Spring 2014

Potential acid mine drainage treatment utilizing acidophilic sulfate reducing bacteria in an upflow bioreactor

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Department:

POTENTIAL ACID MINE DRAINAGE TREATMENT UTILIZING ACIDOPHILIC SULFATE REDUCING BACTERIA IN AN UPFLOW BIOREACTOR

by

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A THESIS

Presented to the Faculty of the Graduate School of the

MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN ENVIRONMENTAL ENGINEERING

2014

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ABSTRACT

During coal and iron mining, pyrite is often exposed to oxygen, causing acid mine drainage (AMD). Acid mine drainage has characteristic traits of: a rust color, low pH levels (around 3 or 4) and high concentrations of sulfate, metal sulfates and heavy metals.

Sulfate reducing bacteria (SRB) are often utilized in acid mine drainage treatment by implementing them into biochemical reactors (BCR). As SRB break down various carbon sources, bicarbonate is produced, raising the pH and generating hydrogen sulfide which reacts with numerous metals. This approach can be troublesome, as SRBs do not thrive at low pH levels often associated with AMD.

Previous studies have found acidophilic sulfate reducing bacteria (aSRB) able to reduce sulfate and remove metals at pH values as low as 3.25. However these studies often use easily degradable carbon sources like ethanol, lactic acid and glycerol. In the present study, various solid carbon sources at a pH range of 3.0 to 6.0, high and low sulfate concentration, and media that provided either sulfate or iron as an electron acceptor were tested. Of the five carbon sources, sweet potato and horse manure resulted in black precipitate, indicating possible sulfate reduction. To mimic a BCR, column studies were conducted. After flowing pH 3.5 to 4.0 synthetic AMD through the upflow columns for 117 days, pH was raised to between 6.0 and 7.0. Sulfate reduction was evident in one column containing sweet potato and inoculum, but no others were active in this ongoing study. A leading hypothesis is that complete reduction was inhibited by the presence of fermenting bacteria.

ACKNOWLEDGEMENTS

This research would not have been possible without the support and help of several individuals. Dr. Burken, who was constantly full of knowledge and help but also put up with me being heavily involved in on campus organizations and varsity softball. Not only did he support and encourage me to follow my dreams, but also pushed me to become the leader I am today. Dr. Fitch, who was a wealth of knowledge on biochemical reactors and I could always count on for a challenging question. Dr. Mormile, whose expertise in anaerobic technique and anaerobic bacteria made this project possible and always had words of encouragement. Dr. Wronkiewicz is thanked for his review of this work.

Supplies, funding and support came from the Missouri S&T Environmental Research Center and Dr. Joel Burken's Pocket Fund.

A giant thank you goes out to all of my fellow researches and lab mates: Matt Limmer, who had to answer my endless questions and put up with me sitting next to him the past 3 years. Katie Bartels, for her never ending enthusiasm to make synthetic acid mine drainage and test column samples. Kristia Parker, who helped with testing but was always there for the occasional venting session.

Last but definitely not least, my amazingly supportive family who encouraged me to go to school 20 hours away, dealt with my 2 am phone calls, and short week long visits home because the bacteria were always calling my name. There is no possible way I could have done this without your love and support.

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NOMENCLATURE

Symbol	Description
AMD	Acid mine drainage
SRB	Sulfate reducing bacteria
aSRB	acidophilic Sulfate Reducing Bacteria
BCR	Biochemical Reactor

1 INTRODUCTION

1.1 ACID MINE DRAINAGE

Early mining efforts have left a legacy of abandoned sites that dot our landscape which are often left unattended. Between these sites and naturally exposed sulfide deopsits, acid mine drainage (AMD) is produced.

Acid mine drianage is characterized by low pH levels (around 1 to 3) and high concentrations of sulfates and heavy metals. Pyrite, an iron sulfide, becomes oxidized when exposed to chemical or biological weathering or when in contact with surface or groundwater. The process generates hydrogen ions and consumes hydroxides, causing a strong acidification process (Neculita et al. 2007).

$$4FeS_2 + 14O_2 + 4H_2O \rightarrow 4Fe^{2+} + 8SO_4^{2-} + 8H^+ \quad (1)$$

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

$$4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_{3\,(S)} + 12H^+ \quad (3)$$

These processes produce an increased concentration of sulfate, iron oxyhydroxide precipitates (Figure 1.1) and a decrease in pH (Neculita et al. 2007).

The U.S. Forest Service estimates that around 20,000 to 50,000 mines are currently generating acid on lands managed by them and these mines are negatively affecting about 8,000 to 16,000 km of streams. In EPA Region 8 (Colorado, Montana,

North Dakota, South Dakota, Utah and Wyoming), mining activities have left an estimated 51,700 abandoned mine areas that are dispersed and remotely located (Association 1998). As these tens of thousands of small sites are dispersed and often escape treatment as there are no utilities located nearby, and without direct human impact, the often notable ecological impacts are not a priority. Many of these mines are small, abandoned facilities created before modern environmental controls located in remote areas of the western United States. Several large mines were developed towards the end of the twentieth century, are located near population centers and have human-health impacts. Many of these mines have been abandoned and left for tax payers to cleanup (Group 2008). The cost of cleanup of environmental damage caused by AMD is great. The Canadian mining industry has identified acidic drainage as a considerable environmental liability, with an estimated cost of $2 to $5 billion dollars required for proper remediation (Group 2008). Due to the cost of active treatment systems, natural treatment systems are desired because they do not require electrical power, mechanical equipment, buildings or daily operation and maintenance (Behum et al. 2011).

Figure 1.1 Typical acid mine drainage. The color indicates active iron hydroxide formation.

1.2 HEALTH EFFECTS

Mining activities target accessing and harvesting mineral resources. In doing so, the mining, handling, and processing result in fugitive emissions of the minerals and co-located minerals and metals. The processes can also change the hydrology and chemistry of the mining sites and lead to ecological and health impacts.

Acid mine drainage presents longer-term, and 'legacy' scale issues. Acid mine drainage can last for undetermined lengths of time and contamination is often not apparent until after a site is abandoned for a long period of time. Therefore the need for robust, self-sustainable solutions is required.

Acid mine drainage contains high levels of many ions including heavy metals and semi-metal ions, like arsenic, lead, cadmium and chromium. Local surface and ground

water can become exposed and contaminated by the metals in AMD. Arsenic, found at concentrations ranging from detection limits, <1 µg/L, to as high as 340,000 µg/L, has many health effects. Non-cancer effects include stomach pain, nausea, vomiting and diarrhea. Partial paralysis, thickening and discoloration of the skin, and numbness in hands and feet are also associated with arsenic exposure. Arsenic is linked to cancer of the prostate, liver, lungs, bladder, kidney, skin and nasal passages. The arsenic standard in drinking water is 0.010 parts per million.

High levels of lead are also associated with AMD. Lead can cause nerve damage to the sense organs and controlling the body, increased blood pressure, hearing and vision impairment, reproductive problems and retarded fetal development (which can occur even at low levels of exposure). In children, lead poisoning can cause brain damage or mental retardation, behavioral problems, anemia, liver and kidney damage, hearing loss, hyperactivity and developmental delays. In extreme cases, lead poisoning can cause death (Health 2011). Elderly people with inheritable genetic diseases, alcoholics and smokers, and people with neurological dysfunction or kidney disease are also susceptible to lead poisoning (Lovley and Phillips 1986).

Exposure to increased levels of cadmium can cause nausea, vomiting, diarrhea, muscle cramps. It can also cause kidney, liver, bone and blood damage. Exposure to increased levels of chromium can cause allergic dermatitis and is a possible carcinogen.

Acid mine drainage also causes considerable ecological impact on aquatic resources. Fish, for example, are exposed to metals and hydrogen ions directly through their gills. This causes impaired respiration and can result in large fish kills. Fish are also exposed to metals through ingestion of contaminated sediments and food. The decrease

in pH of the water due to AMD altering gill membranes or changes in gill mucus results in death caused by hypoxia. One investigation found that streams with a pH of 4.5 and total acidity of 15mg/L had a fish loss of 90% (Metals 2011). Decreased diversity of fish species and poor taxa richness and abundance are also associated with AMD entering surface waters. Iron hydroxide can coat the surface of stream sediments destroying the habitat, decreasing clean gravel fish use for spawning and reducing fish food items like benthic macroinvertebrates.

Fish kills due to AMD has occurred worldwide. As fish consume benthic organisms and other food, the toxins can bioaccumulate and biomagnify. Not only is AMD detrimental to fish and other organisms found in the streams, but humans can also be impacted by consuming the fish that contain the high concentrations of contaminants.

2 LITERATURE REVIEW

2.1 ACID MINE DRAINAGE TREATMENT AND TECHNOLOGY

2.1.1 History. Traditionally, acid mine drainage (AMD) treatment is completed through a chemical process. To treat AMD chemically, the system typically consists of an inflow pipe or ditch, a storage tank or bin to hold the neutralizing agent, a way to control the chemical application rate, a settling pond to capture the precipitated metal oxyhydroxides, and a discharge pit. To choose the correct chemical and the amount needed, acidity level, flow, type and concentration of metals present, rate and degree of chemical treatment needed, desired final water quality, price of the agent, labor, machinery, equipment cost, number of years treatment is needed, and risk factors must be taken into account. Common neutralizing agents used to treat AMD are limestone ($CaCO_3$), hydrated lime ($CaOH_2$), pebble quick lime, soda ash (Na_2CO_3), caustic soda (NaOH), and 20% or 50% liquid caustic. Common chemical agents include ammonia (NH), potassium hydroxide (KOH), magnesium hydroxide ($Mg(OH)_2$) and magna lime (MgO). Selecting the appropriate neutral agent or chemical to treat with depends on both the oxidation state and concentrations of metals in the AMD (EPA 1983).

