 | -56 | -87 | 0 | 56 | -33 |
| | Sorghum Syrup | -147 | | -37 | 64 | 58 | -16 |
| | Soybean Oil | -75 | 230 | 174 | -152 | 252 | 86 |
| | Wood Mulch | -294 | -320 | -232 | -249 | -302 | -279 |
| | None | -1 | 17 | -49 | -21 | -19 | -15 |
| | Mean | -102 | -8 | -70 | -111 | -4 | |

Control: -24

Table 2.6 Mean pH of each treatment combination (n=2). The pH was measured in each flask daily.

| | | Substrate | | | | |
|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand |
| Amendment | Biosolid | 12.1 | 11.6 | 11.7 | 11.4 | 11.3 |
| | Corn Mash | 5.6 | 6.5 | 5.6 | 6.2 | 5.8 |
| | Sorghum Syrup | 5.1 | | 6.1 | 5.1 | 4.5 |
| | Soybean Oil | 6.1 | 6.0 | 8.1 | 6.2 | 5.7 |
| | Wood Mulch | 8.3 | 8.1 | 8.3 | 8.3 | 8.4 |
| | None | 7.2 | 7.4 | 6.7 | 6.5 | 6.5 |
| | Control | 6.3 | | | | |

Table 2.7 Substrate and amendment data.

| Sample | pH (S.U.) | CEC (meq/ 100 g) | Total P (%) | Total N (%) |
|---|---|---|---|---|
| Biosolid | 11.5 | 3.8 | 0.76 | 1.75 |
| Corn Mash | 6.3 | 12.7 | 0.63 | 5.21 |
| Mulch | 8.3 | 53.2 | 0.93 | 2.14 |
| Sorghum Syrup | NA | NA | 0.03 | 0.07 |
| Soybean Oil | NA | NA | 0.00 | 0.00 |
| Creek Sediment | 6.2 | 0.188 | - | - |
| Limestone | 7.8 | 0.080 | - | - |
| Marble | 8.5 | 0.018 | - | - |
| River Gravel | 4.8 | 0.039 | - | - |
| Sand | 9.3 | 0.049 | - | - |

Table 2.8 Treatments with a significant ($p<0.001$) decrease in Mn concentration during the treatment period. Y = Yes N = No

| | | Substrate | | | | |
|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand |
| Amendment | Biosolid | Y | Y | Y | Y | Y |
| | Corn Mash | N | Y | N | N | Y |
| | Sorghum Syrup | Y | | Y | N | N |
| | Soybean Oil | Y | N | N | N | Y |
| | Wood Mulch | Y | Y | Y | Y | Y |
| | None | Y | Y | Y | N | Y |

Control: N

Table 2.9 Significant differences between treatments for Mn removal ($p<0.001$). The differences between amendments (horizontal) are indicated by letters and the differences between substrates (vertical) are indicated numerically.

| | | Substrate | | | | | |
|---|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand | Control |
| Amendment | Biosolid | A 1 | A 14 | A 12 | A 1 | A 13 | B |
| | Corn Mash | AB 2 | AB 23 | AB 124 | AB 1 | AB 145 | A |
| | Sorghum Molasses | AC 3 | | BC 24 | AC 1 | BC 246 | BC |
| | Soybean Oil | AC 2 | C 2 | C 234 | C 2 | AC 145 | BC |
| | Wood Mulch | A 1 | A 14 | A 12 | B 1 | A 136 | B |
| | None | AC 1 | C 134 | AC 23 | BC 2 | C 235 | BC |
| | Control | 2 | 2 | 24 | 2 | 2 | |

Table 2.10 Percent of Mn removal within the first day of treatment.

| | | Substrate | | | | |
|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand |
| Amendment | Biosolid | 100.0 | 97.5 | 99.6 | 99.7 | 97.2 |
| | Corn Mash | 7.9 | 38.3 | 42.7 | 30.6 | 27.6 |
| | Sorghum Syrup | -7.1 | | 2.0 | 6.8 | 3.3 |
| | Soybean Oil | 44.0 | -2.0 | 11.8 | 2.0 | 22.2 |
| | Wood Mulch | 88.9 | 93.3 | 90.4 | 92.1 | 94.3 |
| | None | 51.7 | 20.4 | 3.2 | -4.0 | 25.3 |

Control: 0.3

Table 2.11 Treatments with a significant ($p<0.001$) decrease in $SO_4^{2-}$ concentration during the treatment period. Y = Yes N = No

| | | Substrate | | | | |
|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand |
| Amendment | Biosolid | Y | Y | Y | Y | N |
| | Corn Mash | N | N | N | N | N |
| | Sorghum Syrup | Y | | N | N | N |
| | Soybean Oil | Y | N | N | N | N |
| | Wood Mulch | N | N | N | Y | Y |
| | None | N | N | N | N | Y |

Control: N

Table 2.12 Significant differences between treatments for sulfate removal ($p<0.001$). The differences between amendments are indicated by letters (horizontally) and the differences between substrates are indicated numerically (vertically).

| | | Substrate | | | | | |
|---|---|---|---|---|---|---|---|
| | | Creek Sediment | Limestone | Marble | River Gravel | Sand | Control |
| Amendment | Biosolid | A 13 | B 13 | AB 12 | ABC 12 | B 13 | C |
| | Corn Mash | A 2 | A 13 | A 1234 | A 12 | A 23 | A |
| | Sorghum Molasses | AC 12 | | BC 24 | AC 12 | A 23 | A |
| | Soybean Oil | A 3 | B 23 | A 1234 | B 3 | B 23 | B |
| | Wood Mulch | A 23 | A 13 | A 1234 | B 12 | B 13 | A |
| | None | A 23 | A 13 | A 34 | A 2 | A 23 | A |
| | Control | 12 | 23 | 34 | 2 | 23 | |

Figure 2.1 Schematic diagram of the batch experiments.

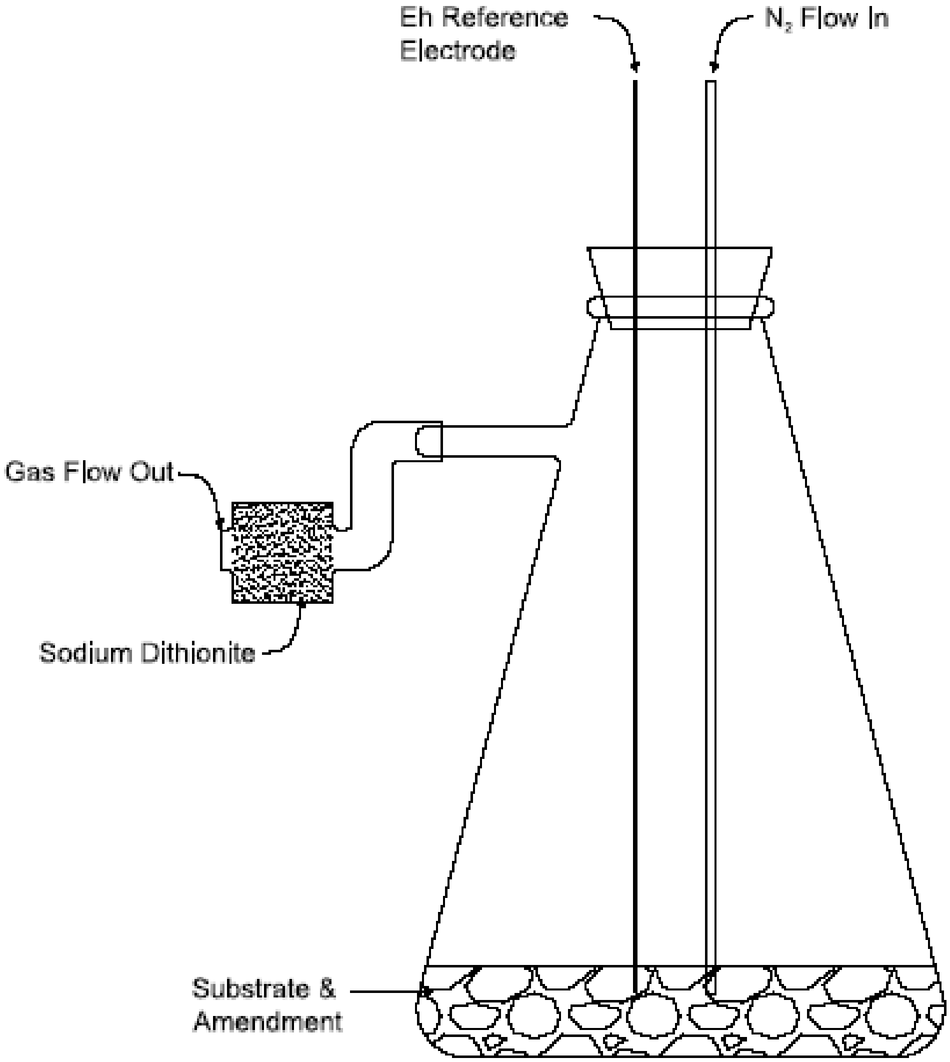


Figure 2.2 Final Mn removal capacity in percentage.

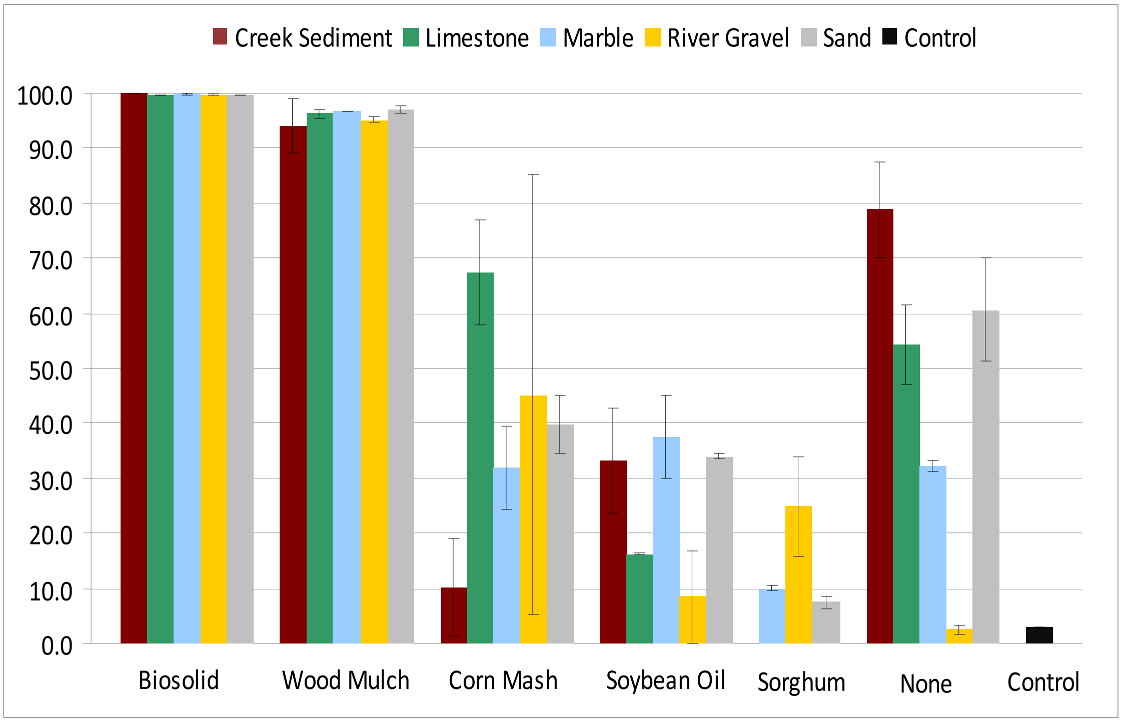


Figure 2.3 Manganese Eh-pH diagram. Notice that increasing Mn concentration decreases the area of $Mn^{2+}$ solubility. Areas in red indicate the locations of the treatments on the diagram based on pH and Eh data. 1. Biosolid treatments, 2. Wood Mulch treatments, 3. Corn Mash, Sorghum Syrup, Soybean Oil, and No Amendment treatments. From Stumm & Morgan, 1996.

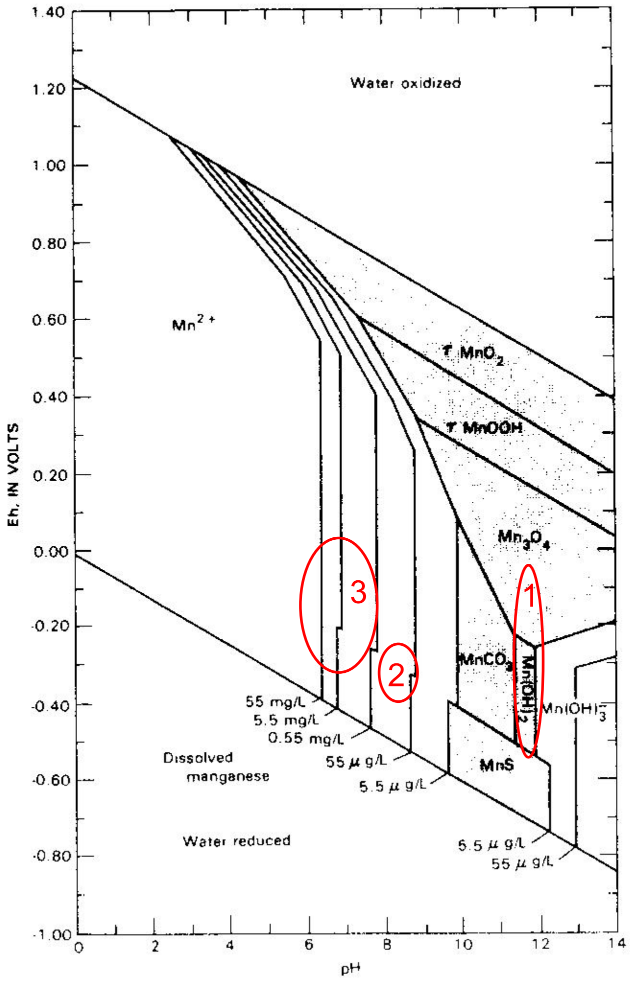


Figure 2.4 Correlation of the cation exchange capacity and the Mn removal for the substrate treatments without and amendment. The correlation coefficient is 0.53. (p=0.16)

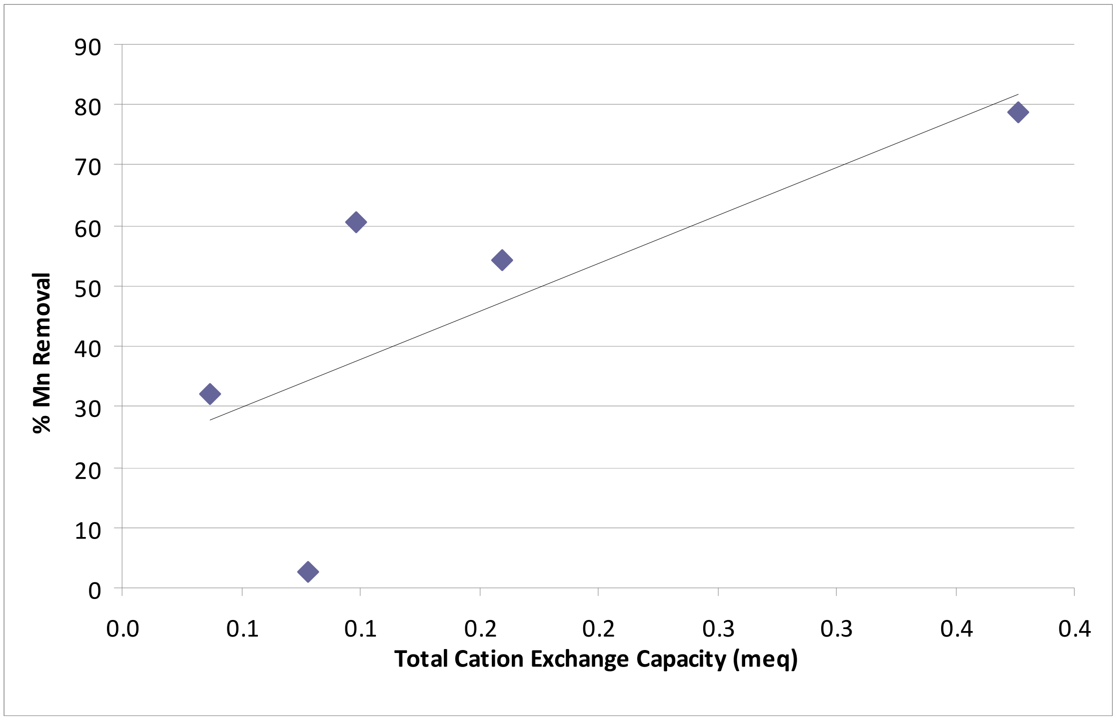


Figure 2.5 Correlation of the cation exchange capacity and Mn removal efficiency for all treatments. ($r^2$=0.27, p<0.01)

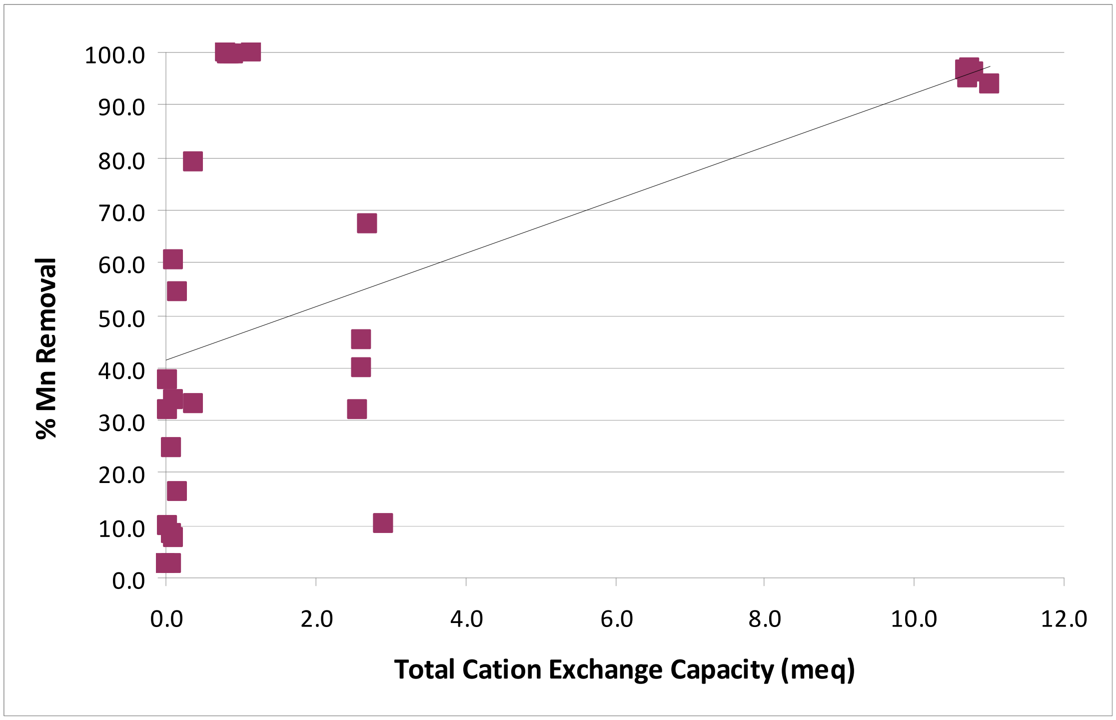


Figure 2.6 Mn removal capacity in percentage of each treatment organized by substrate.