Using neutralizing and chemical agents is known to be problematic. Limestone tends to have a low solubility and develops an armor of iron hydroxide when added to AMD. The limestone is most effective when the water is anoxic. When the neutralized water is exposed to oxygen, the ferrous iron oxidizes, hydrolyzes, precipitates, and coats the limestone, slowing the rate of dissolution and buffering and limits the effectiveness of the limestone (Gazea et al. 1996). Hydrated lime is problematic because it requires

extensive mechanical mixing, where as soda ash is troublesome because when exposed to moisture, it will stick to the machinery. Caustic soda is very water soluble and disperses rapidly, raising the pH of water quickly, but it is also dangerous to handle and very resource intensive as large tanks and platforms are required. Caustic soda can freeze during the winter so it must be stored undergound, have a tank heater or use a lower percentage of caustic soda in the mixture. Ammonia is dangerous to handle, requiring specialized training to use it safely. Ammonia can also easily raise the pH above neutral and has potential biological implications downstream. Overall, chemical reactors are not a preferred method to treat AMD because of the high cost of chemical agents, inefficient removal of sulfate, the production of bulky sludge which must be disposed of, and useful and valuable metal resources cannot be recovered (Kaksonen et al. 2006; Ňancucheo and Johnson 2012). Acid mine drainage is often located in remote locations, making it difficult to replace the agents and armored lime, as well as finding a method to dispose of the bulky sludge.

2.1.2 Biochemical Reactor. A biochemical reactor (BCR) is an engineered treatment system. Biochemical reactors utilize a consortia of bacteria including cellulose degraders, fermentative bacteria and sulfate reducing bacteria and substrate to remove metals from contaminated water. There are several ways BCRs can be designed. Each process (bioprocesses, chemical reactions, and solid separation) can take place in a separate tank, which includes pre and post-treatment units, or can all be incorporated within an organic substrate such as wood chips or manure in one unit. If incorporated with an organic substance, limestone is often included for buffering capacity and substrate permeability (ITRC 2013).

A BCR has both chemical and biological reactions. Chemically, as alkalinity is added to or generated in the BCR, the pH drives the formation of metal sulfide solids. Increase in pH often lowers the solubility of many metals and the metals will precipitate as solids and become trapped in the solid substrate or captured in downstream sedimentation cells. Reducing conditions are now needed to move sulfate to the sulfide state. Biologically, SRBs, cellulose degraders and fermenters are present in the BCR. Cellulose degraders, such as *Bacteroids* and *Clostridium* (Pereyra et al. 2008) are responsible for degrading substrate, typically a complex carbohydrate into simple carbon compounds, are necessary in the BCR as SRBs depend on them to provide simpler carbon sources (Neculita et al. 2007). Cellulose degraders can survive in aerobic or anaerobic conditions. Within a BCR for sulfate reduction, fermentative anaerobes will predominate. Fermenters and clostridia degrade amino acids, sugars, and fatty acids to simpler organic compounds, like propionic acid and alcohols. The simpler organic compounds can also be used by the SRBs. Methanogens are also present in BCRs but SRBs out-compete them for the hydrogen available. In a mature BCR, methanogenic activity is limited (ITRC 2013).

A BCR typically consists of a free water zone, which is the surface water adjacent to the media, and three separate reactive zones: oxidative zone, transitional zone, and sulfide zone (Figure 2.1). The transitional zone is anoxic to slightly anaerobic, iron becomes oxidized, and the organic matter is degraded as microbial activity is very high here. The sulfide zone is anaerobic and highly reduced, and microbial activity is also high. The drainage system typically consists of crushed gravel and perforated pipes.

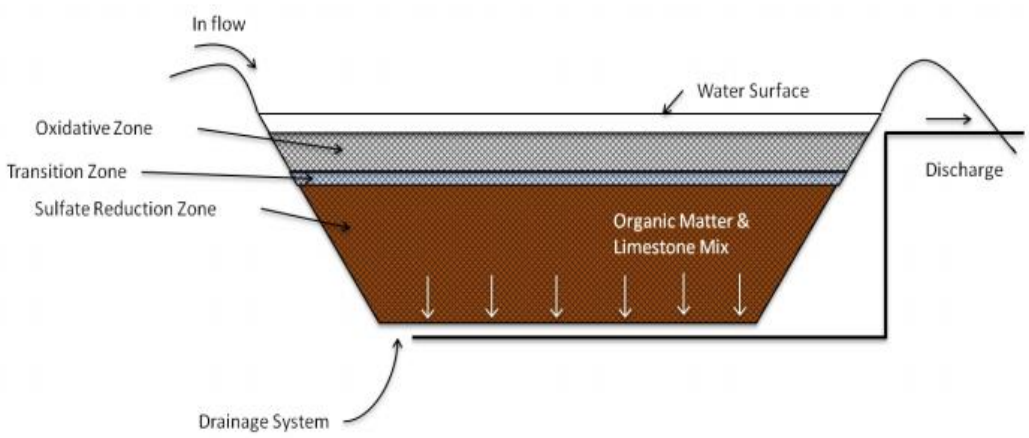

Figure 2.1 Typical setup of a biochemical reactor.

Biochemical reactors can utilize local materials for substrate, making the initial cost of material for construction low and the construction practices basic. Biochemical reactors have low operational and maintenance requirements and do not require electrical power. A BCR is known to function for years without having to refurbish or replace the organic substrate, which is very beneficial as they are often located in remote areas with limited access. However, when treating AMD, BCRs can be problematic as they often require pre- and post-treatment and therefore are not standalone systems. Biochemical reactors effluent may not consistently meet water quality standards as organics and nutrients can be released, elevated biological oxygen demand and color may be present. The effective design of a BCR may be limited by space. Overtime, the organic substrate will need to be replaced and the permeability of the BCR will change. As permeability changes, the BCR can develop paths of preferential flow or plugging. This short

circuiting causes a decreased retention time, leading to decreased performance of metal removal within the BCR (ITRC 2013).

2.1.3 Sulfate Reducing Bacteria Sulfate reducing bacteria are critical within the BCRs treating AMD. SRBs carry out the critical process steps by reducing sulfate to hydrogen sulfide:

$$2\ CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (4)$$

The HCO_3^- (bicarbonate) buffers total acidity and helps to neutralize low pH. Hydrogen sulfide dissolves readily in water (along with ionic species HS^-) and can form metal sulfide precipitates:

$$H_2S + M^{+2} \rightarrow MS_{(s)} \downarrow + 2H^+ \quad (5)$$

where M^{+2} is a cationic metal like Cd^{+2}, Fe^{+2}, Ni^{+2}, Pb^{+2}, or Zn^{+2} (Neculita et al. 2007).

Sulfate reducing bacteria will first utilize easily degradable organic matter, typically low molecular weight compounds with simple structures (methanol, ethanol, lactate, and polylactic acid) and then utilize complex organic carbon sources (cellulosic wastes and organic wastes like sawdust, hay, alfalfa, woodchips, manure, sewer sludge). Complex carbon sources are favorable in BCRs because they consist of less expensive waste material but can be problematic because when used alone, the complex solid organics often do not directly support the activity of SRBs. A consortia of bacteria,

including fermenters and cellulolytic microbes, are needed to help break down the complex carbon sources into short-chain organic carbon compounds to be utilized by the SRBs, as shown in Figure 2.2 (Neculita et al. 2007).

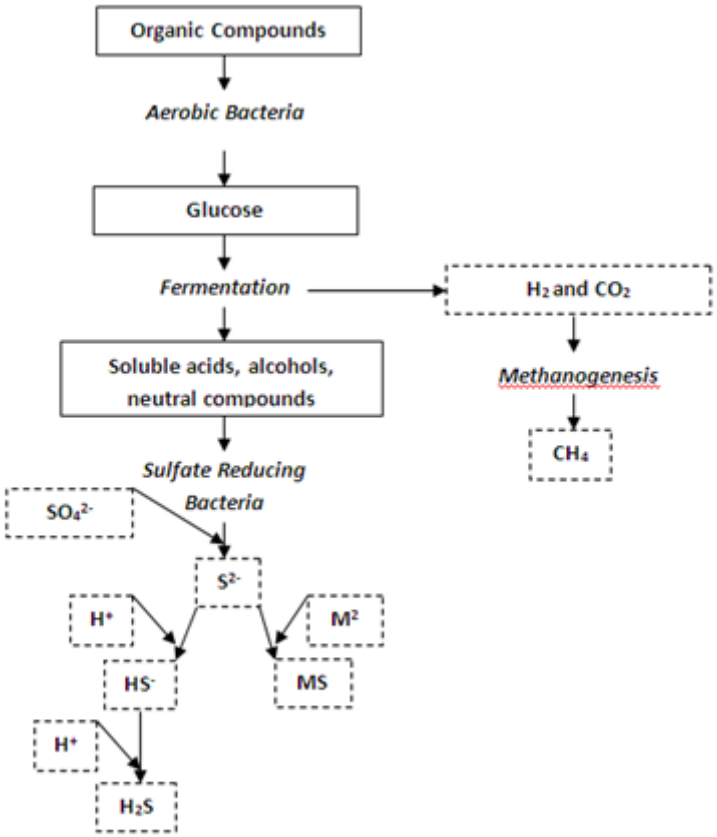

Figure 2.2 Breakdown of carbon sources within a biochemical reactor by a consortia of microbes (ITRC 2013; Díaz 2004).

The rate at which substrates become available to SRB may be limited by the anaerobic degradation of complex organic carbon compounds to simpler molecules by the consortia of microbes present (Figure 2.2) (Logan et al. 2005). The selection of the type of carbon source to use is usually made based on availability and cost of the added electron donor per unit of reduced sulfate. Implementing additional carbon sources into the biochemical reactor (BCR) is often necessary because AMD contains relatively low concentrations of dissolved organic carbon, less than 10 mg/L, and therefore the availability of carbon from additional organic sources is a common limiting factor (Kolmert and Johnson 2001). The amount of sulfate reduced by SRBs is also controlled by the available surface area, hydraulic retention time, and the initial concentration of the AMD (Neculita et al. 2007). Many studies have been conducted using a wide variety of carbon sources in different set-ups and different conditions, which are summarized in Table 2.1. Sulfate reducing bacteria can be problematic because they do not thrive at a low pH. When using SRBs in a BCR, limestone is often required to buffer the pH. Limestone can be troublesome because over time it becomes chemically depleted or armored in iron hydroxide and would need to be replaced. Replacement can be costly and problematic, as BCRs are often located in remote locations and hard to access.

Table 2.1 Carbon sources used in different lab studies (Neculita et al. 2007).

			pH		SO_4^{2-} (mg/L)		
Reactor Type	**Total Volume (L)**	**Organic Matter Source**	**Inf.**	**Eff.**	**Inf.**	**EFf.**	**Ref.**
Field bioreactor	765,600	mixture of softwood, dust, hay, cattle manure	4.0-5.5	5.0-6.0	175-260	200-275	Johnson and Hallberg (20005)
Field bioreactor	92,000-108,000	mixture of cow manure and cut straw	3.3-7.5	6.5-7.5	70-229	---	Zaluski et al. (2003)
Pilot-scale bioreactor	20,000	cattle manure, sawdust, hay and alfalfa	3.5-7.5	6.5-7.5	<60	<40	Reisinger et al. (2000)
Pilot-scale bioreactor	200-4,500	spent mushroom compost (manure, hay, straw, corn cobs, and wood chips)	3.2-6.2	6.4-7.1	1002-2997	831-2,387	Dvorak et al. (1992)
Pilot-scale bioreactor	3,900	methanol	2.9-3.2	6.9	1900-2100	832	Glombitza (2001)
Pilot-scale bioreactor	570	rice stalks, cow manure, and limestone	3.6	6.2	---	---	Cheong et al. (1998)

Table 2.1 Carbon sources used in different lab studies cont. (Neculita et al. 2007).

			pH		SO_4^{2-} (mg/L)		
Reactor Type	**Total Volume (L)**	**Organic Matter Source**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**Ref.**
Bench-scale bioreactor	200	shredded wood chips, sawdust, alfalfa hay, and cow manure	3.0-3.5	5.5-7.0	3,000-3,500	2,500-4,500	Reisman et al. (2003)
Laboratory bioreactor	45	cow manure, sawdust, and whey	2.5-3.5	6.5	<1,000	<200	Drury (1999)
Laboratory bioreactor	25-29	livestock manure	2.7-6.2	6.3-7.1	1,000	922-970	Willow and Cohen (2003)
Laboratory bioreactor	17	alfalfa, hay, timothy hay and cereal straw	3.5	6.5	1,010	420-960	Bechard et al. (1994)
Laboratory bioreactor	4.8	lactate	4.52	7.2	2,280-2,315	<400	Jong and Parry (2003)
Column	9	wood chips, leaf compost, and poultry manure	3.8-4.0	7	1,500	1,220	Beaulieu et al. (2000)

Table 2.1 Carbon sources used in different lab studies cont. (Neculita et al. 2007).

			pH		SO_4^{2-} (mg/L)		
Reactor Type	**Total Volume (L)**	**Organic Matter Source**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**Ref.**
Column	4.7-7.8	spent manure revitalized with methanol/ ethanol	2.5-4.2	5.4-7.3	900	400-500	Tsukamoto et al. (2004)
Column	0.25	spent mushroom compost, oak chips, spent oak, sludge, and organic-rich soil	6.8	7.0-8.5	2,580	200-650	Change et al. (2000)
Column/ Batch	Column: 0.12 Batch: 0.5	compost, oak leaf, poultry manure, and sheep manure	2.4	6.0-7.5	540 column 1040 batch	<850 column <200 batch	Gibert et al. (2004)
Batch	31	whey	3.0-4.4	3.5-6.0	857-936	715-5,390	Christensen et al. (1996)
Batch	25	bedded cattle manure and mixture of cattle slurry screenings, and green waste compost	4.2	5.9-6.3	14,752	---	Amos and Younger (2003)

Table 2.1 Carbon sources used in different lab studies cont. (Neculita et al. 2007).

			pH		SO_4^{2-} (mg/L)		
Reactor Type	**Total Volume (L)**	**Organic Matter Source**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**Ref.**
Batch	25	bedded cattle manure and mixture of cattle slurry screenings, and green waste compost	4.2	5.9-6.3	14,752	Not reported	Amos and Younger (2003)
Batch	2	single source or mixture of maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure, and conifer sawdust	4	6.5-8.5	4,244	163-5,575	Zagury et al. (2006)
Batch	1	several barks and wood chips	1.6	5.0-6.0	2,500	750-1,250	Tasse and Germain (2002)

Table 2.1 Carbon sources used in different lab studies cont. (Neculita et al. 2007).

			pH		SO_4^{2-} (mg/L)		
Reactor Type	**Total Volume (L)**	**Organic Matter Source**	**Inf.**	**Eff.**	**Inf.**	**Eff.**	**References**
Batch	0.5-1	single source or mixtures of sewage sludge, leaf mulch, wood chips, sheep manure, sawdust, and cellulose	2.6-6.0	6.5-7.0	1,200-4,800	<35	Waybrant et al. (1998)
Batch	0.5	mixture of leaf compost, poultry manure, and wood chips	5.5-6.0	7.9	2,000-3,200	<90	Cocos et al. (2002)

2.1.4 Acidophilic Sulfate Reducing Bacteria. Characterized species of SRB are very sensitive to even mild acidity and do not grow at pH<5.5, implicating the use of biological reactors to treat AMD without utilizing a buffering agent (Koschorreck et al. 2003). However, novel species of acid tolerant or acidophilic sulfate reducing bacteria (aSRBs) such as *Desulfosporosinus acidiphilus* have been identified and may be promoted in BCRs (Alazard et al. 2010). Acidophilic sulfate reducing bacteria are often extracted from AMD impacted sites or geothermal environments and have shown sulfate reduction in laboratory conditions at pH values of 3.0 to 4.0. Several articles have shown significant sulfate reduction and pH increase by using aSRB. Fixed-bed reactors utilizing aSRB from two derelict mine sites in Wales showed a sulfate conversion rate of 26.0 to 31.2 mmol m^{-3} d^{-1} at a pH as low as 4 with glycerol, lactic acid, and ethanol as carbon sources (Kolmert and Johnson 2001). At an initial pH of 3.2, sulfate reduction rates from 250 mmol m^{-3} d^{-1} to 120 mmol m^{-3} d^{-1} and a pH increase to 6.5 was achieved in a solid substrate reactor containing cow manure, sawdust and supplemental whey additions as carbon sources (Drury 1999). An anaerobic bioreactor enriched with aSRB isolated from sediment samples taken from Dawsley Creek, South Australia found significant pH increase, from 3.25 to 5.82, and 38.3% sulfate removal (Elliott et al. 1998). In another study, 90% of dissolved metals and 11% of sulfate was removed in a down flow column with an initial pH of 4.8 (Lyew and Sheppard 1999). It was found at the Woodcutters mine site in Northern Territory, Australia that an upflow, anaerobic backed bed bioreactor, could increase pH from 4.0 to 6.93 and the rates of sulfate reduction was 553 mmol m^{-3} d^{-1}, with 80% sulfate removal (Jong and Parry 2006).

Traditional BCRs utilize SRBs that require a neutral pH to survive. A limestone drain must be implemented into the BCR to neutralize the AMD. However, this can be costly and troublesome because overtime it becomes armored and requires replacement. Acidophilic sulfate reducing bacteria can possibly eliminate the need for limestone in these systems. Acid mine drainage sites are often located in remote areas and access is limited. By using aSRBs in a BCR, less maintenance would be required because the limestone will not have to be replenished or replaced. The cost of the BCR will also decrease.

3 GOALS AND OBJECTIVES

The primary goal of this work overall is to determine how acidophilic sulfate reducing bacteria function at various pH levels, sulfate concentrations and with different carbon sources as electron donors. The secondary goal is to then determine aSRBs treatment potential in column studies, which mimic a biochemical reactor. To reach this goal, specific objectives were formed:

- Objective: Identify appropriate solid carbon source for the consortia of bacteria found at Red Lake, including acidophilic sulfate reducing bacteria (aSRBs).
    - Hypothesis: The consortia of bacteria will utilize the most readily available carbon source and carbon sources that break down most easily into simple sugars or related organics.

- Objective: Evaluate the activity of the aSRBs across a wide pH and various sulfate concentrations
    - Hypothesis: Sulfate reduction and pH increase will occur at all pH ranges, with a slower rate of sulfate reduction and pH neutralization at pH values below 5. The greater amount of sulfate present, the greater the pH change within the batch systems.