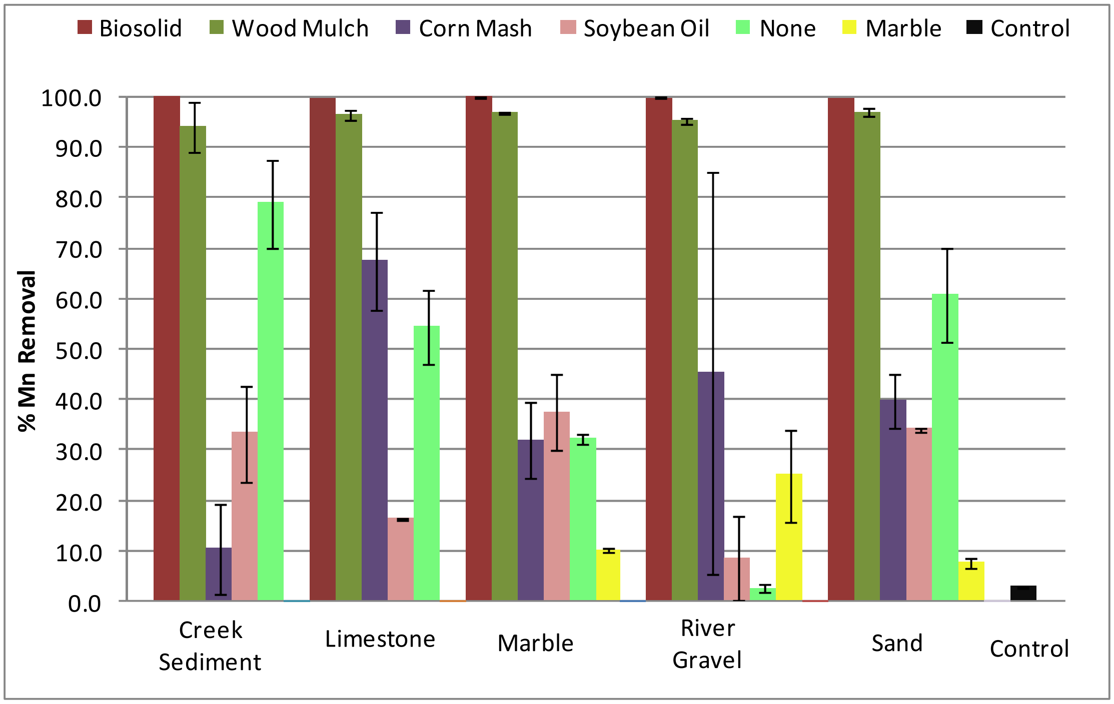


Figure 2.7 Total percent of sulfate removal for all treatments.

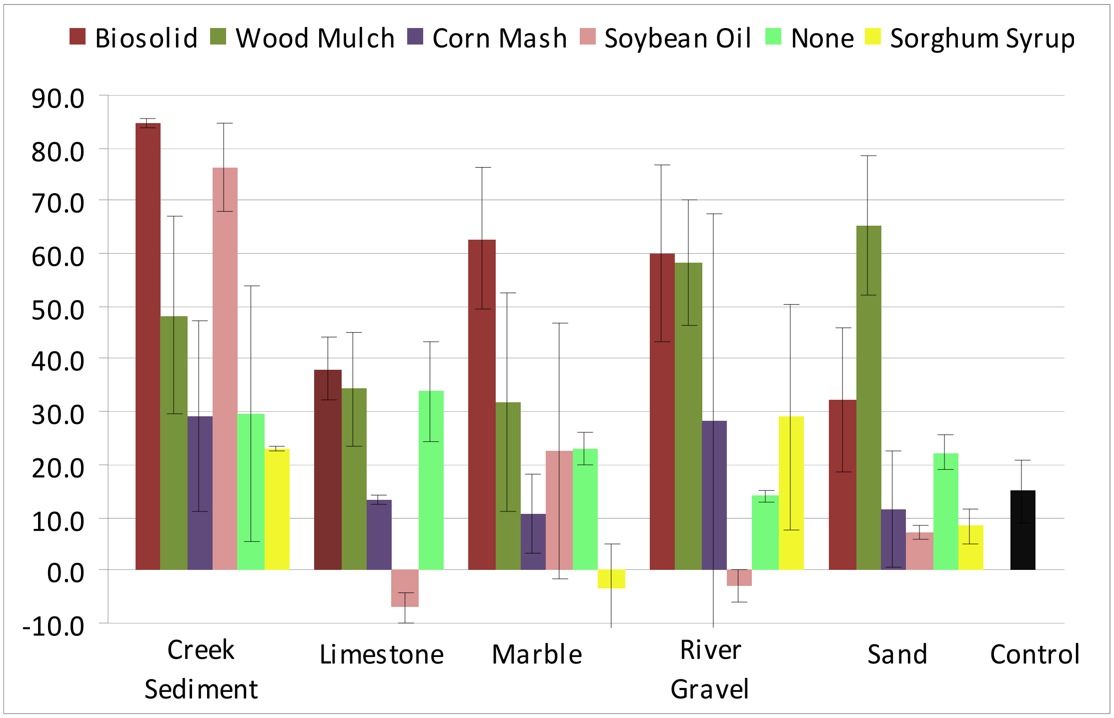
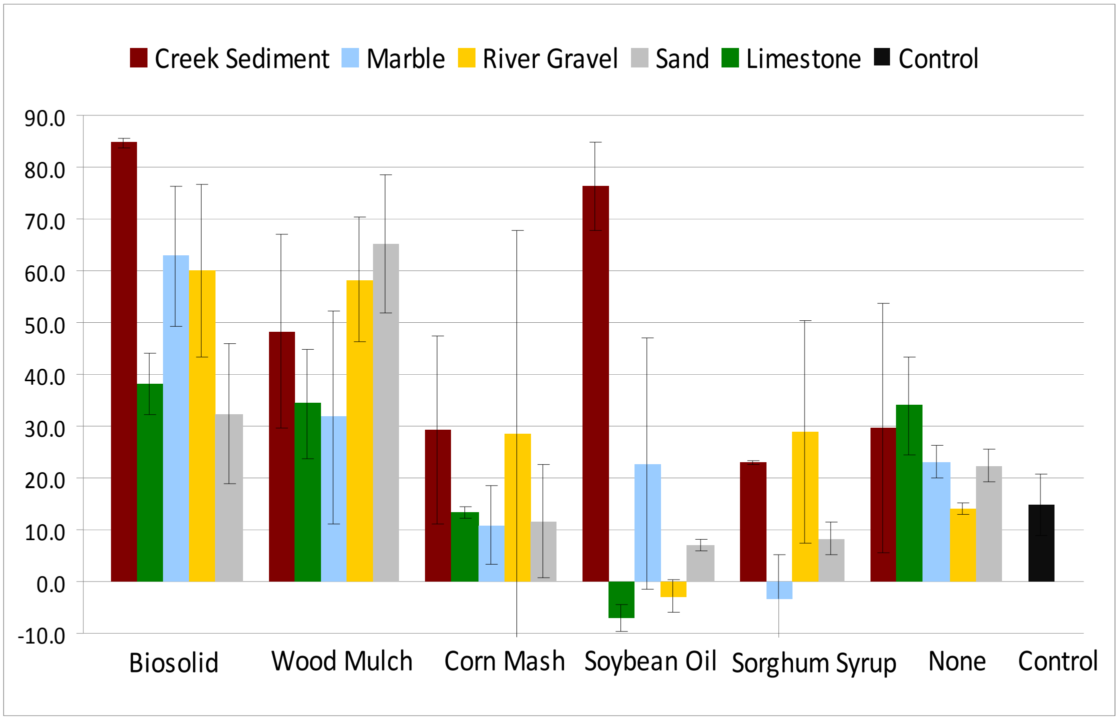


Figure 2.8 Percent of sulfate removal arranged by amendment.



# CHAPTER 3

# A Small Scale Sulfate Reducing Bioreactor for Manganese Removal

## *3.1 Background*

In the 1990s several square miles of the University of Kentucky's Robinson Forest (Fig. 3.1), located in the Cumberland Plateau region of Eastern Kentucky were surface mined for coal. Unlike many mined areas of the world, the drainage from this site is naturally buffered to a neutral to alkaline pH, subsequently removing most dissolved metals from solution through precipitation. Of the earth metals common in mine drainage leachate, manganese (Mn) is one of the most difficult to treat due to its relatively high solubility (Table 3.1). In addition, Mn does not auto-oxidize until pH reaches approximately 8 under normal oxidized conditions (Fig. 3.2). The mine drainage also contains high concentrations of dissolved sulfate ($SO_4^{2-}$) due to the exposure and oxidation of pyrite during the mining process. This research focuses on $SO_4^{2-}$ and Mn contaminated drainage discharging into a small tributary to Laurel Fork in Breathitt County, Kentucky in a valley known as Guy Cove (Fig. 3.3). Water quality data was collected monthly from the Guy Cove Branch for approximately two years and compared to the water quality data from the Little Millseat Branch, a reference reach stream for Kentucky (Tables 3.2 and 3.3). Manganese and sulfate concentrations in the mine drainage far exceed those observed in the Little Millseat Branch. An ideal treatment option for the Guy Cove drainage is a sulfate reducing bioreactor to convert the $SO_4^{2-}$ to sulfide ($S^{2-}$) and precipitate MnS. The goal of this research was to identify an ideal mixture of an inorganic substrate and an organic amendment to enhance naturally occurring microbial reduction of $SO_4^{2-}$. Our hypothesis was that under reducing conditions, $SO_4^{2-}$ would transform to $S^{2-}$ and precipitate with the Mn to form insoluble MnS, thus removing both the excess $SO_4^{2-}$ and Mn from solution.

Of the many metals and metalloids commonly found in acid mine drainage, Mn is one of the more difficult to remove due to the complexity of the interactions governing Mn solubility. Manganese precipitation is inhibited if the Fe: Mn ratio is too high (Karathanasis & Barton, 1997), Mn precipitates dissolve if $Fe^{2+}$ concentrations are too high (Evangelou, 1998; Stumm & Morgan, 1996), and most other metals preferentially complex with sulfide before Mn (Stumm & Morgan, 1996). Research has shown widely variant success in Mn removal. Tabak et al. (2003) successfully removed 95% of the Mn load from AMD, while Machemer and Wildeman (1992) were only able to see a temporary decrease in Mn concentrations. Machemer and Wildman also observed a relationship between metal and $SO_4^{2-}$ concentrations over time, suggesting that following the saturation threshold stage, sulfide precipitation can be a significant metal removal mechanism.

Research utilizing sulfate reduction to encourage metal and sulfate removal has found widely varying efficiencies. Waybrant et al. (1998) attained greater than 99% $SO_4^{2-}$ removal, while Champagne (2005) observed 73% removal of $SO_4^{2-}$. Several field based experiments in either permeable reactive barriers or constructed wetlands reached $SO_4^{2-}$ removal efficiencies approximating 70% (McIntire & Edenborn, 1990; Benner et al., 1999; Sass et al., 2001). As evidenced by the variability in removal efficiencies, there are several factors impacting the efficacy of sulfate-reducing treatments including the initial $SO_4^{2-}$ concentration, temperature, residence time (Waybrant et al., 2002) and the carbon source (Gibert et al., 2002). The source and characteristics of the organic matter have a significant impact on the removal efficiency of a sulfate-reducing system. Factors such as the particle size of the organic matter, the labile to recalcitrant carbon ratio, and the availability of nitrogen and phosphorus are perhaps the most important (Waybrant et al., 2002; Gibert et al., 2002). The ideal metal removal mechanism in a bioreactor is metal sulfide precipitation due to their insolubility and stability (Stumm & Morgan, 1996). However, there are several coinciding mechanisms occurring in a fully functioning treatment system, which are difficult to separate and include metal sulfide precipitation, hydroxide and carbonate formation, simple sorption, and physical filtration (Wildeman and Updegraff, 1997; Neculita et al., 2007). In addition, removal mechanisms may change over time. When a bioreactor is first installed and begins to function, sorption seems to predominate until sorption sites are filled (Machemer & Wildeman, 1992; Gibert et al., 2005). As the system matures and microbial populations flourish in the system, metal sulfide precipitation begins to predominate.

A sulfate-reducing bioreactor (SRB) requires four conditions to be appropriate to successfully function (Boudreau & Westrich, 1984; Ludwig et al., 2002; Neculita et al., 2007). First, there must be a consistent source of $SO_4^{2-}$ to feed the SRB and metals to complex the generated sulfide. Secondly, the bacteria need carbon and nutrients. Thirdly, the bacteria need a substrate for physical support. The precipitation of metal-sulfides will also be greatly enhanced if an adhesion surface is available. Lastly, chemical conditions must be appropriate. For example, SRB function best at near-neutral pH and in the absence of non-sulfate electron acceptors, including nitrate and oxygen. The second and third criteria are typically combined; thus, in essence, every bioreactor requires $SO_4^{2-}$ and contaminant metals, a substrate, and appropriate chemical conditions. The goal of this study was to find a suitable substrate for Mn removal. In much of the previous research, Mn has not been the primary removal target and was predominately removed via co-precipitation with other metals.

## *3.2 Materials and Methods*

### *3.2.1 Research Materials*

The bioreactor was tested using a synthetic drainage solution to simplify the chemistry and minimize interferences. While the average Mn and $SO_4^{2-}$ concentrations in the mine effluent are approximately 30 and 1,300 mg $L^{-1}$, respectively, the maximum observed concentration has ranged significantly beyond that concentration (Tables 3.2-3.3). Consequently, the synthetic mine drainage was mixed to a final concentration of approximately 90 mg $L^{-1}$ Mn and

1,500 mg $L^{-1}$ $SO_4^{2-}$. Reagent grade manganese sulfate ($MnSO_4$) and calcium sulfate dihydrate ($CaSO_4$ $2H_2O$) were used as the $Mn^{2+}$ and $SO_4^{2-}$ sources for the synthetic drainage solution (Fisher Scientific). The pH of the $MnSO_4$ solution was approximately 5.8 with an electrical conductivity of approximately 1,900 µS. The creek sediment used in the bioreactors was collected from the mine site in Robinson Forest. There are several weirs installed in the streams to measure flow rates and volume where, periodically, the University is required to dredge the material from the basin behind the weir to remove accumulated material. The samples collected for use in the bioreactors were selected from the material dredged from the stream bed. The sediment was collected in five gallon buckets and sealed to prevent moisture loss until use. The mulch used in the bioreactors was identical to that used in the first phase of the experiment. The mulch was collected from a University of Kentucky research farm and is composed of the woody debris on the farm. The debris was chipped and mixed thoroughly and stored until it was needed. As with the sediment, several five gallon buckets were used to collect the mulch and store it until it was used in the bioreactor. The biosolids were also identical to those used in the batch experiment phase of the research. They were collected from the Winchester, Kentucky wastewater treatment plant and stored in sealed five gallon buckets.