- Objective: Implement findings into upflow column reactors, mimicking a biochemical reactor

- Hypothesis: The consortia of bacteria found at Red Lake will utilize the specified carbon sources under acidic conditions to reduce sulfate and increase pH with rate limitations being observed in the porous column design.

Each objective was assessed in the research covered herein. Data and conclusions generally supported hypothesis, however, aSRBs were able to utilize the various carbon sources across all pH ranges and sulfate concentrations within the batch experiments. When implemented into column studies, sulfate reduction only occurred in the second column containing sweet potato and inoculum, but pH increase appears to be inhibited by fermenting bacteria and the products they produce (volatile fatty acids such as acetic acid, propionic acid and butyric acid). Through this research, knowledge of aSRBs ability to produce sulfate under acidic conditions utilizing solid carbon sources was gained.

4 MATERIALS AND METHODS

4.1 RED LAKE

Red Lake (Figure 4.1) is located in the Rocky Fork Lakes Conservation Area approximately six miles north of Columbia, Missouri. Historically, this area was owned by Peabody Coal Company from 1963 to 1972 where they strip mined approximately 1,150 acres of land covered mostly by oak-hickory forest and upland fields. Prior to acquisition, the Missouri Department of Conservation purchased the Rocky Fork Lakes Conservation Area. The land is now reclaimed and covered with a mixture of trees, shrubs and fescue. Even with the land reclamation, Red Lake is still impacted by the effects of acid mine drainage. There are two visible seeps colored rust orange with traits characteristic of AMD that run directly into the lake (Table 4.1).

Figure 4.1 Photo of Red Lake in the strip coal mine area of central Missouri and the collection source of the aSRBs.

A metagenomic study of Red Lake conducted in Dr. Michael Sadowsky's lab at the University of Minnesota showed the presence of sulfate reducing bacteria at a pH around 3.7. Below a layer of decomposing biomass, black mud that smells of sulfide is present. The organisms in this layer can be a key player in lessening the impact of AMD at Red Lake, as it is interesting to note a thriving ecosystem (trees, cattails, frogs, insects, and spiders) surrounds the lake in spite of its pH (usually about 3.9) and heavy metal content.

Table 4.1 Conditions at AMD seep flowing into Red Lake.

pH	2.5-3.7
Sulfate Concentration	1,500 to 3,300 ppm
Iron Concentration	1.5 to 17 ppm

4.2 BATCH EXPERIMENT ONE

Sediment samples were obtained from the outer bank of Red Lake located in the Rocky Forks Conservation Area in Columbia, Missouri. Samples were taken approximately 18 inches down in the clay layer and were assumed anaerobic. Samples were stored and sealed in paint cans and placed in the fridge at 5°C once returning back to Rolla.

Five organic substrates were individually evaluated in 160 mL serum bottles to determine the most readily available carbon source. Substrates tested included Whatman filter paper number 42 (i.e. cellulose), sorghum chips, switch grass, sweet potato, and horse manure. 0.5 grams of carbon source were anaerobically added to 50 mL of acidophilic sulfate reducing bacteria (aSRB) media and then autoclaved at 120°C for 30 minutes. Experimental bottles were made in triplicates. The bottles containing the horse manure were autoclaved three times to attenuate bacteria present. The media is a modified version from Bernardez to match the sulfate concentration of the lake and was made anaerobically and consisted of 0.5 g/L KH_2PO_4, 1 g/L NH_4Cl, 1 g/L Na_2SO_4, 1 g/L $CaCl_2$, 1.83 g/L$MgCl_2$*$6H_2O$, 0.1 g/L ascorbic acid, 0.013 g/L sodium thioglycollate, 0.035g/L NaCl, 1.59 g/L $FeSO_4$*$7H_2O$, and 0.0001 g/L resazurin (Bernardez et al. 2012). After cooling to room temperature, 22°C, the experimental bottles were anaerobically inoculated with 5.0 g of sediment sample and pH was adjusted with 0.5N HCl. Bottles were stored in a dark cabinet at room temperature. Sulfate reduction was visually evaluated based on the presence of black metal precipitation.

4.3 BATCH EXPERIMENT TWO

A second batch experiment consisting of two matrices was also conducted. The first matrix was to determine if aSRBs were responsible for the presence of black precipitate and to assess what conditions (sulfate concentration, pH, and carbon source) promote or attenuate sulfate reduction. The second matrix was to determine if acidophilic iron reducing bacteria (aFRBs) were responsible for the presence of black precipitate and what pH conditions and carbon source allowed for optimum iron

reduction. Molybdate was added to the aFRB batch experiment to prohibit the aSRBs from reducing sulfate. A summary of the two matrices is described in Table 4.2

Utilizing an anaerobic glove bag, 20 mL of modified media from Bernardez, the same make up as batch experiment one, was anaerobically added to 30 mL serum bottles. To each bottle, 0.2 grams of respective carbon source was anaerobically added as well. The serum bottles were autoclaved three times for 20 minutes at 120 psi to ensure all unwanted bacteria was killed. The bottles were cooled to room temperature and 2 grams of collected Red Lake sediment was anaerobically added to each experimental bottle, which were done in triplicate. The control bottles did not contain any Red Lake sediment. pH was then adjusted with 0.5N HCl. The pH of both the experimental and control bottles were measured under anaerobic conditions with Scientific Instruments IQ150 portable pH probe that was calibrated prior to testing and checked with every 10 samples. The serum bottles were left in a dark cabinet for 60 days, being visually inspected every two days. At the end of the 60 days, the pH values were tested in the aSRB bottle and aFRB bottles under anaerobic conditions. The sulfate concentration was measured in the aSRB bottles using the bench top Hach DR/2400 Spectrophotometer and US EPA accepted Hach SulfaVer 4 Method 8051. Prior to each test, the Hach DR/2400 was calibrated and standards were checked with every 10 samples.

To ensure the carbon source did not limit the amount of sulfate reduced in the bottles containing sweet potato, 5 mL of glucose (1,000mg BOD/L) was anaerobically added to both the experimental and control bottles. After sitting for 30 days in a dark cabinet the sulfate concentration was tested using Hach Method 8051.

Table 4.2 Batch experiment two setup.

	aSRB Batch Experiment	aFRB Batch Experiment
Sulfate Concentration	High concentration: 2670 mg/L Low concentration: 1530 mg/L	700 mg/L
Iron Concentration	N/A	10 mg/L
pH levels	3.0, 3.5, 4.0, 4.5, 4.5, 5.0, 5.5, 6.0	3.0, 3.5, 4.0, 4.5, 4.5, 5.0, 5.5, 6.0
Carbon Sources	Sweet potato Horse manure	Sweet potato Horse manure
Molybdate Present	No	Yes

4.4 COLUMN EXPERIMENT

Six columns were set up to mimic a BCR. The columns were constructed from clear polyvinyl chloride (PVC) pipe with an overall height of 12 inches and an inner diameter of 2 inches. Columns had a total of two ports at 4 inches and 8 inches, fitted with mininerts for sampling. Each column had four inches of 3/8 inch diameter inert gravel at the influent end, followed by 9 inches of an inoculum and carbon source mixture (0.65 inches of sediment and 8.35 inches of carbon source) and then 4 more inches of gravel at the effluent end. The various make up of carbon sources and inoculum found within each column are as follows:

1. Sweet potato and inoculum
2. Sweet potato and inoculum
3. Horse manure and inoculum
4. Horse manure and inoculum
5. Autoclaved horse manure and inoculum
6. Horse manure only

The columns were constructed in an anaerobic glove bag and filled with synthetic AMD and rubber stoppers were put onto each end. A half-inch hole was drilled into each stopper to allow for ventilation of gases. The synthetic AMD, which was adapted from Choudhary was comprised of .0015 g/L $FeSO_4*7H_2O$, 0.0574 g/L $CuSO_4*5H_2O$, 0.0943 g/L $ZnSO_4*7H_2O$, 0.098 g/L $MgSO_4*H_2O$, 0.0467 g/L $NiSO_{4*}6H_2O$, 0.0059 g/L $CoSO_4*7H_2O$, 2.07 g/L Na_2SO_4, and 0.0001 g/L resazurin (Choudhary and Sheoran 2012). The synthetic AMD was made anaerobically and autoclaved for 30 minutes at 120 psi. The pH was adjusted to between 3.5 and 4 with 1 N HCl. The columns remained in the anaerobic glove bag for 30 days to allow for bacteria growth and adaptation to conditions. They were periodically rotated and flipped to ensure the sediment did not accumulate in one spot of the column.

Following the 30 day incubation, the upflow columns were setup (Figure 4.2). While setting up the columns, the second column containing sweet potato and inoculum fell over and had to be remade and sit in the glove bag for another 30 days. Using a peristaltic pump, Masterflex 06404-16 Norprene tubing and Masterflex 06416-16 Tygon tubing, the media flowed through the bottom of the columns at a hydraulic retention time of 30-39 hours. The effluent from the columns was collected in corresponding 50 mL Erlenmeyer flask. Samples of the influent media, the two sampling ports, and the effluent in the Erlenmeyer flasks were tested for pH, oxidation reduction potential (ORP), and sulfate concentration. The pH and ORP were measured using a Sper Scientific pH SD Card Logger 850060. The logger was calibrated prior to each test and a standard was checked as well. Sulfate was tested using the bench top Hach DR/2400 Spectrophotometer and US EPA accepted Hach SulfaVer 4 Method 8051.

To determine if aSRBs were possibly being inhibited by the acidic products produced by fermentative bacteria, the presence of volatile fatty acids was tested for. Volatile fatty acids were tested using Agilent Technologies 6890N Network GC System and a DB-FFAP (30m x 0.249mm x 0.25 µm) column, specifically for volatile fatty acids. Standards were made using acetic acid, propionic acid and butyric acid.

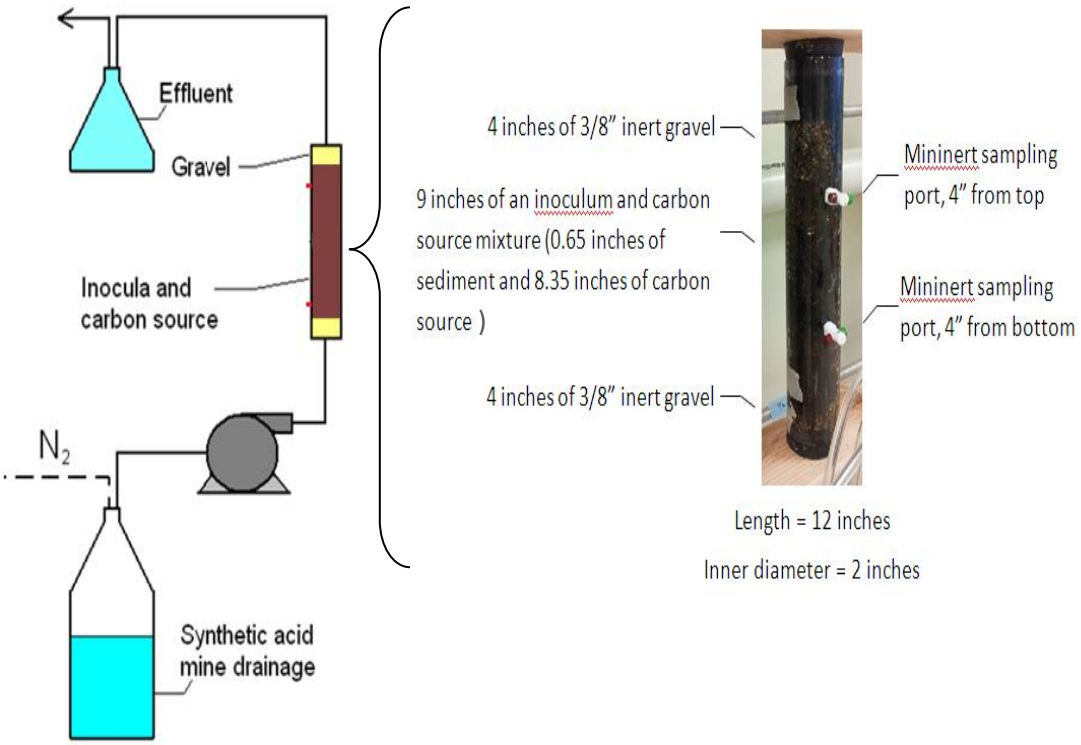

Figure 4.2 Schematic and photo of upflow column arrangement, showing the feed solution and sample collection vessel.

5 RESULTS AND DISCUSSION

5.1 BATCH EXPERIMENT ONE

After 30 days the serum bottles were visually inspected for the presence of black precipitate, indicative of sulfate reduction. Black precipitate was present in bottles containing sweet potato and horse manure but not in bottles containing Whatman filter paper number 42 (cellulose), sorghum chips, and switch grass (Figure 5.1). From this evidence, the aSRBs were able to utilize sweet potato and horse manure as carbon sources to reduce sulfate. Whatman filter paper number 42, sorghum chips, and switch grass carbon sources did not support sulfate reduction.

Figure 5.1 Left: Serum bottle without black precipitate (switch grass) Right: Serum bottle with black precipitate (sweet potato).

5.2 BATCH EXPERIMENT TWO

5.2.1 aSRB Batch Experiment and Sweet Potato. By day 15 in the second batch experiment, the sweet potato was shown to serve as a carbon source for aSRBs at low and high sulfate concentration and across all pH values (3.0 to 6.0) as black precipitate was observed in all reactors. At day 33, reactors were observed to be completely black with visible precipitate accumulation. The pH value and sulfate concentration in the bottles were analyzed on day 37. For all experimental bottles, pH 3.0 through 6.0, at a high sulfate concentration, the pH increased to around 6.5, while the control bottles, that had no inoculum, remained around the initial pH (Figure 5.2, Figure 5.3) Consistent results were recorded in all low sulfate concentration bottles, indicating the aSRBs were able to buffer the synthetic AMD to a pH of 6.5 when using sweet potato biomass as a carbon source.

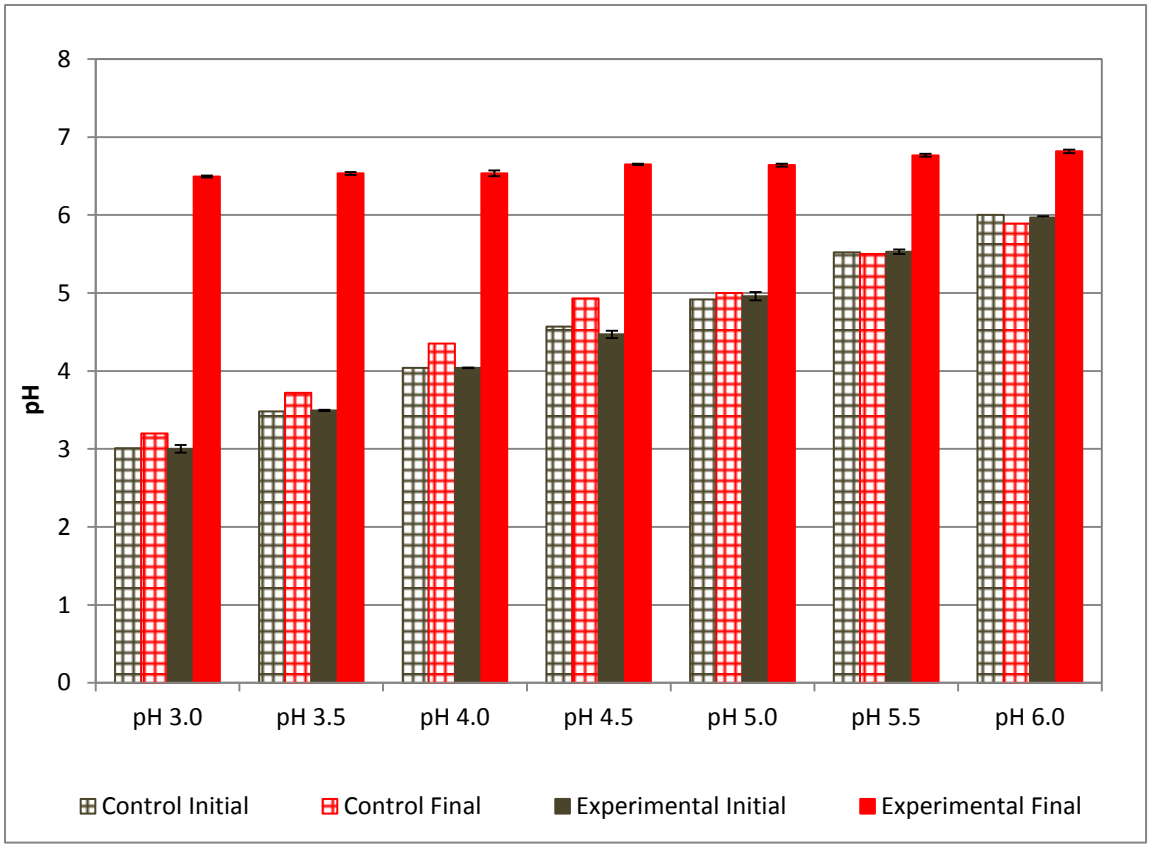

Figure 5.2 pH in aSRB bottles containing sweet potato and high sulfate concentration increased in experimental bottles but remained the same in control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