This study was conducted in a University of Kentucky greenhouse and was thus not exposed to temperature variations or weathering conditions. The temperature was approximately 25° C for the duration of the experiment. The bioreactors used in the study were 15 gallon (57 L) plastic tanks ordered from US Plastics Corp. (Lima, OH, USA) and were manufactured by Ace Roto-Mold. Each tank was plumbed such that it would be an upflow treatment system. The plumbing used in the system was one-half inch diameter (½”) PVC tubing purchased from a retail hardware store and all joints were sealed with thread tape, PVC cement, and/or caulk, as necessary. Figure 3.4 shows the portion of the plumbing inside the tanks. The solution flowed into the tanks via an adapter from the tubing to the PVC and flowed down to the bottom of the tank. The solution then flowed along a section of PVC with holes drilled along the entire length, spaced approximately one to two inches (1-2”) apart, to resemble a section of perforated pipe. The solution then flowed upward through the treatment material and drained out through another piece of PVC. The outlet section and the perforated section were both wrapped in cheesecloth to prevent clogging due to the material. A gas trap filled with a saturated sodium thiosulfate solution was attached to the system to allow gases to escape and prevent oxygen from entering the system. The system is shown prior to being filled in Fig. 3.5. After the material was mixed, approximately 2/3 of each tank was filled with the substrate and amendment mixture and a redox probe installed (Fig. 3.6). The systems were then attached via flexible plastic tubing to a peristaltic pump and filled with the synthetic drainage solution at a rate of approximately 5-10 mL per minute. Once breakthrough was achieved, the pumping rate was decreased to approximately 1 mL per minute.

The treatment material was mixed as in the first phase of the experiment. The substrate (creek sediment) was added in a 10:1 ratio to the amendment (wood mulch and biosolids) on a mass basis. Three tanks were used to replicate one treatment, consisting of only wood mulch as the amendment. Three other tanks were used to replicate the second treatment with a combination of the wood mulch and approximately 15% biosolids.

### *3.2.2 Research Methods*

A sample from each tank was collected every day for analysis. Each sample was analyzed for Mn, $SO_4^{2-}$, pH, redox status (Eh), and electrical conductivity (EC). In addition, the redox status within each tank was measured daily and the amount of solution collected in a 24 hour period was recorded. Electrical conductivity was measured using a Hanna Combo pH & EC unit. The pH and redox status were measured with a Fisher Scientific Accumet AP62 pH/mV meter using an Accumet pH probe and a silver/silver chloride Mettler Toledo InLab Redox Probe, respectively. The Eh values were corrected to reflect a standard hydrogen electrode. Manganese concentrations were tested using a Solaar M5

ThermoElemental Atomic Absorption Spectrometer. Sulfate-sulfur ($SO_4$-S) concentrations were analyzed by a variation of APHA Method 4500-$SO_4^{2-}$ E (APHA, 1998). The method has been adapted for use on a Molecular Devices Versamay Tunable Plate Reader, analyzed at 450 nm. The samples for $SO_4^{2-}$ analysis were diluted as necessary using one normal hydrochloric acid. A sample volume of 130 µL was placed in a sterile plate with an equivalent amount of a barium chloride and carboxy-methyl cellulose reagent. The samples were stirred for 45 minutes and then analyzed on the Plate Reader at 450 nm. The Mn samples were diluted and analyzed via atomic absorption. Due to the highly acidic nature of the samples, they were each neutralized using sodium hydroxide prior to disposal. The remainder of the solution drained from each tank was diluted and discarded.

The tanks were filled at a rate of approximately 10 mL per minute, resulting in a filling period of 12 days. In order to preserve the anoxic environment, the tanks were not opened to obtain a sample of the solution. Consequently, data is unavailable for the first 12 days, prior to effluent leaching from the tanks. Five of the tanks began draining either day 11 or 12, but the final tank did not begin draining until day 19. After the tanks were filled, the pumping rate was decreased to approximately 1.0 mL per minute, providing a theoretical treatment time of approximately 20 days. However, calculation of the pumping rate based on the amount of effluent collected each day indicated a pumping rate of approximately 0.8 mL per minute. The difference in pumping rates calculated based on the influent flow and the effluent flow may have been caused by leakage or spillage of the effluent prior to measuring the volume.

### *3.2.3 Statistical Analysis*

The data was statistically analyzed using SAS 9.1. Concentrations of $SO_4^{2-}$ and Mn and the redox status were analyzed to determine statistical differences. The “PROC MIXED” procedure with the LS-Means statement was used for the analysis based on the nature of the data and the correlation between each sampling point, or day, with those coming before and after it. The maximum probability allowed to indicate significant differences is 0.05 ($\alpha=0.05$). The Satterthwaite approximation was utilized to calculate the degrees of freedom for the analysis.

## *3.3 Results & Discussion*

The bioreactors successfully removed greater than 90% of the Mn from solution and more than 70% of the $SO_4^{2-}$ from solution over the 65 day treatment period. There was no indication of a decline in either Mn or $SO_4^{2-}$ removal rates, indicating the Mn removal mechanism was precipitation via carbonate or sulfide formation. The redox status within the reactors and the pH of the effluent indicated that $MnCO_3$, MnS, and Mn-oxides could be stable within the bioreactor, thus accomplishing the primary goal of this research.

### *3.3.1 Manganese Removal*

Both treatments significantly reduced Mn and $SO_4^{2-}$ concentrations for the duration of the 65 day trial period ($p<0.01$). Mn decreased from an average concentration of 85 mg $L^{-1}$ to approximately 7 mg $L^{-1}$ (Fig. 3.7). The Mn concentration varied considerably from day to day in both treatments, with a slightly smaller range for the mulch treatment (1.0-8.5 mg $L^{-1}$) compared to the range of the combined treatment (1.9-12.9 mg $L^{-1}$). In addition, the mulch treatment appeared to consistently produce a lower Mn concentration than the combined treatments; however, the difference was not statistically significant. In Phase I of the research, the biosolid treatments removed more Mn than the mulch treatment; however, the combination of the mulch and biosolids in this phase did not significantly enhance Mn removal. The treatment mixture without the addition of biosolids removed 92.5% of the Mn from solution; whereas the treatment with biosolids removed 93.1% of the Mn. The difference was not statistically significant ($p<0.05$).

In addition to Mn and $SO_4$-S concentrations, pH, Eh, and EC were measured in each sample. Analysis of the Mn concentrations as a function of the electrical conductivity, pH, and redox potential of the samples indicated poor relationships between these parameters and Mn concentrations. The correlation coefficients for all comparisons are presented in Tables 3.4 and 3.5. Figure 3.8 shows the Mn concentrations of both treatments as a function of the mean electrical conductivity for the replicates. The effluents consistently had a color associated with dissolved organic amendments. Filtering the samples through activated carbon removed some of the color, but did not completely remove the dissolved organic components in solution. Electrical conductivity is associated with dissolved ions and solids in solution, indicating that a small proportion of the Mn may be sorbed to organics in solution or may be precipitated and remain in suspension.

Surprisingly, Mn was not associated with either the pH of the effluent (Fig. 3.9) or the redox status within the treatment tank (Fig. 3.10). Comparison of the mean pH and Eh values of the effluent and the stability diagram (Fig. 3.2) indicated that the Mn would most likely be in solid form, whether it be a carbonate, oxide, or sulfide. There was an apparent difference between the combined treatment and the mulch treatment with regards to the redox status; however, this was caused by one replicate of the mulch treatment not maintaining strongly reducing conditions consistently (Fig. 3.11). Following the trend of the other five tanks, the second mulch replicate slowly reached an Eh of approximately -200 mV, but then oxidized. There was no apparent cause for this change, as all six tanks were built, filled, and maintained identically; however, after a period of approximately 45 days, the Eh slowly decreased and maintained an equivalent Eh level to the end of the trial period. The differences in Eh did not affect the final Mn concentration, as shown in Fig. 3.12. While the second replicate appeared to have slightly higher Mn concentrations, they were generally interspersed with the first replicate, suggesting the third replicate achieved a higher level of Mn removal than would generally be expected for this treatment.

The removal efficiency of Mn was also correlated with the EC, Eh, and pH of the samples to determine potential relationships in Fig. 3.13-3.15, respectively. As with the correlations between Mn concentration and the same parameters, none of the correlations with removal efficiency are statistically significant. Although the correlations between Mn removal efficiency and Eh for both treatments were better (Tables 3.4 and 3.5), the values are too low (0.15) to suggest a meaningful relationship between the two variables.

Contrary to the Phase I results, the rate of Mn removal is unknown in these treatments due to the delay caused by filling the tanks. However, the results suggest a consistent removal of Mn over a period of 65 days. The mean influent Mn concentration was approximately 85 mg $L^{-1}$, thus over a period of 65 days approximately 8 g of Mn were added to each replicate. A portion of the Mn was anticipated to have sorbed to the treatment matrix. However, the consistency of the Mn removal over the entirety of the treatment period points to a second functioning removal mechanism. The creek sediment is composed of carbonaceous parent material and the biosolids used in the research are lime-stabilized, thus, especially in the combination treatment, there is a substantial concentration of carbonates, in addition to the sulfide generated by $SO_4^{2-}$ reduction. Given the pH of the treatments (7.5) and the Eh (-250 mV) as shown in Fig. 3.9 and 3.10, MnS could be a stable precipitate in the treatment tanks (Fig. 3.2). However, given that neither the Mn concentration nor the Mn removal rate is correlated with the Eh or pH, it is likely other removal mechanisms are at least partially responsible for Mn removal, including carbonate precipitation, oxide formation, or sorption to the treatment matrix. The mulch and creek sediment used in the bioreactors is identical to that used in Phase I of the study. Consequently, there is an extremely high cation exchange capacity of approximately 53 meq per 100 grams of material. The creek sediment is also derived from carbonitic parent material and the biosolids were lime-stabilized, accounting for an extremely substantial source of carbonates in the reactor matrix. Due to the consistent removal of Mn during the entirety of the treatment period, sorption is not likely to be the principle method of Mn removal; however, it is possible that the sorption capacity of the matrix was not exceeded during the trial period. The consistency of the Mn removal indicates multiple coinciding removal mechanisms, which is ideal for any treatment system as it increases the effectiveness and the life span of the system.

### *3.3.2 Sulfate Removal*

Both treatments successfully enhanced $SO_4^{2-}$ removal, accounting for greater than 70% reduction in $SO_4^{2-}$ concentrations during the treatment period. The addition of the biosolids to the treatment mixture did not significantly impact the removal capacity of the matrix, accounting for a slight decrease (6%) in $SO_4^{2-}$ removal. The influent $SO_4$-S concentration averaged 457 mg $L^{-1}$. By day 15, mean $SO_4$-S concentrations had decreased to approximately 275 mg $L^{-1}$ in both treatments, showing a distinctive development of $SO_4^{2-}$ reducing conditions during the greater portion of the treatment period (Fig. 3.16). Sulfate concentrations stabilized at approximately 150 mg $L^{-1}$ around day 60, providing a final removal efficiency of approximately 70%. As with Mn, $SO_4$-S concentrations were correlated with electrical conductivity (Fig. 3.17) and pH (Fig. 3.18) of the effluent and Eh within the reactors (Fig. 3.19). As expected, there was a moderate correlation between the redox status and $SO_4^{2-}$ concentrations for the combined treatment (Tables 3.4-3.5). However, there was no correlation between Eh and $SO_4$-S concentrations for the mulch treatment. As explained previously, one of the mulch treatment replicates did not maintain strongly reducing conditions for approximately 30 days during the treatment problem. Consequently, the $SO_4^{2-}$ removal efficiency from that replicate was much lower than the other two replicates as shown in Fig. 3.20. The biosolids added to the treatment mixture may have reduced the solution more rapidly than the mulch alone; however, it is unclear due to the mulch replicate which did not maintain reducing conditions. The disparity in $SO_4$-S concentration and Eh between the three mulch treatment replicates may have contributed to the lack of correlation between mean Eh and mean $SO_4$-S concentration for the treatment.

In contrast to the Eh-$SO_4^{2-}$ relationship, the mulch treatment showed a stronger correlation between $SO_4^{2-}$ concentrations and pH than the combined treatment, though still insignificant. All six of the replicates maintained a constant pH of approximately 7.8 throughout the duration of the experiment. Consequently, the difference in the correlations between the treatments and the pH is due to the variability in the $SO_4^{2-}$ concentration. There was also a moderate correlation between the electrical conductivity and $SO_4^{2-}$ concentration, which was expected. The electrical current is transmitted via solid particles and dissolved salts, such as $SO_4^{2-}$ salts. A portion of the Mn may have complexed with the $SO_4^{2-}$ to form $MnSO_{4(aq)}$, which would be drained from the system in the effluent due to its high solubility. In addition, many other ions may have complexed with the $SO_4^{2-}$, such as $Ca^{2+}$ or $K^+$, both of which are common in the treatment matrix. The removal efficiency of the $SO_4^{2-}$ and the pH, Eh, and EC were also correlated to detect correlations in Fig. 3.21-3.23. Similarly to the previously discussed correlations, none indicated a significant relationship between any parameter and $SO_4^{2-}$ removal.

## *3.4 Conclusions*

The use of a bioreactor to enhance $SO_4^{2-}$ reducing conditions and Mn removal appears to be a valid treatment system for alkaline mine drainage. In the absence of Fe, Mn can be readily removed via precipitation with carbonate, sulfide, or oxide formation. The addition of biosolids to the treatment mixture did not significantly enhance the benefits observed for the mulch treatment without biosolids. The lifespan of the treatment matrix is unknown at this time as the reactors showed no decrease in removal efficiency of either Mn or $SO_4^{2-}$ during the treatment period. This was expected, however, as previous research has shown bioreactors to function after a period of more than two years without regeneration of the matrix (Benner et al., 2002; Waybrant et al., 2002). Our findings suggest that by using waste materials, which are readily available and inexpensive for the treatment matrix, we could enhance optimal treatment conditions to remove both Mn and excessive $SO_4^{2-}$ from solution, providing a method for an inexpensive and effective treatment technique for alkaline mine drainage in Eastern Kentucky.

Table 3.1 Solubility products of common metal compounds. All values are the negative log of the solubility product (-log $K_{so}$). Data derived from McBride, 1994.

**Carbonates**

| Pb | Cd | Fe | Mn | Zn | Ca |
|---|---|---|---|---|---|
| 13.1 | 11.7 | 10.7 | 10.4 | 10.2 | 8.42 |

$K_{so} = (M^{2+})(CO_3^{2-})$

**Oxides and Hydroxides**

| $Fe^{3+}$ | $Al^{3+}$ | $Hg^{2+}$ | $Cu^{2+}$ | $Zn^{2+}$ | $Pb^{2+}$ | $Fe^{2+}$ | $Cd^{2+}$ | $Mn^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|---|---|
| 39 | 31.2 | 25.4 | 20.3 | 16.9 | 15.3 | 15.2 | 14.4 | 12.8 | 11.2 |

$K_{so} = (Mn^{+})(OH^{-})_n$

**Sulfides**

| Hg | Cu | Pb | Cd | Zn | Fe | Mn |
|---|---|---|---|---|---|---|
| 52.1 | 36.1 | 27.5 | 27 | 24.7 | 18.1 | 13.5 |

$K_{so} = (M^{2+})(S^{2-})$

Table 3.2 Average cation and anion concentrations in water samples from restoration sites.

| *Site** | EC | Cl | $SO_4^{2-}$ | Mg | Ca | K | Na |
|---|---|---|---|---|---|---|---|
|  | µS | ----------------------------mg $L^{-1}$-------------------------------- |  |  |  |  |  |
| LM (30yr)† | 46 | 0.6 | 10 | 1.8 | 2.3 | 1.3 | 1.1 |
| LMp^Δ | 46 | 1.4 | 10 | 1.5 | 1.4 | 1.5 | 1.3 |
| LMi^Δ | 24 | 1.7 | 10 | 1.7 | 1.4 | 1.5 | 1.4 |
| GC 1‡ | 478 | 1.3 | 225 | 43 | 47 | 8.9 | 7.6 |
| GC 2 | 1732 | 2.2 | 1280 | 205 | 125 | 7.3 | 8.4 |
| GC 3 | 1440 | 2.1 | 1018 | 162 | 109 | 11.4 | 10.6 |
| GC 4 | 1723 | 2.3 | 1293 | 204 | 137 | 11.4 | 13.1 |
| LF up | 1692 | 2.5 | 1299 | 196 | 136 | 12.4 | 15.4 |
| LF down | 1685 | 2.4 | 1344 | 190 | 138 | 11.2 | 16.7 |

*LM =Little Millseat reference stream; GC = Guy Cove restoration stream; LF = Laurel Fork stream, upstream and downstream of the Guy Cove outlet.

†Average from weekly samples collected over a thirty year period.

‡Guy Cove samples collected monthly starting June, 2004.

^Δp = perennial weir, I = intermittent flume.

Table 3.3 Average nutrient and metal concentrations in water samples from the restoration sites.

| *Site** | pH | $NO_3$ | $NH^4$ | TOC | Alk | Fe | Mn |
|---|---|---|---|---|---|---|---|
| | S.U. | ----------------------------mg $L^{-1}$-------------------------------- | | | | | |
| LM (30yr)$^{\dagger}$ | 6.46 | 0.13 | NA | 4.99 | 17 | -- | -- |
| LMp$^{\Delta}$ | 6.53 | 0.14 | 0.04 | 4.83 | 25 | 0.12$^{\Psi}$ | 1.1$^{\Psi}$ |
| LMi$^{\Delta}$ | 6.72 | 0.09 | 0.04 | 5.31 | 20 | 0.15$^{\Psi}$ | 0.9$^{\Psi}$ |
| GC 1$^{\ddagger}$ | 7.94 | 0.09 | 0.07 | 30.1 | 324 | 0.28 | 4.0 |
| GC 2 | 6.35 | 0.02 | 0.47 | 12.1 | 88 | 2.96 | 26.0 |
| GC 3 | 6.46 | 0.02 | 0.10 | 15.2 | 115 | 3.49 | 23.5 |
| GC 4 | 7.04 | 0.01 | 0.09 | 9.6 | 79 | 0.69 | 21.4 |
| LF up | 7.03 | 0.02 | 0.17 | 9.5 | 79 | 2.48 | 19.5 |
| LF down | 6.87 | 0.03 | 0.21 | 9.4 | 72 | 3.56 | 17.6 |

*LM =Little Millseat reference stream; GC = Guy Cove restoration stream; LF = Laurel Fork stream, upstream and downstream of the Guy Cove outlet.
$^{\dagger}$Average from weekly samples collected over a thirty year period.
$^{\ddagger}$Guy Cove samples collected monthly starting June, 2004.
$^{\Psi}$Samples collected monthly in 2006.
$^{\Delta}$p = perennial weir, I = intermittent flume
TOC = Total organic carbon; Alk = Alkalinity

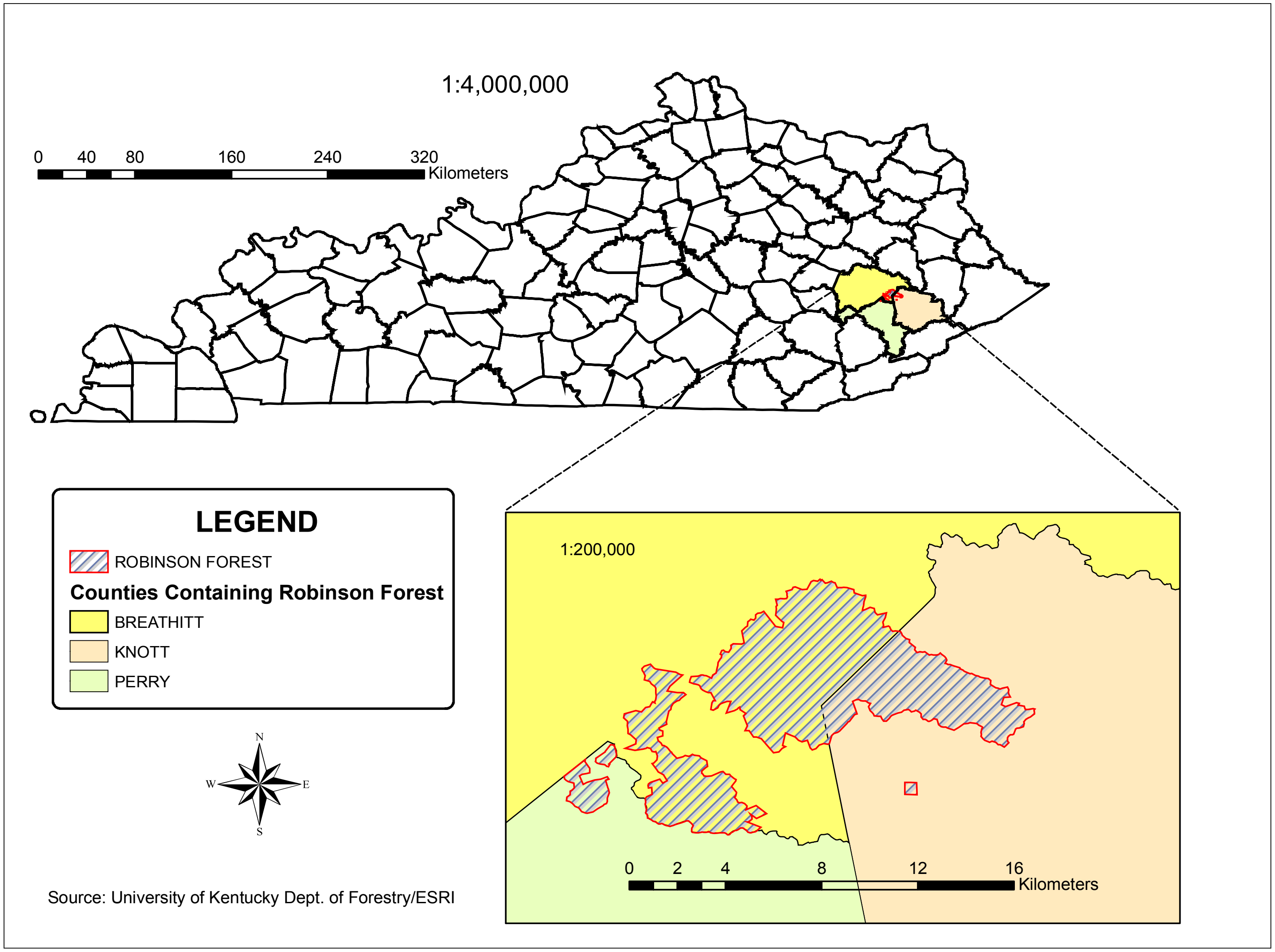
Table 3.4 Correlation coefficient values for the relationship between the primary contaminants and the indicator parameters for the combined mulch and biosolid treatment. None of the correlations were statistically significant.

| | Mn (mg $L^{-1}$) | $SO_4$-S (mg $L^{-1}$) | % Mn Removal | % $SO_4$-S Removal |
|---|---|---|---|---|
| Electrical Conductivity | 0.08 | 0.27 | 0.02 | 0.27 |
| pH | 0.01 | 0.00 | 0.13 | 0.00 |
| Redox Status | 0.00 | 0.24 | 0.13 | 0.24 |

Table 3.5 Correlation coefficient values for the relationship between the primary contaminants and the indicator parameters for the mulch treatment. None of the correlations were statistically significant.

| | Mn (mg $L^{-1}$) | $SO_4$-S (mg $L^{-1}$) | % Mn Removal | % $SO_4$-S Removal |
|---|---|---|---|---|
| Electrical Conductivity | 0.15 | 0.19 | 0.12 | 0.19 |
| pH | 0.01 | 0.13 | 0.01 | 0.00 |
| Redox Status | 0.09 | 0.02 | 0.15 | 0.07 |

Figure 3.1 Map showing the location and boundaries of the University of Kentucky's research forest, Robinson Forest.



Source: University of Kentucky Dept. of Forestry/ESRI

Figure 3.2 Manganese stability diagram, from Stumm & Morgan, 1996. A greater Mn concentration increases the region of stability of the Mn precipitates.

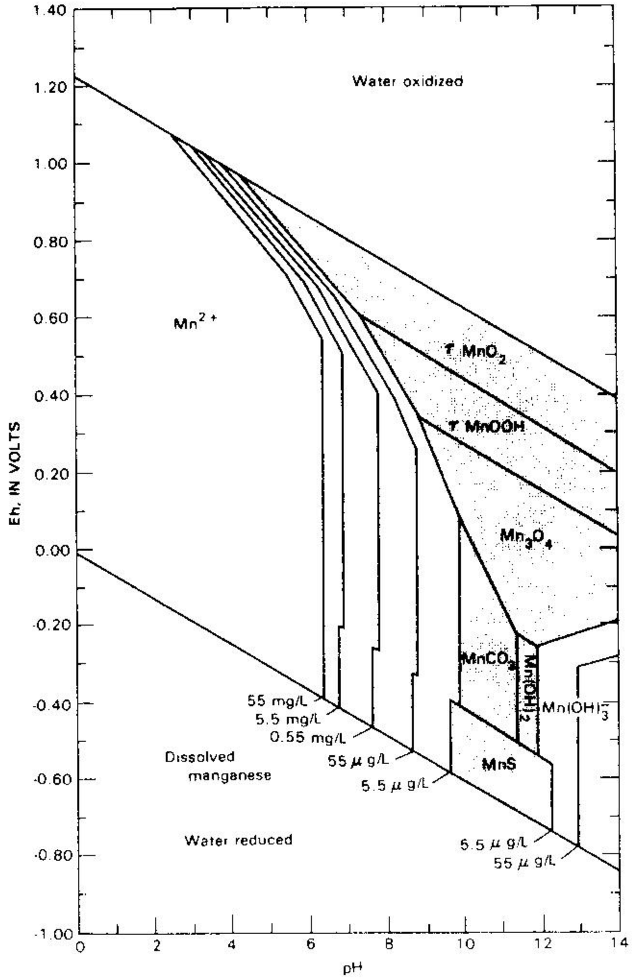


Figure 3.3 Map indicating the location of Guy Cove within Robinson Forest.

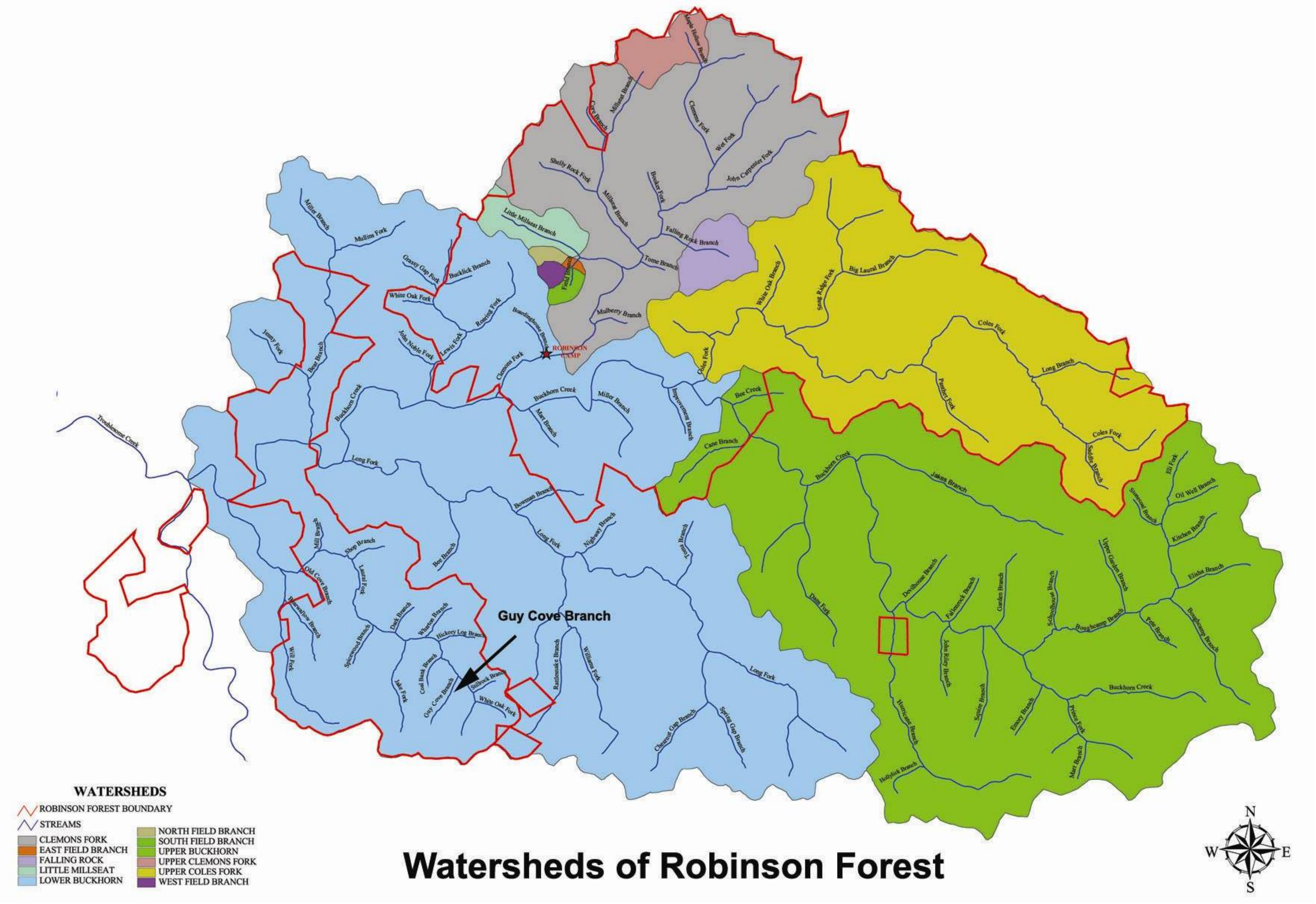


Figure 3.4 Photograph of the section of the plumbing inside the tank. Holes drilled in the horizontal piece of pipe allowed influent to drain uniformly into the treatment matrix.

Figure 3.5 Picture of the tank without the redox probe installed.

Figure 3.6 Picture of the tanks after being filled with the treatment material and the installation of the redox probe.

Figure 3.7 Manganese concentrations during the trial period. Influent Mn concentration averaged 85 mg L$^{-1}$. Each value is the mean of three replicates.

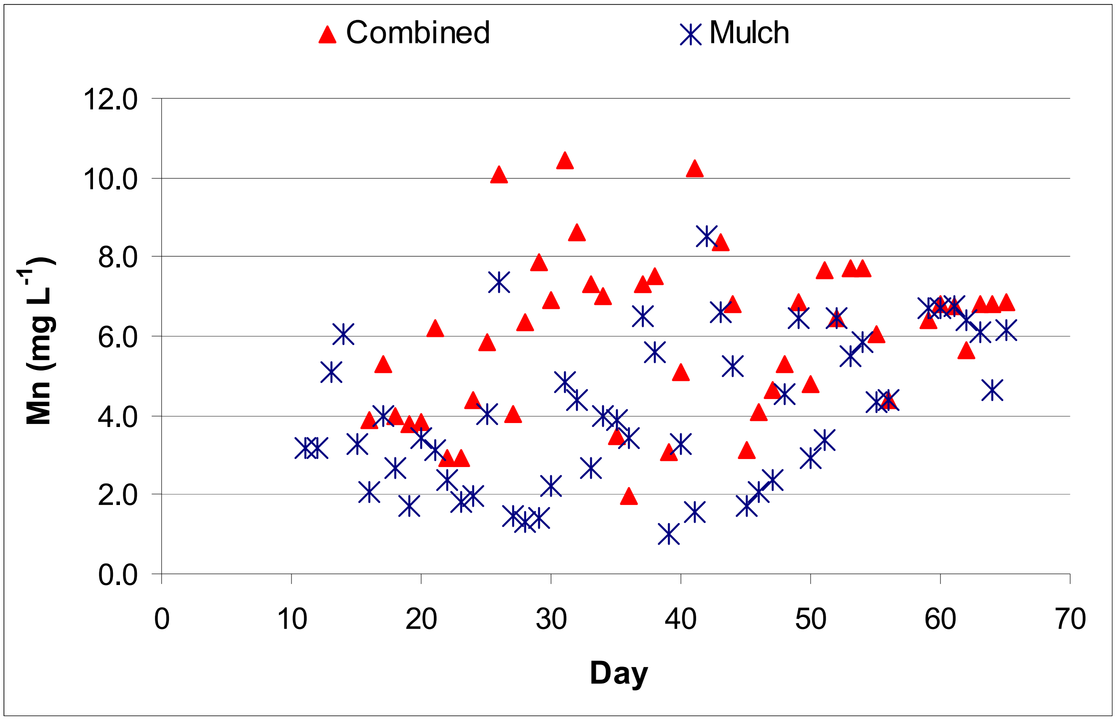


Figure 3.8 Relationship of the Mn concentrations (mg L$^{-1}$) and electrical conductivity (µS) of the effluent and correlation coefficients for each treatment.