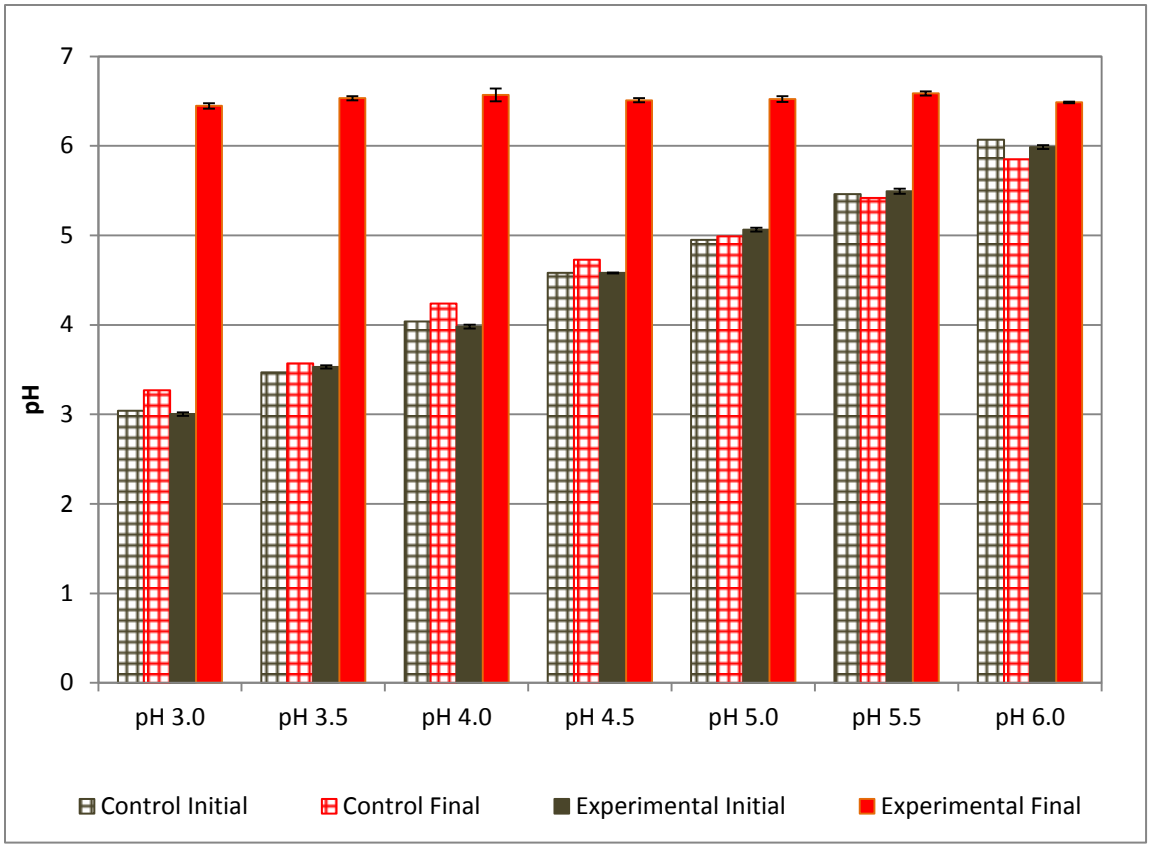

Figure 5.3 pH in aSRB bottles containing sweet potato and low sulfate concentration increased in experimental bottles but remained the same in control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

Sulfate reduction was limited in reactors contatining the high sulfate concentration, at all pH values tested. Reduction halted around 500 mg/L SO_4^{2-}. After adding the glucose and having an additional 30 day incubation (day 68), the aSRB bottles containing the high sulfate concentration were tested and sulfate was reduced down to around 200 mg/L to 0 mg/L in all of the experimental bottles (Figure 5.4, Figure 5.5).

The sulfate concentration in the control bottles, which contained no inoculum, was not depleted. In all low sulfate concentration reactors, sulfate was reduced down to 100 mg/L to 0 mg/L after 37 days. In the control bottles, sulfate concentration was not reduced.

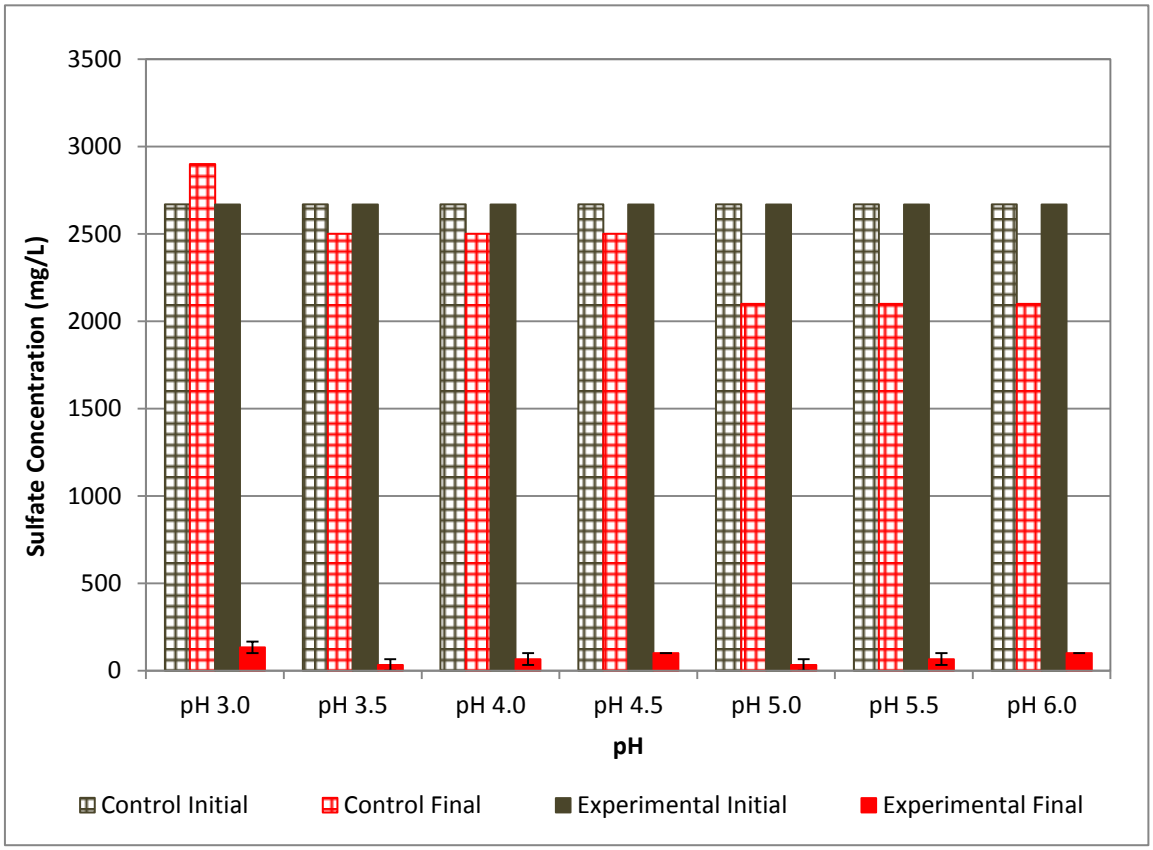

Figure 5.4 Sulfate concentration in aSRB bottles containing sweet potato at high sulfate concentration decreased in experimental bottles but remained the same in control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

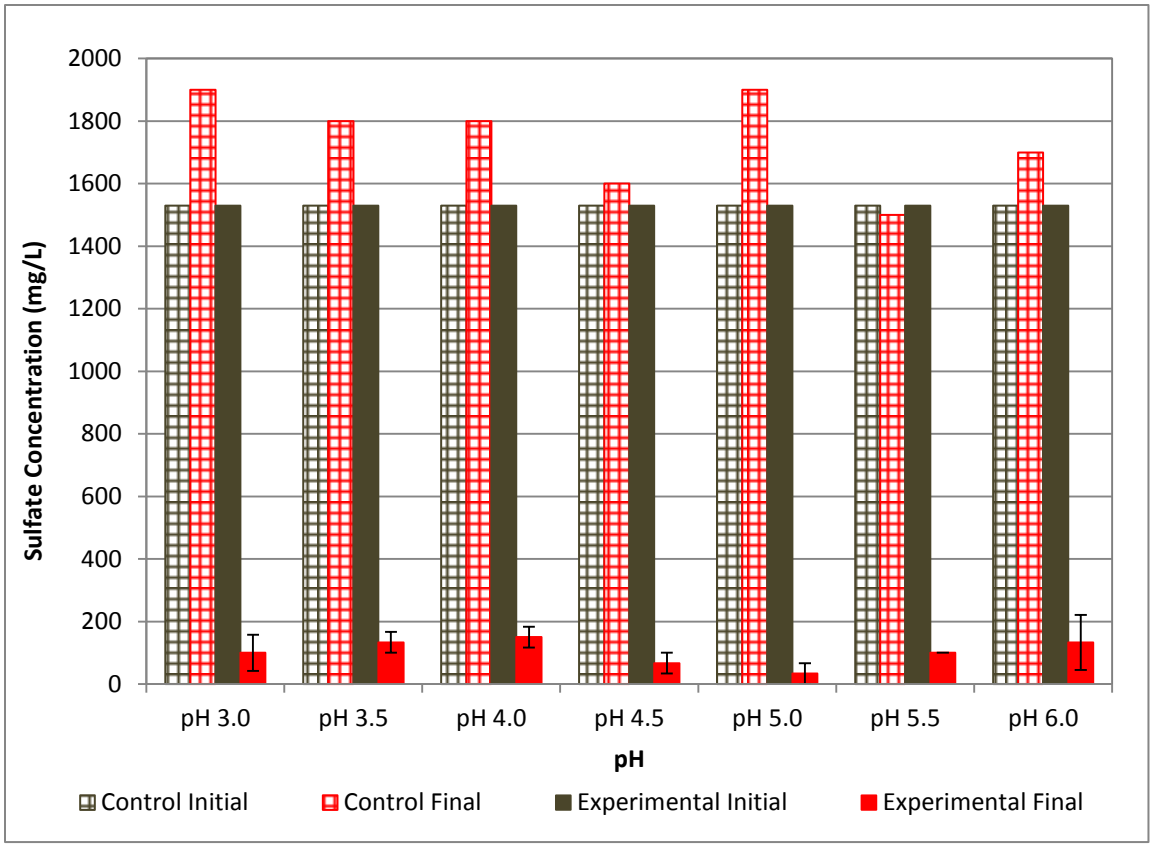

Figure 5.5 Sulfate concentration in aSRB bottles containing sweet potato at low sulfate concentration decreased in experimental bottles but remained the same in control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

5.2.2 aSRB Batch Experiment with Horse Manure. At 15 days the experimental bottles with horse manure at high and low sulfate concentration and across all pH levels had a slight deposition of black precipitate at the interface of the sediment and media, but the sediment remained gray. At day 33 all experimental bottles were mostly gray with small pockets of black precipitate. After 37 days the pH value in all experimental bottles at high and low sulfate concentration increased to a pH value around 6.4 (Figure 5.6, Figure 5.7). The pH in all control bottles increased to between 5 and 6, except for control bottle at pH 3 and high sulfate concentration, which increased to 4.17. The increase of pH in both experimental and control bottles indicates the horse manure itself has some acid neutralizing capacity.

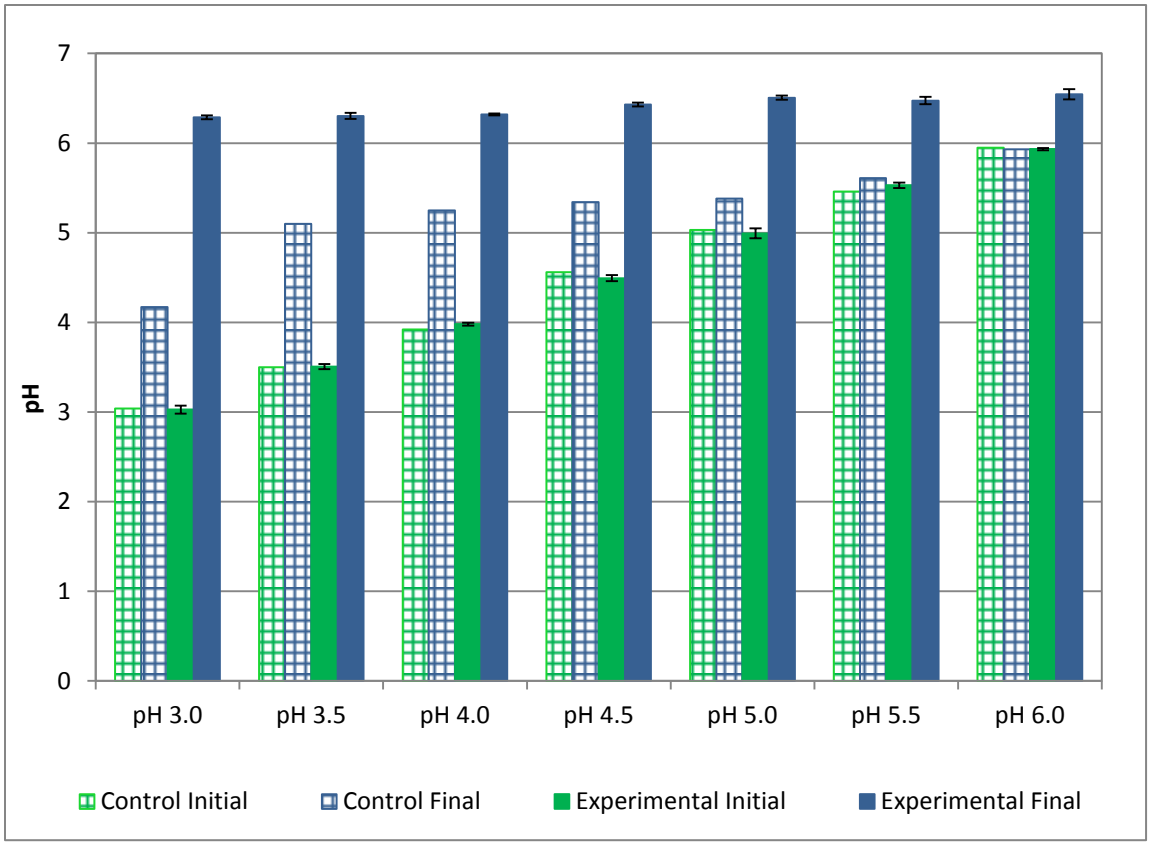

Figure 5.6 pH in aSRB bottles containing horse manure and high sulfate concentration increased in both experimental bottles and control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

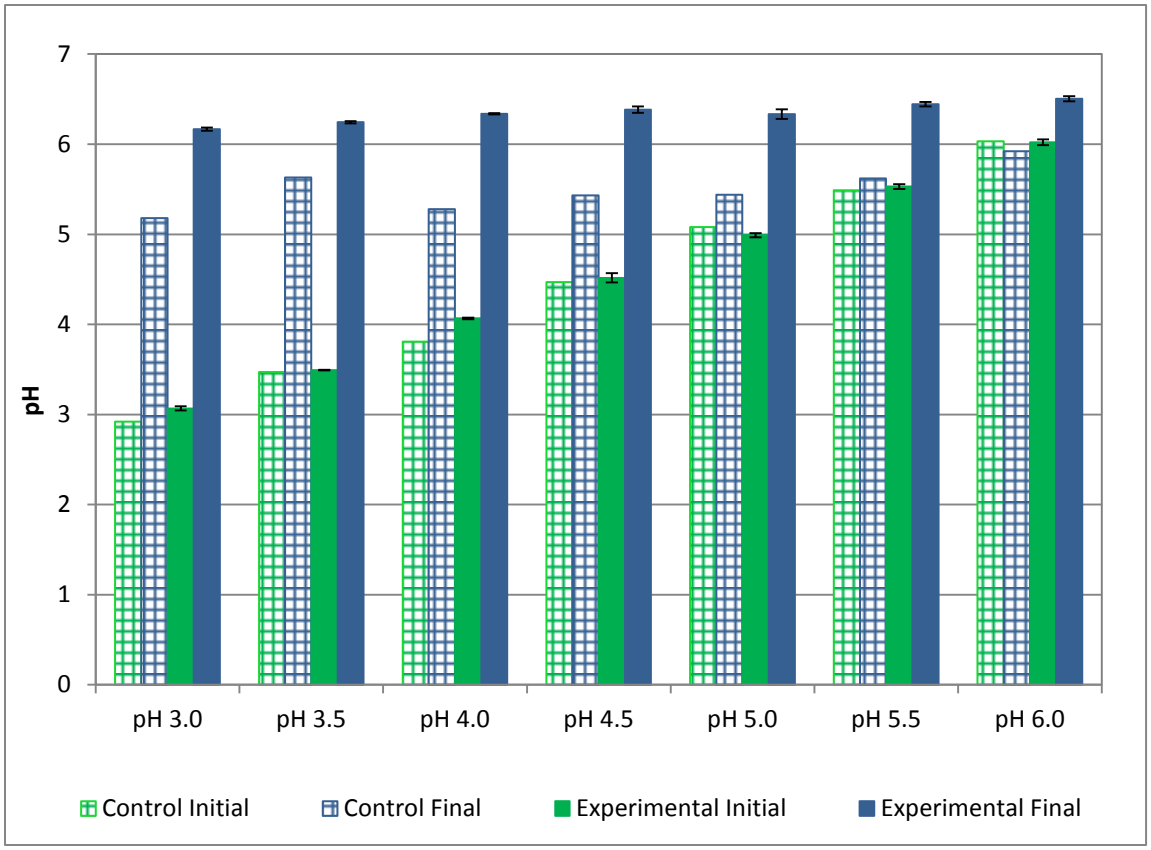

Figure 5.7 pH in aSRB bottles containing horse manure and low sulfate concentration increased in both experimental bottles and control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

In reactors containing a high sulfate concentration and horse manure, sulfate was not reduced but the sulfate concentration increased after 37 days at all pH values. Modest sulfate increase was also measured in low sulfate concentration experimental and control bottles at all pH values (Figure 5.8, Figure 5.9). The observed pH increase indicates the horse manure contained soluble sulfate, causing an increase in sulfate concentration. The increase in pH also appears to be directly due to the manure, not due to aSRBs activity.

The combined results indicate that aSRBs are not able to utilize horse manure as a carbon source.

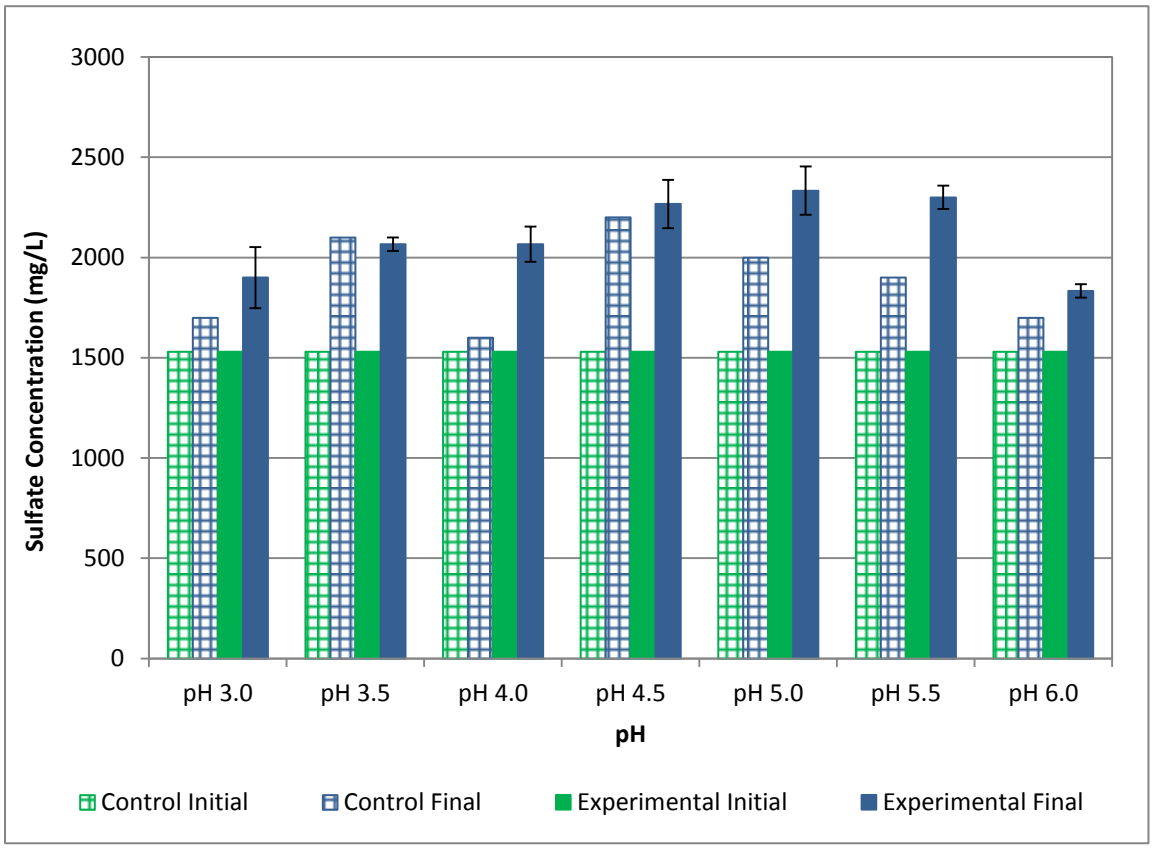

Figure 5.8 Sulfate concentration in aSRB bottles containing horse manure and high sulfate concentration increased in both experimental bottles and control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