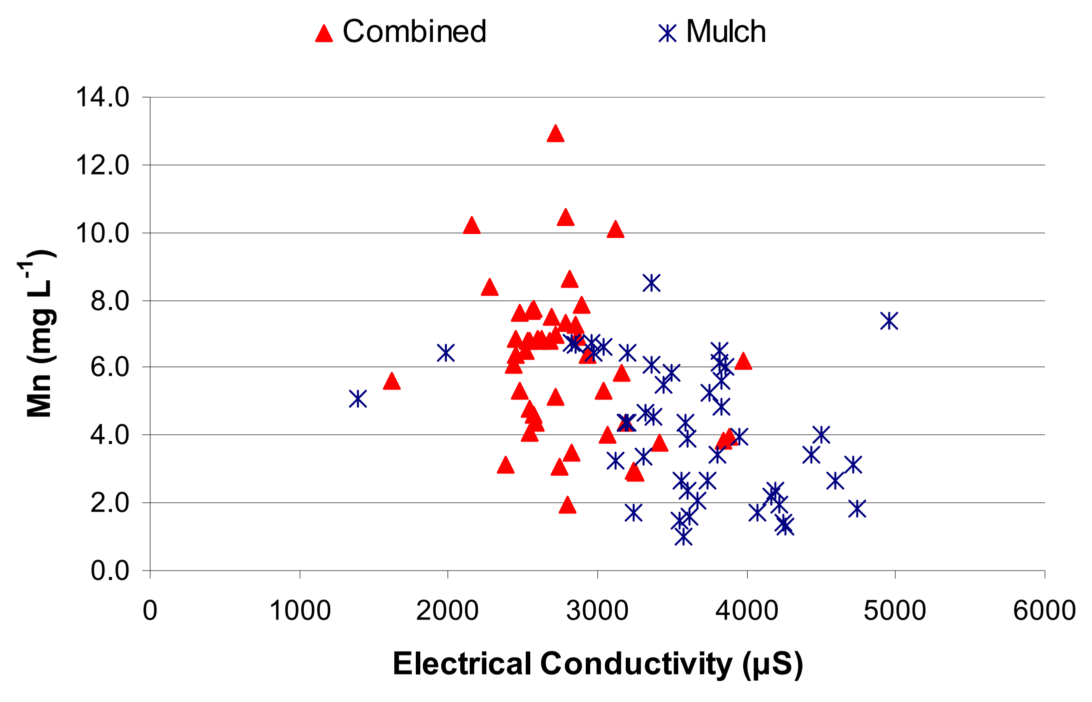


Figure 3.9 Correlation of Mn concentrations (mg L$^{-1}$) and pH of the effluent for both treatments.

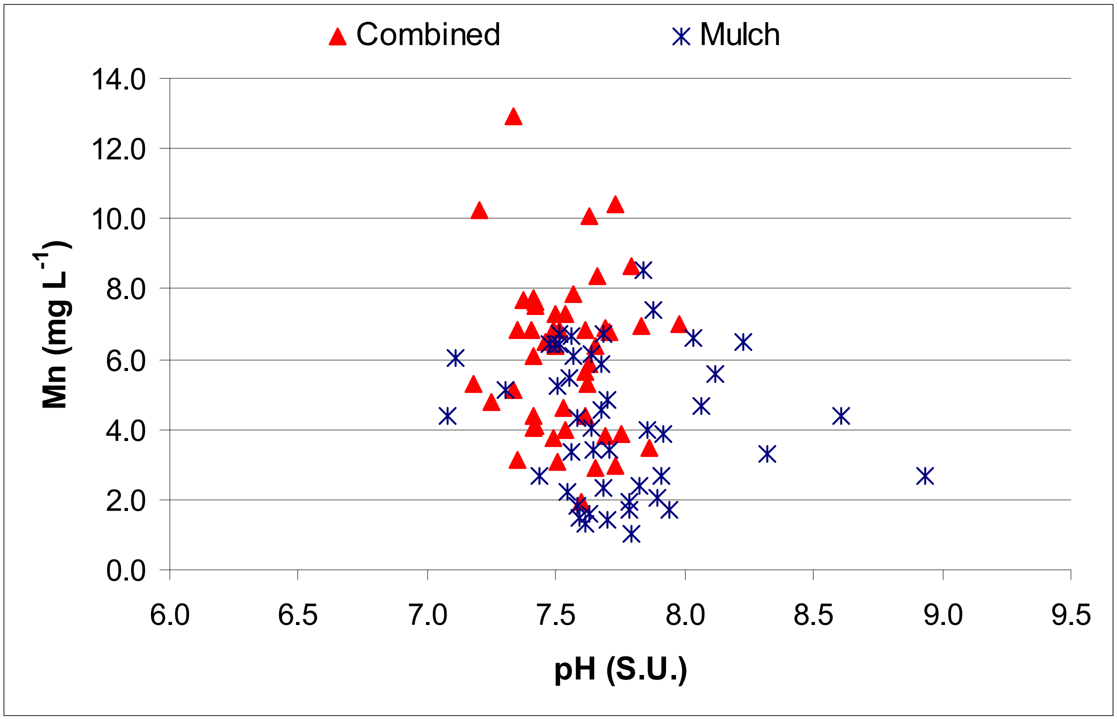


Figure 3.10 Mn concentrations (mg $L^{-1}$) as a function of Eh (mV) within the treatment tank.

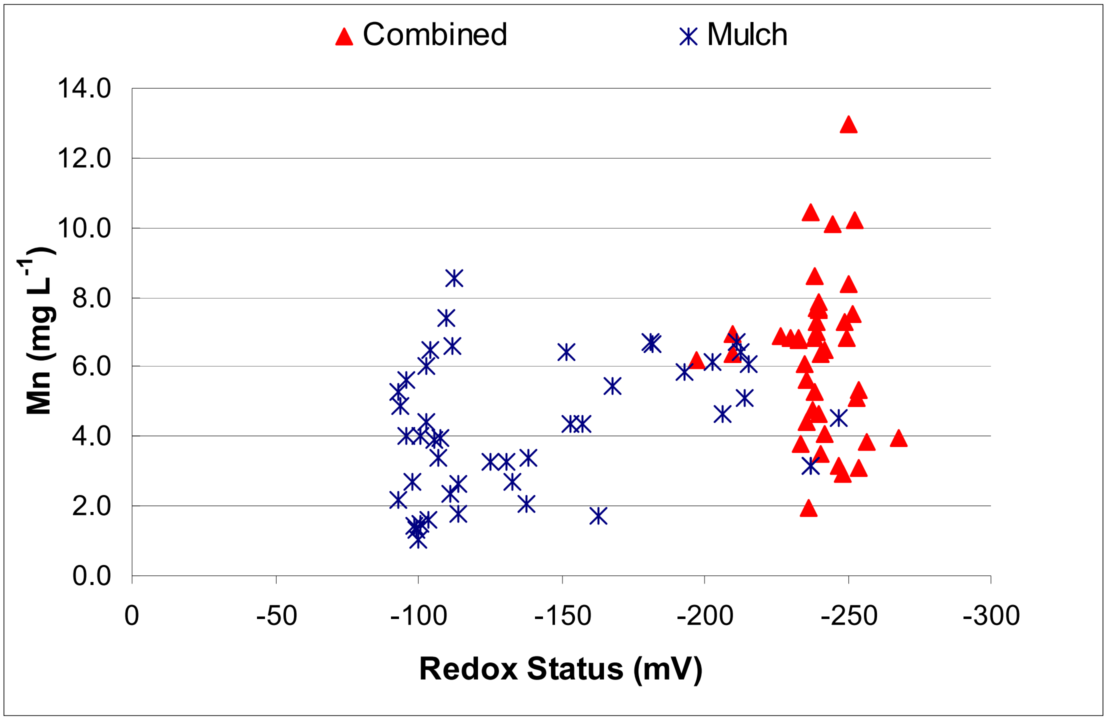


Figure 3.11 Redox status (mV) of the mulch treatment replicates during the trial period.

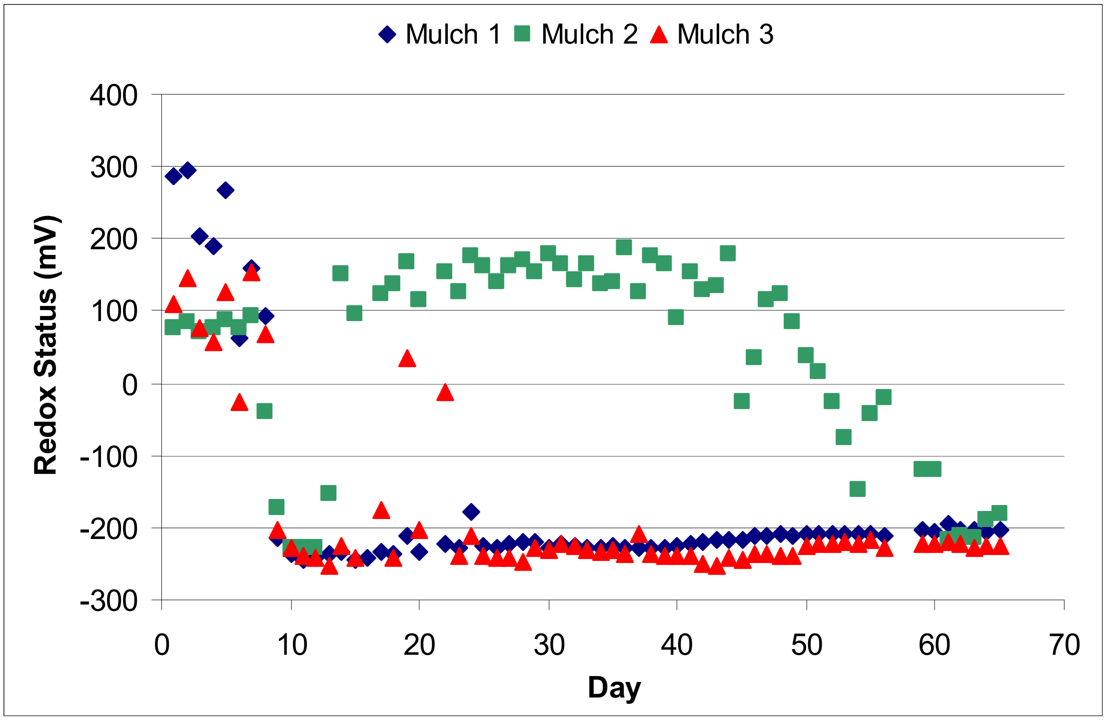


Figure 3.12 Mn concentrations (mg $L^{-1}$) of the three mulch treatment replicates. Influent Mn concentrations averaged 85 mg $L^{-1}$.

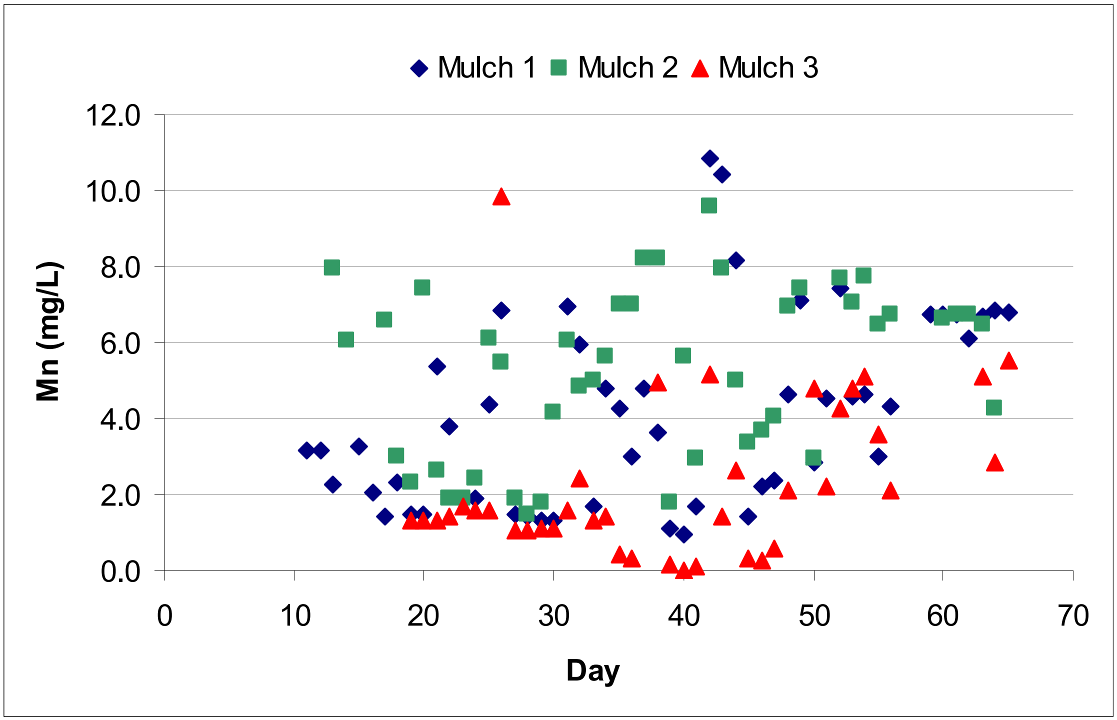


Figure 3.13 Mn removal efficiency (%) correlated with the electrical conductivity (µS) of the samples. Neither treatment resulted in a significant correlation.

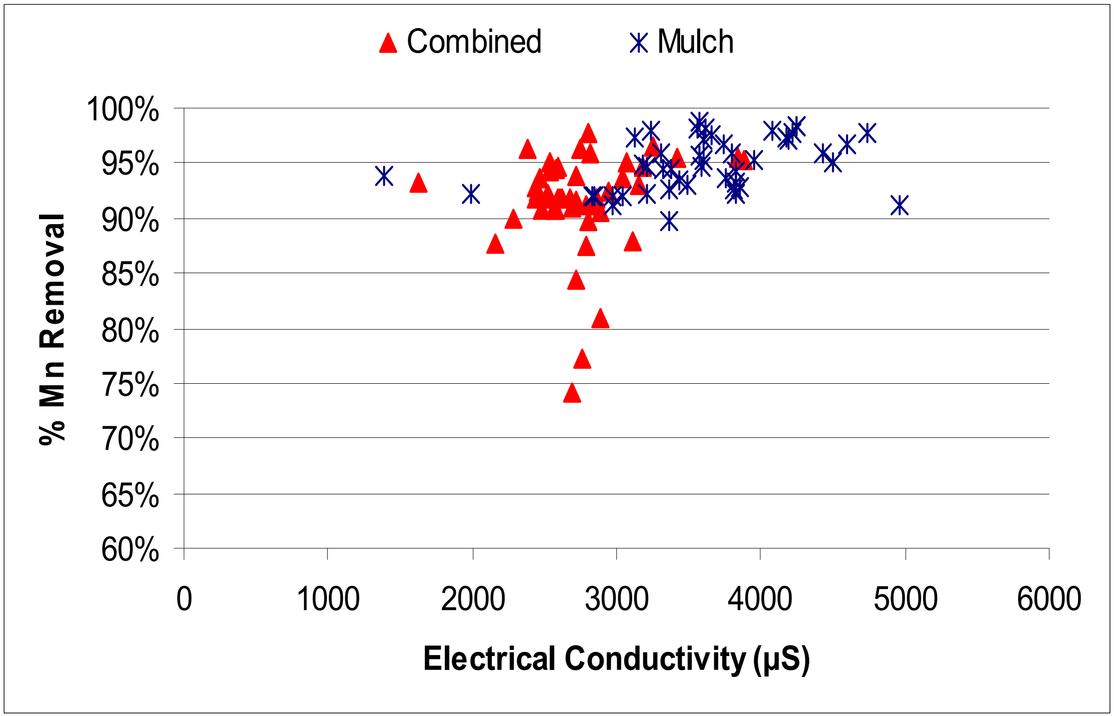


Figure 3.14 Correlation of Mn removal efficiency (%) and redox status (mV).

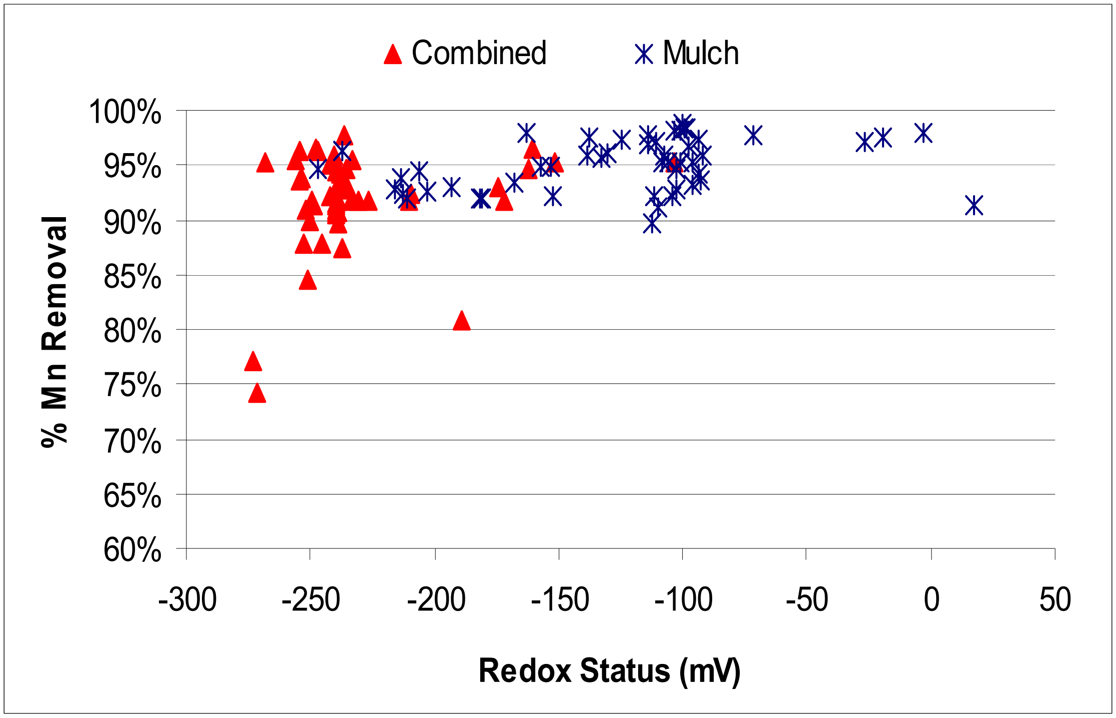


Figure 3.15 Mn removal efficiency (%) correlated with pH. Neither correlation is significant.

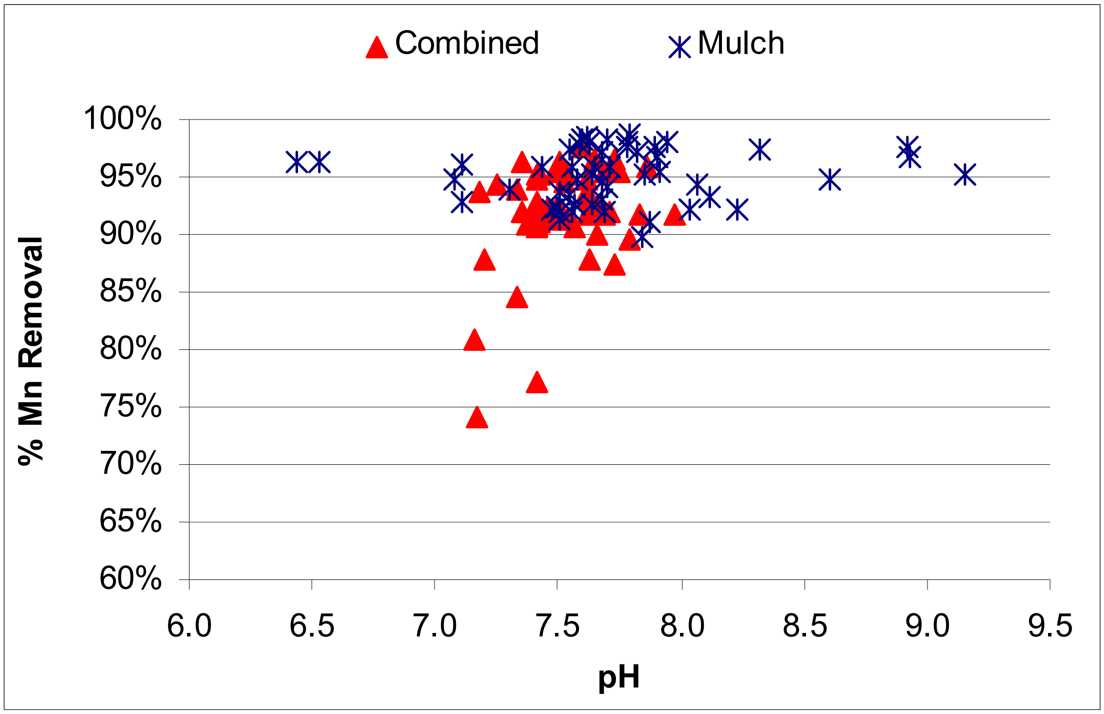


Figure 3.16 Mean sulfate concentrations (mg L$^{-1}$) during the trial period for each treatment. Influent $SO_4$-S concentration averaged 450 mg L$^{-1}$. (n=3)

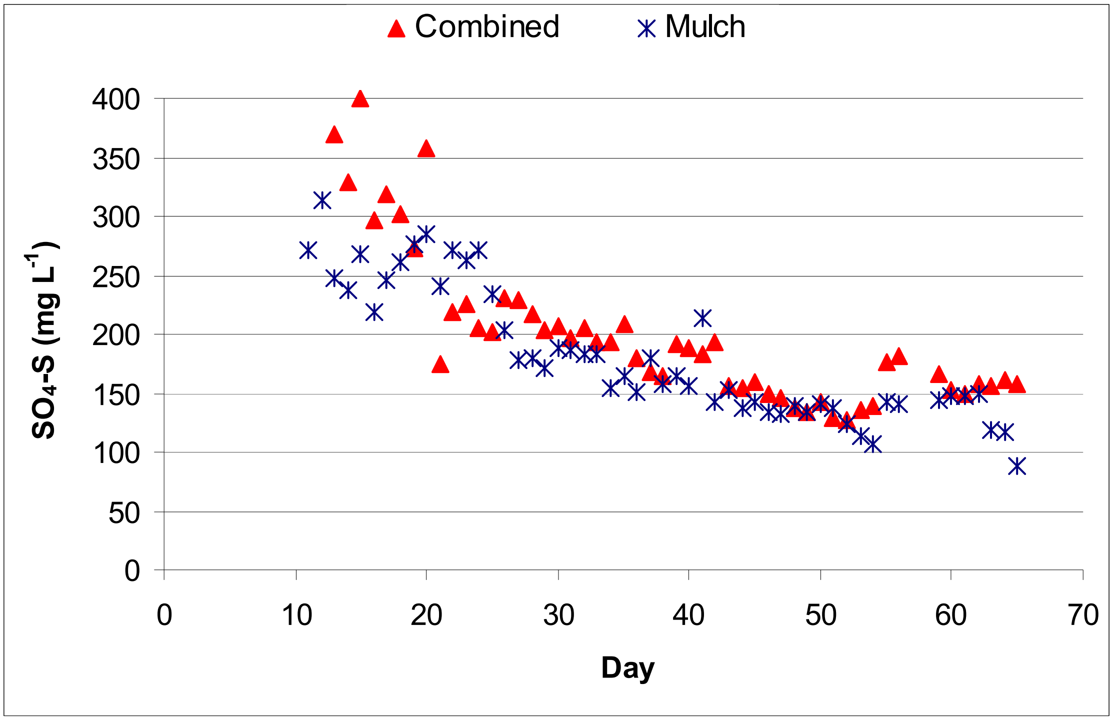


Figure 3.17 Correlation of the mean electrical conductivity (µS) and the mean sulfate concentrations (mg L$^{-1}$) of each treatment.

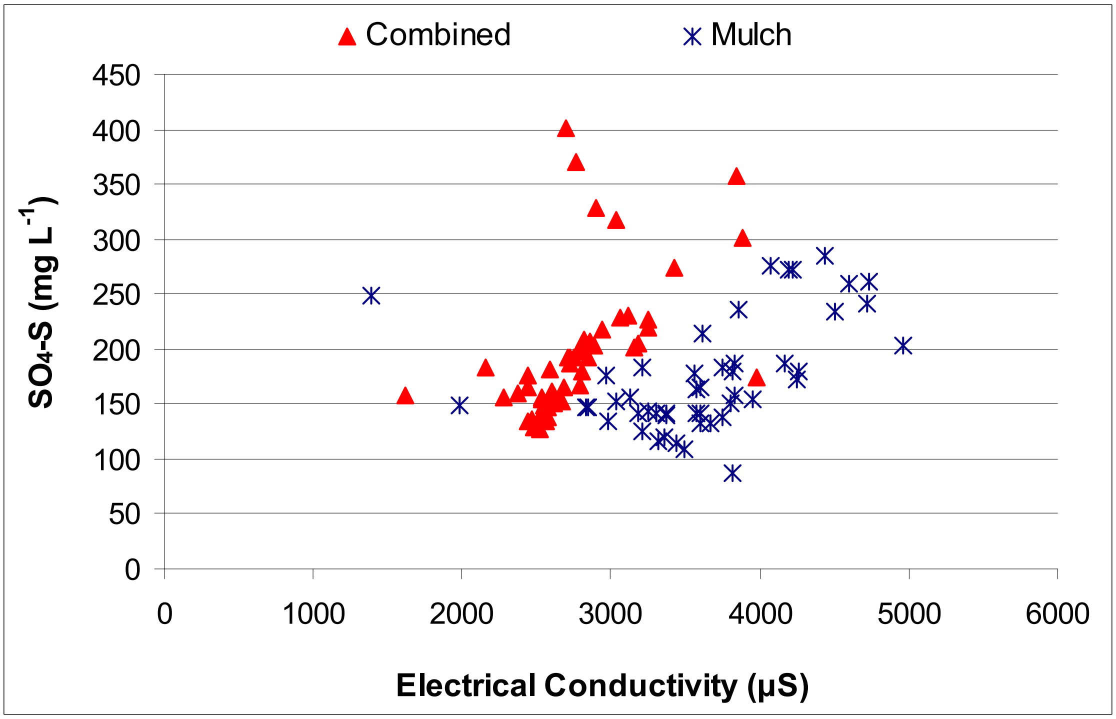


Figure 3.18 Correlation of mean sulfate concentrations (mg L$^{-1}$) and pH.

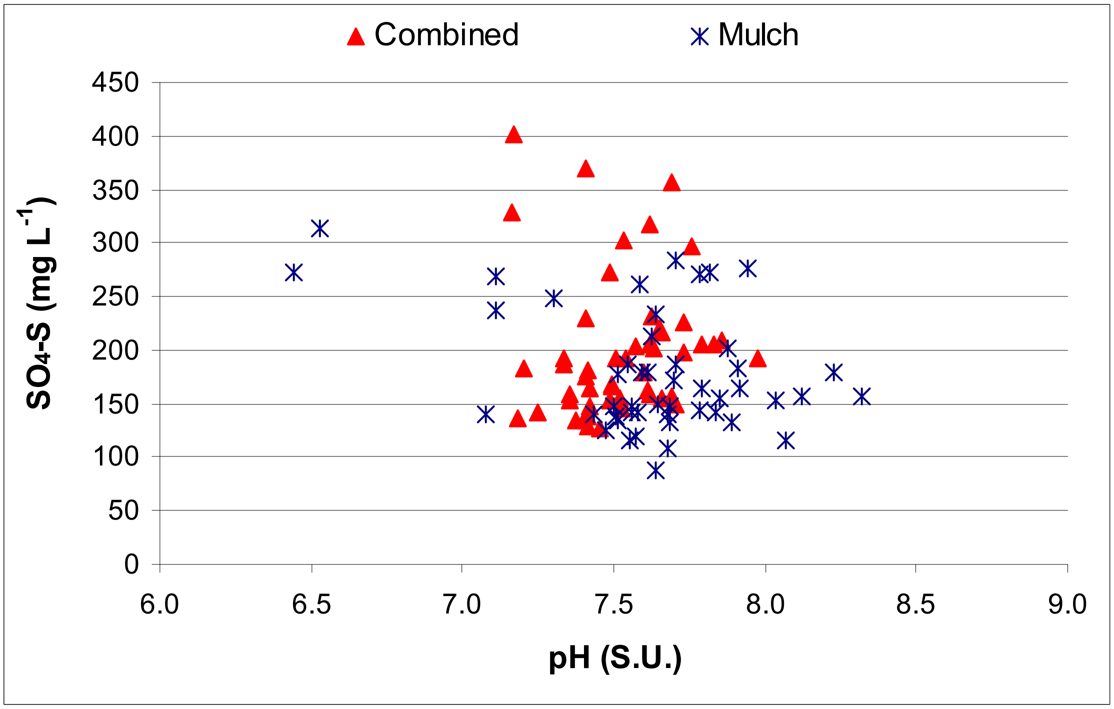


Figure 3.19 Correlation of mean redox status (mV) and the mean sulfate concentrations (mg L$^{-1}$) for each treatment.

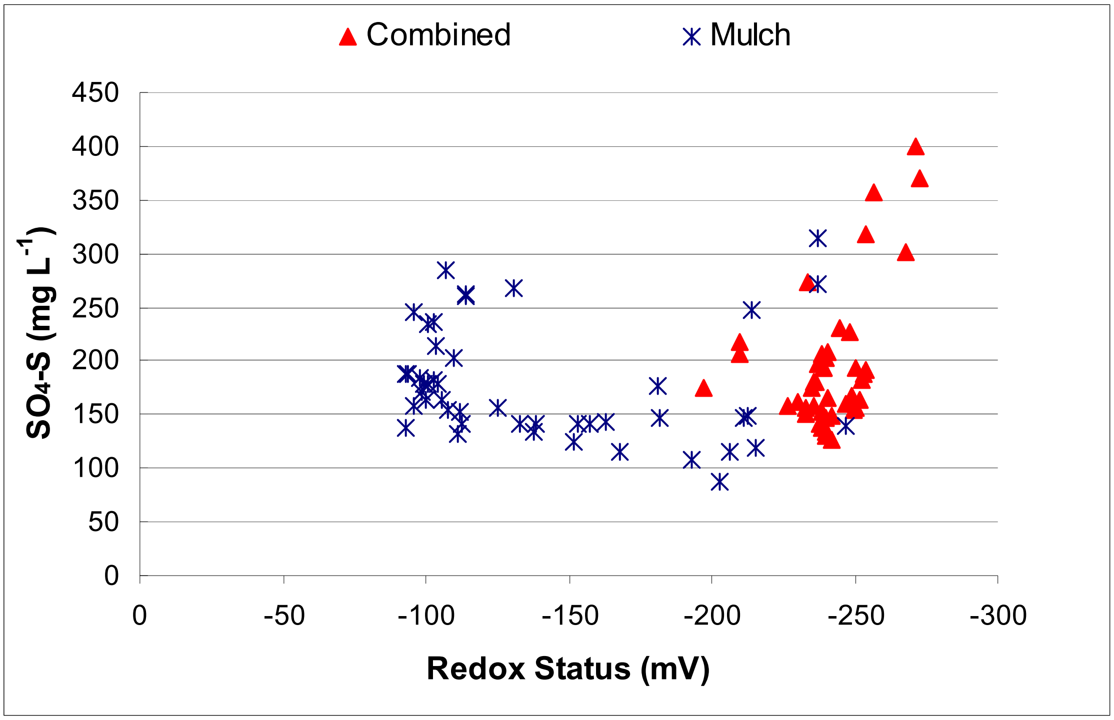


Figure 3.20 Effluent $SO_4$-S concentrations (mg $L^{-1}$) of the three mulch treatment replicates. Influent $SO_4$-S concentration averaged 450 mg $L^{-1}$.

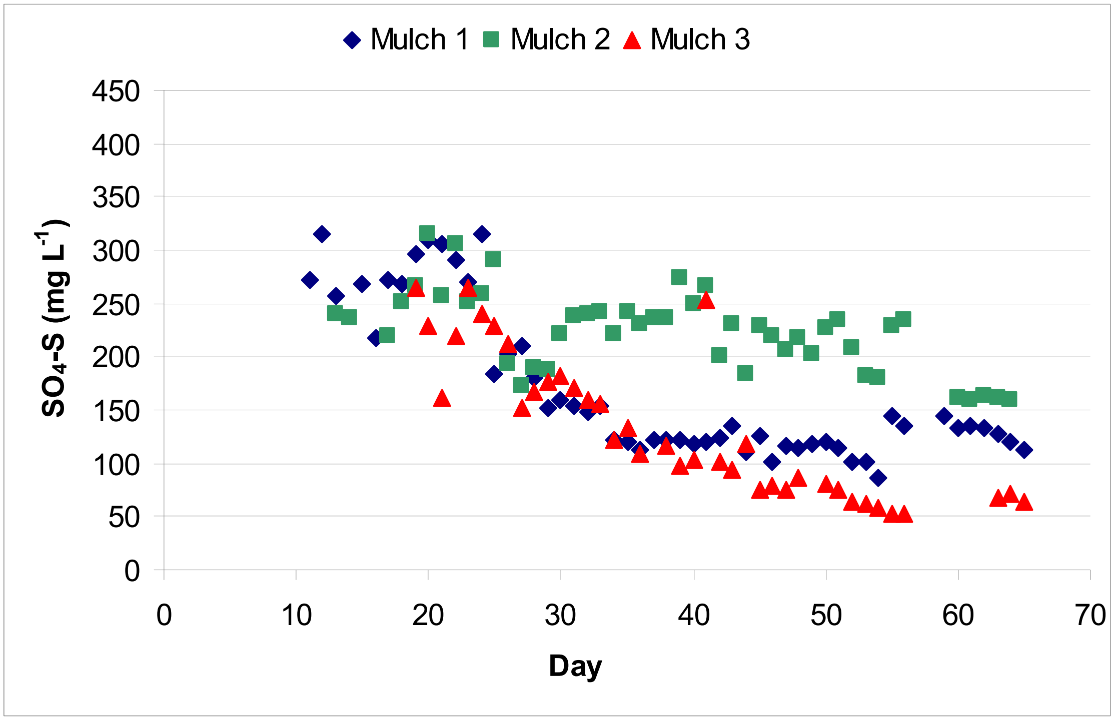


Figure 3.21 Correlation of $SO_4^{2-}$ removal efficiency (%) and pH.

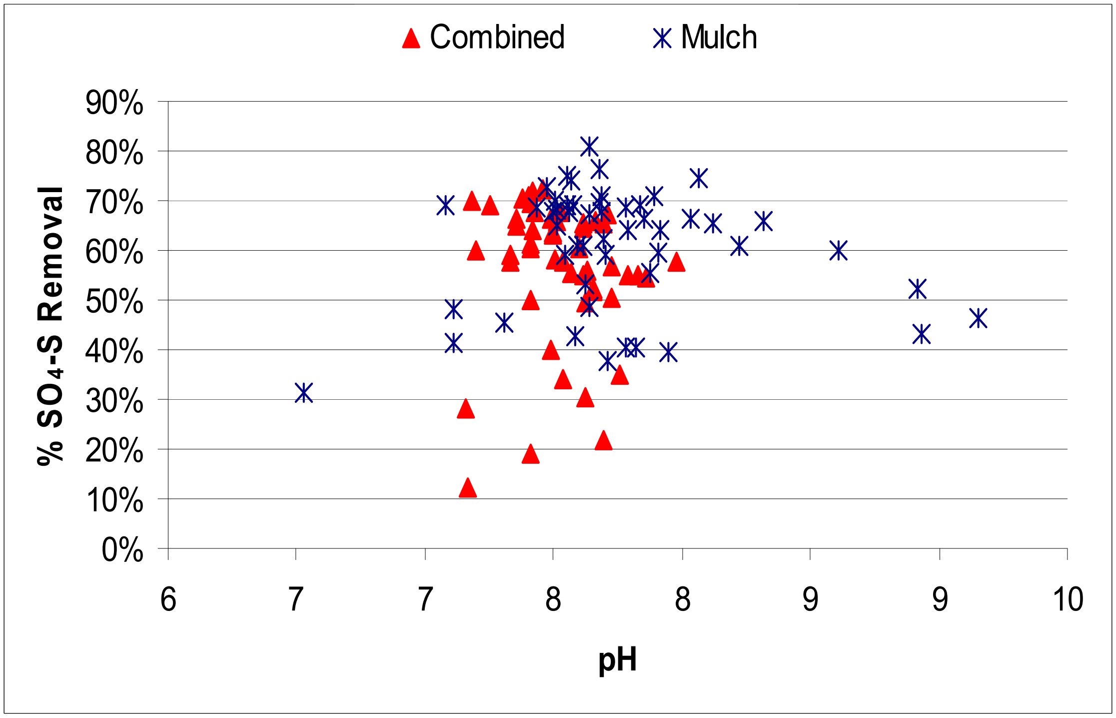


Figure 3.22 Sulfate removal efficiency (%) and redox status (mV) correlation.

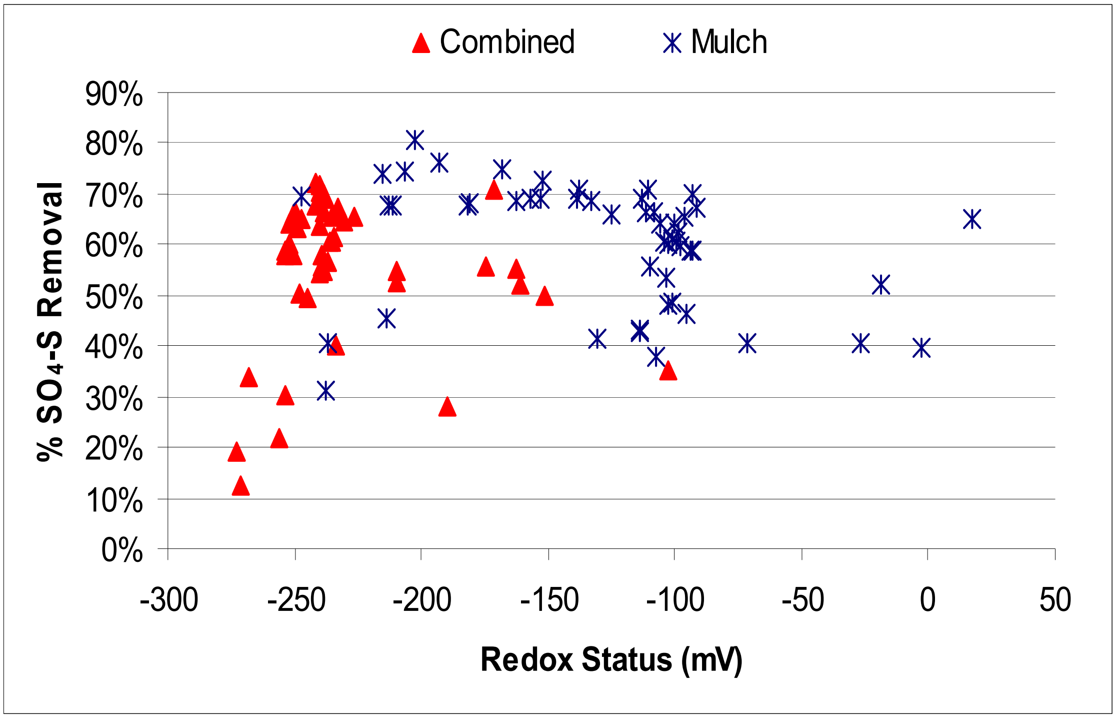
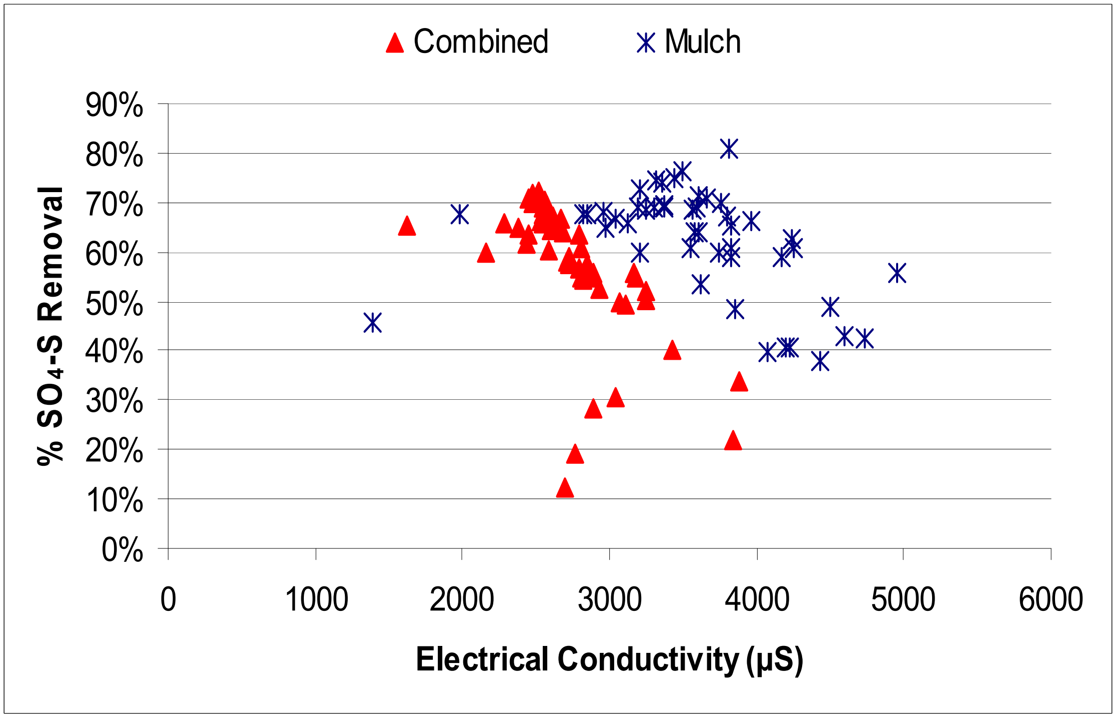


Figure 3.23 Correlation of the sulfate removal efficiency (%) and electrical conductivity (µS).



# CHAPTER 4

# Summary and Conclusions

The primary goal of this study was to develop a bioreactor capable of efficiently reducing $SO_4^{2-}$ to $S^{2-}$ and removing Mn from solution associated with alkaline mine drainage. The anticipated primary Mn removal mechanism was MnS precipitation. However, mineralogical analysis of the substrates utilized in the batch experiment portion of the study (Phase I, Ch. 2) indicated other coinciding Mn removal mechanisms such as simple sorption, $MnCO_3$ formation, and Mn-oxyhydroxide precipitation. Several of the treatment combinations, primarily those involving either biosolids or wood mulch, resulted in greater than 95% removal of the Mn with concomitant reduction of $SO_4^{2-}$ concentrations by 50% or more. The most effective treatment, utilizing creek sediment as the substrate with biosolids as the amendment, resulted in 100% removal of Mn and 85% removal of $SO_4^{2-}$ over the trial period. The results of Phase I also showed Mn being removed very rapidly from solution. Since previous research has shown that several days are generally required for $SO_4^{2-}$ reducing conditions to develop, there was some concern regarding the long-term capability of a bioreactor system to treat Mn contamination. Subsequently, the two most effective treatment combinations (biosolids with creek sediment and wood mulch with creek sediment) detected in the batch experiments were tested in a small scale bioreactor (Phase II).

Unlike the Phase I experiments, where Mn and $SO_4^{2-}$ were added in a single dose, Mn and $SO_4^{2-}$ were added constantly in the latter phase of the experiment in an effort to mimic the conditions found in a normal mine drainage situation. Due to the inherent problems of using a large mass of biosolids, the biosolids were mixed with the wood mulch in one treatment and the wood mulch was tested in another treatment. The results indicated no significant benefit of adding biosolids to the treatment mixture at the level tested. Both treatments removed approximately 93% of the Mn and 65-70% of the $SO_4^{2-}$, with no statistical differences detectable. There was also no detectable decrease in the removal efficiency of either contaminant during the 65 day trial period. Our results from both Phases of our study indicate Mn may be removed from solution via $SO_4^{2-}$ reduction and subsequent precipitation. In addition, this method should require minimal maintenance and is relatively inexpensive. Though the bioreactors were able to significantly reduce Mn and $SO_4^{2-}$ concentrations during the entirety of the trial period, the long term efficacy of the system is unknown. One of the main reasons the biosolid treatments were so effective in Phase I is most likely due to the extremely high concentration of $CO_3$. The creek sediment utilized in the bioreactors contained approximately 2% $CO_3$. Thus, one potential strategy to increase the longevity of the matrix would be to mix in some portion of small limestone pieces to increase carbonate concentrations. Another potential strategy would be to add either sorghum syrup or soybean oil to the matrix as the effectiveness begins to decrease. Though neither amendment was effective at enhancing the treatment of Mn or $SO_4^{2-}$ solely, in combination with other amendments (i.e. wood mulch) and a stabilized and fully-functioning microbial community, either could be beneficial as a source of additional carbon. Addition of a liquid amendment would also not require the excavation of the extant matrix and subsequent stabilization period with a new matrix.

Further research would allow additional interpretations about the effectiveness of treating Mn contaminated mine drainage using sulfate reducing bioreactors under more dynamic conditions. Since the bioreactor phase of this experiment was conducted in a greenhouse, temperature and climate were controlled; thus, it would be beneficial to test the system in the more variable environment of natural conditions. It would also be beneficial to test the efficacy of the bioreactor using natural mine drainage, rather than a synthetic drainage solution as was used in this research. As part of the synthetic solution, Fe was intentionally left out in this study because it inhibits Mn removal. However, in natural drainage many metals, including Fe are generally found in solution, which may cause interference or inhibition of Mn removal. The lifespan of the treatment material is also unknown.

Previous research utilizing bioreactors have found widely variant life spans, ranging from a period of several months to several years.  Consequently, these bioreactors should be monitored regularly to detect a decrease in treatment efficiency and recharge or replace the treatment matrix as necessary.

## Works Cited

### *Chapter 1*

Barton, C. & A.D. Karathanasis. 1999. Renovation of a failed constructed wetland treating acid mine drainage. Environmental Geology. 39:39-50.

Benner, S.G., D.W. Blowes, W.D. Gould, R.B. Herbert, Jr. & C.J. Ptacek. 1999. Geochemistry of a Permeable Reactive Barrier for Metals and Acid Mine Drainage. Environ. Sci. Tech. 33:2793-2799.

Benner, S.G., D.W. Blowes, C.J. Ptacek, & K.U. Mayer. 2002. Rates of sulfate reduction and metal sulfide precipitation in a permeable reactive barrier. Applied Geochemistry. 17:301-320.

Blanton, J.C., J.C. Cobb, M.S. Smith, & P.C. VanBooven. 2003. A Report on the Robinson Forest. University of Kentucky Board of Trustees. Lexington, KY.

Bolis, J.L., T.R. Wildeman, & R.R. Cohen. 1991. The use of bench scale permeameters for preliminary analysis of metal removal from acid mine drainage by wetlands. American Society of Surface Mining and Reclamation, 1991 Annual Meeting, Durango, CO.

Boudreau, B.P. & J.T. Westrich. 1984. The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments. Geochimica et Cosmochimica Acta. 48:2503-2516.

Champagne, P., P. Van Geel, & W. Parker. 2005. A bench-scale assessment of a combined passive system to reduce concentrations of metals and sulphate in acid mine drainage. Mine Water and the Environment. 24:124-133.

Chockalingam, E. & S. Subramanian. 2006. Studies on removal of metal ions and sulphate reduction using rice husk and *Desulfotomaculum nigrificans* with reference to remediation of acid mine drainage. Chemosphere. 62:699-708.

Christensen, B., M. Laake, & T. Lien. 1996. Treatment of acid mine water by sulfate-reducing bacteria; results from a bench scale experiment. Water Research. 30:1617-1624.

Cocos, A.A., G.J. Zagury, B. Clement, & R. Samson. 2002. Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. Water Research. 32:167-177.

Costello, C. 2003. Acid mine drainage: Innovative treatment technologies. United States Environmental Protection Agency, Technology Innovation Office. Washington, D.C.

Dixon, J.B. & H.C.W. Skinner. 1992. Manganese minerals in surface environments. *In* H.C. Skinner & R.W. Fitzpatrick (Eds.), Biomineralization: Processes of Iron and Manganese. Catena, Cremlingen-Destedt, Germany. 1992.

Drury, W.J. 2000. Modeling of sulfate reduction in anaerobic solid substrate bioreactors for mine drainage treatment. Mine Water and the Environment. 19:18-28.

Dvorak, D.H., R.S. Hedin, H.M. Edenborn, & P.E. McIntire. 1992. Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot-scale reactors. Biotechnology and Bioengineering. 40:609-616.

Eastman, J.A. & J.F. Ferguson. 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J. Water Pollution Control Federation. 53:352-366.

Evangelou, V.P. 1998. Environmental Soil and Water Chemistry. John Wiley & Sons, Inc., New York.

Fox, L.E. 1988. The solubility of colloidal ferric hydroxide and its relevance to iron concentrations in river water. Geochimica et Cosmochimica Acta, 52:771-777.

Gammons, C.H. & A.K. Frandsen. 2001. Fate and transport of metals in $H_2S$-rich waters at a treatment wetland. Geochemical Transactions. 2:1.

Ghiorse W.C. & H.L. Ehrlich. 1992. Microbial biomineralization of iron and manganese. *In* H.C. Skinner & R.W. Fitzpatrick (Eds.), Biomineralization: Processes of Iron and Manganese. Catena, Cremlingen-Destedt, Germany. 1992.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2002. Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments. Reviews in Env. Science & Biotechnology. 1:327-333.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2003. Evaluation of municipal compost/limestone/iron mixtures as filling material for permeable reactive barriers for in-situ acid mine drainage treatment. J. Chem. Technol. Biotechnol. 78:489-496.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2005. Municipal compost-based mixture for acid mine drainage bioremediation: Metal retention mechanisms. Applied Geochemistry. 20:1648-1657.

Gusek, J.J. 2004. Design challenges for large scale sulfate reducing bioreactors. Soil and Sediment Contamination. 13:169-180.

Huntsman, B.E., J.B. Solch, M.D. Porter. 1978. Utilization of a Sphagnum species dominated bog for coal acid mine drainage abatement. Abstracts, 91st Annual Meeting of the Geologic Society of America, Ottawa, Ontario, Canada.

Karathanasis, A.D. & C. Barton. 1997. Ameliorative designs to improve the efficiency of constructed wetlands treating high metal load acid mine drainage in the Rock Creek watershed – Final Report. Kentucky Division of Water. Grant No. C9994506-94-0.

Keen, C.L., J.L. Ensunsa, & M.S. Clegg. 2000. Manganese metabolism in animals and humans including the toxicity of manganese. *In* A. Sigel & H. Sigel (Eds.), Manganese and its role in biological processes. Marcel Dekker, Inc., New York. 2000.

Kentucky Office of Mine Safety and Licensing. 2006. 2005 Annual Report.

Knox, A.S., M.H. Paller, E.A. Nelson, W.L. Specht, N.V. Halverson, & J.B. Gladden. 2006. Metal distribution and stability in constructed wetland sediment. J. Environmental Quality, 35:1948-1959.

Kolmert, A. & D.B. Johnson. 2001. Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. J. Chemical Tech. & Biotechnology. 76:836-843.

Kuyucak, N., F. Chabot, & J. Martschuk. 2006. Successful implementation and operation of a passive treatment system in an extremely cold climate, northern Quebec, Canada. Proc. 7th Int. Conf. on Acid Rock Drainage (ICARD), March 26-30, 2006, St. Louis, MO. *In* R.I. Barnhisel (ed.) American Society of Mining and Reclamation, Lexington, KY. 38:3131-3138.

Leopold, L.B. 1964. Fluvial processes in geomorphology. W.H. Freeman, San Francisco, CA.

Ludwig, R.D., R.G. McGregor, D.W. Blowes, S.G. Benner, & K. Mountjoy. 2002. A permeable reactive barrier for treatment of heavy metals. Ground water. 40:59-66.

Machemer, S.D. & T.R. Wildeman. 1992. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J. Contaminant Hydrology. 9:115-131.

McBride, M.B. 1994. Environmental Chemistry of Soils. Oxford University Press, New York.

McIntire, P.E. & H.M. Edenborn. 1990. The use of bacterial sulfate reduction in the treatment of drainage from coal mines. Proc. 1990 Mining and Reclamation Conference.

Morgan, J.J. 2000. Manganese in natural waters and Earth's crust: Its availability to organisms. *In* A. Sigel & H. Sigel (Eds.), Manganese and its role in biological processes. Marcel Dekker, Inc., New York. 2000.

Nagpal, S., S. Chuichulcherm, A. Livingston, & L. Peeval. 2000. Ethanol utilization by sulfate-reducing bacteria: An experimental and modeling study. Biotechnology & Bioengineering. 70:533-543.

Neculita, C., G.J. Zagury, & B. Bussiere. 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. J. Environ. Qual. 36:1-16.

Pond, G.J. 2004. Effects of surface mining and residential land use on headwater stream biotic integrity in the Eastern Kentucky Coalfield region. Kentucky Department of Environmental Protection, Division of Water.

Sass, B., A. Gavaskar, W.S. Yoon, N. Gupta, E. Drescher, & C. Reeter. 2001. Geochemical investigation of three permeable reactive barriers to assess impact of precipitation on performance and longevity. Proceedings of the International Containment & Remediation Technology Conference and Exhibition, Orlando, FL. June 12.

Schwertmann, U. & R.W. Fitzpatrick. 1992. Iron minerals in surface environments. *In* H.C. Skinner & R.W. Fitzpatrick (Eds.), Biomineralization: Processes of Iron and Manganese. Catena, Cremlingen-Destedt, Germany. 1992.

Sikora, F.J., L.L. Behrends, G.A. Brodie, & M.J. Bulls. 1996. Manganese and trace metal removal in successive anaerobic and aerobic wetlands. National Meeting of the American Society of Surface Mining and Reclamation. Knoxville, TN.

Sikora, F.J., L.L. Behrends, G.A. Brodie, & H.N. Taylor. 2000. Design criteria and required chemistry for removing manganese in acid mine drainage using subsurface flow wetlands. Water Environment Research. 72:536-544.

Skousen, J.G. 1995. Douglas abandoned mine land project: description of an innovative acid mine drainage treatment system. Green Lands 25:29-38.

Skousen, J.G., A. Sexstone, & P. Ziemkiewicz. 2000. Acid mine drainage control and treatment. *In* Barnhisel, R.I., R.G. Darmody, & W.L. Daniels (eds.).

Reclamation of Drastically Disturbed Lands. American Society of Agronomy. Madison, Wisconsin.

Sobolewski, A. 1999. A review of processes responsible for metal removal in wetlands treating contaminated mine drainage. International Journal of Phytoremediation. 1:19-51.

Stark, L.R., W.R. Wenerick, F.M. Williams, & P.J. Wuest. 1995. The effects of pH, flow rate, and carbon supplementation on manganese retention in mesocosm wetlands. J. Environ. Qual. 24:816-826.

Stumm, W. & J.J. Morgan. 1996. Aquatic Chemistry: Chemical equilibria and rates in natural waters – 3rd Ed. John Wiley & Sons, Inc. New York, NY.

Suresh R., C.D. Foy & J.R. Weidner. 1987. Effects of excess soil manganese on stomata function in two soybean cultivars. J. of plant nutrition. 10:749-760.

Tsukamoto, T.K., H. A. Killion, and G. C. Miller. 2004. Column experiments for microbiological treatment of acid mine drainage: low-temperature, low-pH and matrix investigations. Water Research 38:1405-1418.

Tuttle, J.H., P.R. Dugan, C.B. MacMillan, & C.I. Randles. 1969. Microbial dissimilatory sulfur cycle in acid mine water. J. of Bacteriology. 97:594-602

United States Environmental Protection Agency. 2002. 2000 National water Quality Inventory Report. EPA-841-R-02-001.

United States Environmental Protection Agency. 2004. Drinking water health advisory for manganese. EPA-822-R-04-003.

United States Environmental Protection Agency. 2005. Mountaintop mining/valley fills in Appalachia final programmatic environmental impact statement. EPA-903-R-05-002.

United States Environmental Protection Agency. 2006. 2006 Edition of the Drinking Water Standards and Health Advisories. EPA-822-R-06-013.

URS Corp. 2003. Passive and semi-active treatment of acid rock drainage – state of the practice. U.S. Army Corp of Engineers, Concord, MA.

Watzlaf, G.R. 1988. Chemical stability of manganese and other metals in acid mine drainage sludge. *In* Mine Drainage and surface Mine Reclamation, Info. Circular 9183. USDI, Bureau of Mines, Pittsburgh, PA.

Waybrant, K.R., D.W. Blowes, & C.J. Ptacek. 1995. Selection of reactive mixtures for the prevention of acid mine drainage using porous reactive walls. 1995 Conf. on Mining and the Environment. Sudbury, Ontario.

Waybrant, K.R., D.W. Blowes, & C.J. Ptacek. 1998. Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environ. Sci. Tech. 32:1972-1979.

Waybrant, K.R., C.J. Ptacek, & D.W. Blowes. 2002. Treatment of mine drainage using permeable reactive barriers: column experiments. Environ. Sci. Tech. 36:1349-1356.

Webb, J.S., S. McGinness, & H.M. Lappin-Scott. 1998. Metal removal by sulphate-reducing bacteria from natural and constructed wetlands. J. Applied Microbiology. 84:240-248.

Webster, J.R., S.W. Golladay, E.F. Benfield, J.L. Meyer, W.T. Swank, & J.B. Wallace. 1992. Catchment disturbance and stream responses: an overview of stream research at Coweeta Hydrologic Laboratory. *in* P.J. Boon, P. Calow, and G.E. Petts (eds). River conservation and management. John Wiley and Sons Ltd., Chichester, UK.

Wellsbury, P., R.A. Herbert, & R.J. Parkes. 1996. Bacterial activity and production in near-surface estuarine and freshwater sediments. FEMS Microbiology Ecology. 19:203-214.

Westrich, J.T. 1983. Consequences and controls of bacterial sulfate reduction in marine sediments. Yale Univ. New Haven, CT.

Wildeman, T., J. Dietz, J. Gusek, & S. Morea. 1993. Handbook for constructed wetlands receiving acid mine drainage. United States Environmental Protection Agency, Office of Research and Development, Cincinnati, OH. EPA-540-93-523.

Wildeman, T.R. & D.M. Updegraff. 1997. Passive bioremediation of metals and inorganic contaminants. *In* D.L. Macalady (ed.) Kluwer Academic Publishers, Dordrecht, the Netherlands.

Willow, M.A. & R.R.H Cohen. 2003. pH, Dissolved Oxygen, and Adsorption Effects on Metal Removal in Anaerobic Bioreactors. J. of Environmental Quality 32:1212-1221.

World Health Organization. 2004a. Manganese in drinking water. Available at www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf. Accessed 15 May 2007.

World Health Organization. 2004b. Sulfate in drinking water. Available at www.who.int/entity/water_sanitation_health/dwq/chemicals/sulfate.pdf. Accessed 15 May 2007.

Younger, P.L. & N.S. Robins. 2002. Mine water hydrogeology and geochemistry. Geological Society of London. London, England.

Zagury, G.J., C.M. Neculita, & B. Bussiere. 2005. Passive biological treatment of acid mine drainage: Challenges of the 21st century. 2nd Symposium sur l'environnement et les mines. Quebec, Ontario.

### *Chapter 2*

American Public Health Association. 1998. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.

Benner, S.G., D.W. Blowes, W.D. Gould, R.B. Herbert, Jr. & C.J. Ptacek. 1999. Geochemistry of a Permeable Reactive Barrier for Metals and Acid Mine Drainage. Environ. Sci. Tech. 33:2793-2799.

Boudreau, B.P. & J.T. Westrich. 1984. The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments. Geochimica et Cosmochimica Acta. 48:2503-2516.

Carey, D.I., Davidson, O.B., and Hiett, J.K., 2001, Kentucky coal production--1790-1999: Kentucky Geological Survey, ser. 12, Information Circular 3.

Champagne, P., P. Van Geel, & W. Parker. 2005. A bench-scale assessment of a combined passive system to reduce concentrations of metals and sulphate in acid mine drainage. Mine Water and the Environment. 24:124-133.

Chockalingam, E. & S. Subramanian. 2006. Studies on removal of metal ions and sulphate reduction using rice husk and *Desulfotomaculum nigrificans* with reference to remediation of acid mine drainage. Chemosphere. 62:699-708.

Cocos, A.A., G.J. Zagury, B. Clement, & R. Samson. 2002. Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. Water Research. 32:167-177.

Evangelou, V.P. 1998. Environmental Soil and Water Chemistry. John Wiley & Sons, Inc. New York, NY.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2002. Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments. Reviews in Env. Science & Biotechnology. 1:327-333.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2005. Municipal compost-based mixture for acid mine drainage bioremediation: Metal retention mechanisms. Applied Geochemistry. 20:1648-1657.

Karathanasis, A.D. & C. Barton. 1997. Ameliorative designs to improve the efficiency of constructed wetlands treating high metal load acid mine drainage in the Rock Creek watershed – Final Report. Kentucky Division of Water. Grant No. C9994506-94-0.

Kentucky Office of Mine Safety and Licensing. 2005. 2004 Annual Report.

Machemer, S.D. & T.R. Wildeman. 1992. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J. Contaminant Hydrology. 9:115-131.

McIntire, P.E. & H.M. Edenborn. 1990. The use of bacterial sulfate reduction in the treatment of drainage from coal mines. Proc. 1990 Mining and Reclamation Conference.

Neculita, C., G.J. Zagury, & B. Bussiere. 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. J. Environ. Qual. 36:1-16.

Phifer, M.A., C.E. Turick, & M.R. Millings. 2001. D-Area coal pile runoff basin sulfate reduction literature review and feasibility report. U.S. Dept. of Energy, Westinghouse Savannah River Company, Aiken, SC.

Pond, G.J. 2004. Effects of surface mining and residential land use on headwater stream biotic integrity in the Eastern Kentucky Coalfield region. Kentucky Department of Environmental Protection, Division of Water.

Sass, B., A. Gavaskar, W.S. Yoon, N. Gupta, E. Drescher, & C. Reeter. 2001. Geochemical investigation of three permeable reactive barriers to assess impact of precipitation on performance and longevity. Proceedings of the International Containment & Remediation Technology Conference and Exhibition, Orlando, FL. June 12.

Skousen, J.G., A. Sexstone, & P. Ziemkiewicz. 2000. Acid mine drainage control and treatment. *In* Barnhisel, R.I., R.G. Darmody, & W.L. Daniels (eds.). Reclamation of Drastically Disturbed Lands. American Society of Agronomy. Madison, Wisconsin.

Stumm, W. & J.J. Morgan. 1996. Aquatic Chemistry: Chemical equilibria and rates in natural waters – 3rd Ed. John Wiley & Sons, Inc. New York, NY.

Tabak, H.H., R. Scharp, J. Burckle, F.K. Kawahara, R. Govind. 2003. Advances in biotreatment of acid mine drainage and biorecovery of metals: 1.Metal precipitation for recovery and recycle. Biodegradation. 14:423-436.

United States Environmental Protection Agency. 2002. 2000 National water Quality Inventory Report. EPA-841-R-02-001.

URS Corp. 2003. Passive and semi-active treatment of acid rock drainage – state of the practice. U.S. Army Corp of Engineers, Concord, MA.

Waybrant, K.R., D.W. Blowes, & C.J. Ptacek. 1995. Selection of reactive mixtures for the prevention of acid mine drainage using porous reactive walls. 1995 Conf. on Mining and the Environment. Sudbury, Ontario.

Waybrant, K.R., D.W. Blowes, & C.J. Ptacek. 1998. Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environ. Sci. Tech. 32:1972-1979.

Waybrant, K.R., C.J. Ptacek, & D.W. Blowes. 2002. Treatment of mine drainage using permeable reactive barriers: column experiments. Environ. Sci. Tech. 36:1349-1356.

Wildeman, T.R. & D.M. Updegraff. 1997. Passive bioremediation of metals and inorganic contaminants. *In* D.L. Macalady (ed.) Kluwer Academic Publishers, Dordrecht, the Netherlands.

Zagury, G.J., C.M. Neculita, & B. Bussiere. 2005. Passive biological treatment of acid mine drainage: Challenges of the 21st century. 2nd Symposium sur l'environnement et les mines. Quebec, Ontario.

### *Chapter 3*

American Public Health Association. 1998. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.

Benner, S.G., D.W. Blowes, W.D. Gould, R.B. Herbert, Jr. & C.J. Ptacek. 1999. Geochemistry of a Permeable Reactive Barrier for Metals and Acid Mine Drainage. Environ. Sci. Tech. 33:2793-2799.

Benner, S.G., D.W. Blowes, C.J. Ptacek, & K.U. Mayer. 2002. Rates of sulfate reduction and metal sulfide precipitation in a permeable reactive barrier. Applied Geochem. 17:301-320.

Boudreau, B.P. & J.T. Westrich. 1984. The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments. Geochimica et Cosmochimica Acta. 48:2503-2516.

Champagne, P., P. Van Geel, & W. Parker. 2005. A bench-scale assessment of a combined passive system to reduce concentrations of metals and sulphate in acid mine drainage. Mine Water and the Environment. 24:124-133.

Evangelou, V.P. 1998. Environmental Soil and Water Chemistry. John Wiley & Sons, Inc., New York.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2002. Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: A review from laboratory to full-scale experiments. Reviews in Env. Science & Biotechnology. 1:327-333.

Gibert, O., J. de Pablo, J.L. Cortina, & C. Ayora. 2005. Municipal compost-based mixture for acid mine drainage bioremediation: Metal retention mechanisms. Applied Geochemistry. 20:1648-1657.

Karathanasis, A.D. & C. Barton. 1997. Ameliorative designs to improve the efficiency of constructed wetlands treating high metal load acid mine drainage in the Rock Creek watershed – Final Report. Kentucky Division of Water. Grant No. C9994506-94-0.

Ludwig, R.D., R.G. McGregor, D.W. Blowes, S.G. Benner, & K. Mountjoy. 2002. A permeable reactive barrier for treatment of heavy metals. Groundwater. 20:59-66.

Machemer, S.D. & T.R. Wildeman. 1992. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. J. Contaminant Hydrology. 9:115-131.

McIntire, P.E. & H.M. Edenborn. 1990. The use of bacterial sulfate reduction in the treatment of drainage from coal mines. Proc. 1990 Mining and Reclamation Conference.

Neculita, C., G.J. Zagury, & B. Bussiere. 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: Critical review and research needs. J. Environ. Qual. 36:1-16.

Sass, B., A. Gavaskar, W.S. Yoon, N. Gupta, E. Drescher, & C. Reeter. 2001. Geochemical investigation of three permeable reactive barriers to assess impact of precipitation on performance and longevity. Proceedings of the International

Containment & Remediation Technology Conference and Exhibition, Orlando, FL. June 12.

Stumm, W. & J.J. Morgan. 1996. Aquatic Chemistry: Chemical equilibria and rates in natural waters – 3rd Ed. John Wiley & Sons, Inc. New York, NY.

Tabak, H.H., R. Scharp, J. Burckle, F.K. Kawahara, R. Govind. 2003. Advances in biotreatment of acid mine drainage and biorecovery of metals: 1.Metal precipitation for recovery and recycle. Biodegradation. 14:423-436.

Waybrant, K.R., D.W. Blowes, & C.J. Ptacek. 1998. Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environ. Sci. Tech. 32:1972-1979.

Waybrant, K.R., C.J. Ptacek, & D.W. Blowes. 2002. Treatment of mine drainage using permeable reactive barriers: column experiments. Environ. Sci. Tech. 36:1349-1356.

Wildeman, T.R. & D.M. Updegraff. 1997. Passive bioremediation of metals and inorganic contaminants. *In* D.L. Macalady (ed.) Kluwer Academic Publishers, Dordrecht, the Netherlands.

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