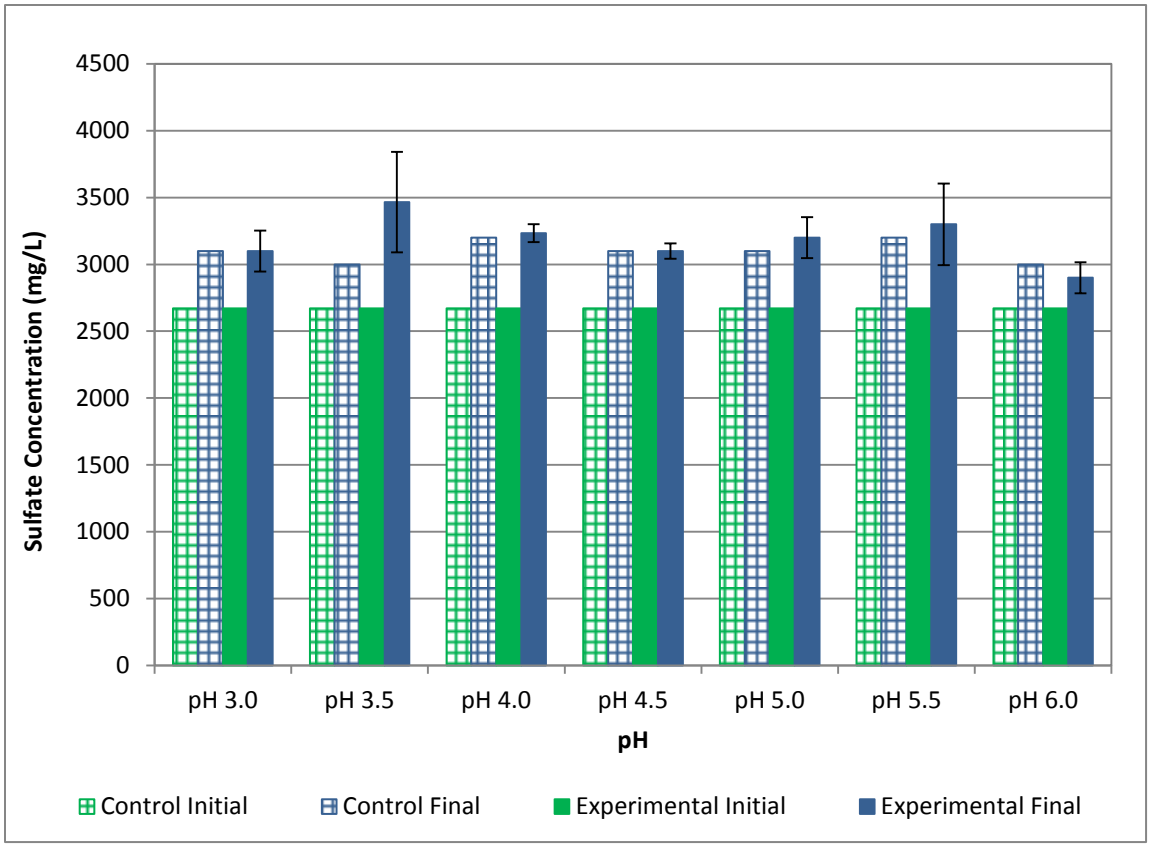

Figure 5.9 Sulfate concentration in aSRB bottles containing horse manure and low sulfate concentration increased in both experimental bottles and control bottles. The error bars represent the variance in samples of the experimental bottles (n=3).

5.2.3 SAS Analysis. Using statistical analysis software (SAS), an analysis of variance (ANOVA) was conducted on the data to determine the interactions between the carbon source, sulfate concentration and pH values. From this ANOVA test, carbon source, initial sulfate concentration in the experimental bottles, and the control bottles all interact to change the final sulfate concentration (P value of 0.0038). The analysis indicates the inoculum did have a factor in changing the sulfate concentration over time when comparing the experimental and control bottles. The control bottles were then removed from the interaction and the model was run using the initial sulfate concentrations and carbon sources, assessing the effect on final concentration (Figure 5.10). A P value of <0.0001 was achieved, indicating the initial pH value did not cause the change in final sulfate concentration, but the carbon source of sweet potato versus horse manure was the primary cause of difference in final sulfate concentration.

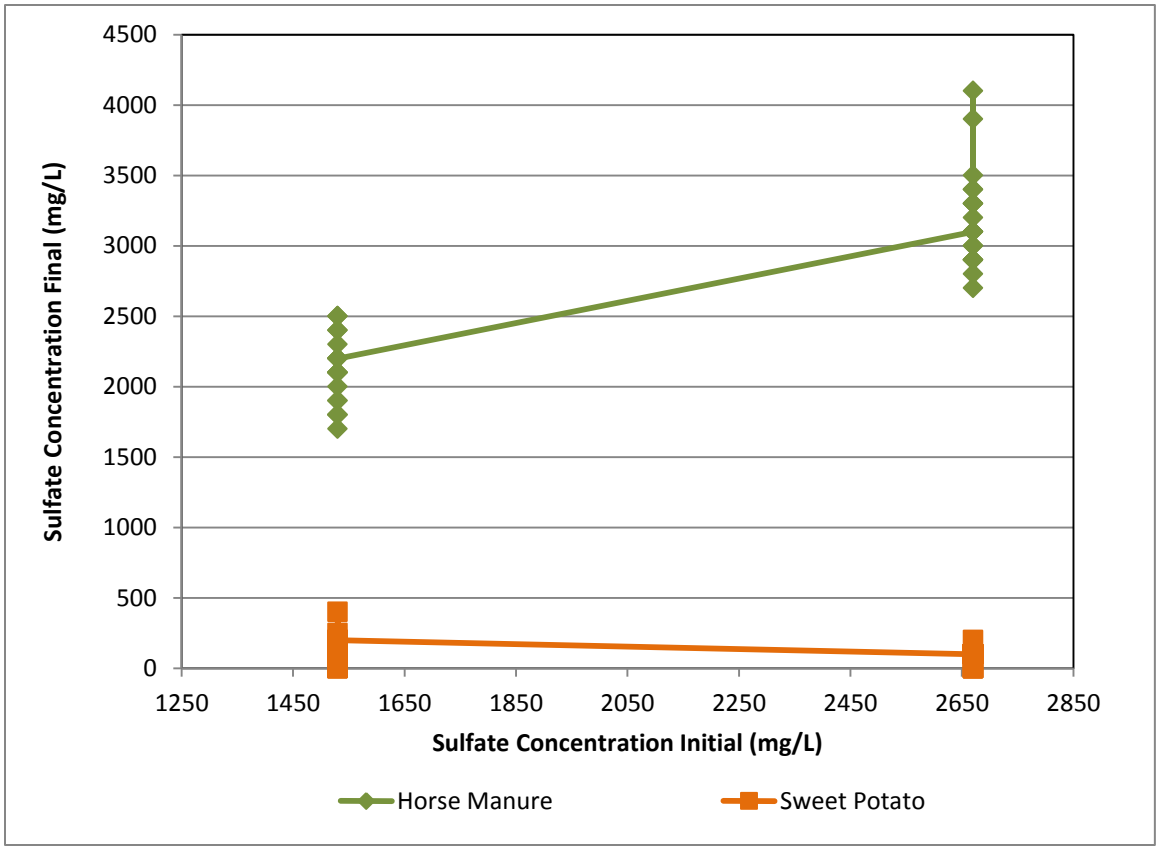

Figure 5.10 SAS Analysis. Significant decrease in sulfate concentration found in bottles containing sweet potato but not in bottles contatining horse manure.

5.2.4 aFRB Batch Experiment. After 37 days, the reactors containing sweet potato as a carbon source and iron reducing media appeared to have no black precipitate present. Reactors at pH 3.0, 3.5, 4.0, and 4.5 showed a similar increase in pH value between the control and experimental bottles (Figure 5.11). Reactors at pH 5.0, 5.5, and 6.0 showed no increase from the initial pH values. At pH 3.0, 3.5 and 4.0, the bottles containing horse manure had a similar increase in both experimental and control bottles (Figure 5.12). Bottles at pH 4.5 through 6.0 containing horse manure did not have a significant increase in pH. Collectively, the data indicates that acidophilic iron reducing bacteria are not responsible for the black precipitate present in the first batch experiment.

Based on the ANOVA analysis of the aFRB, the final pH was the only significant factor causing an increase in pH initial (P value <0.0001), the carbon source was not significant.

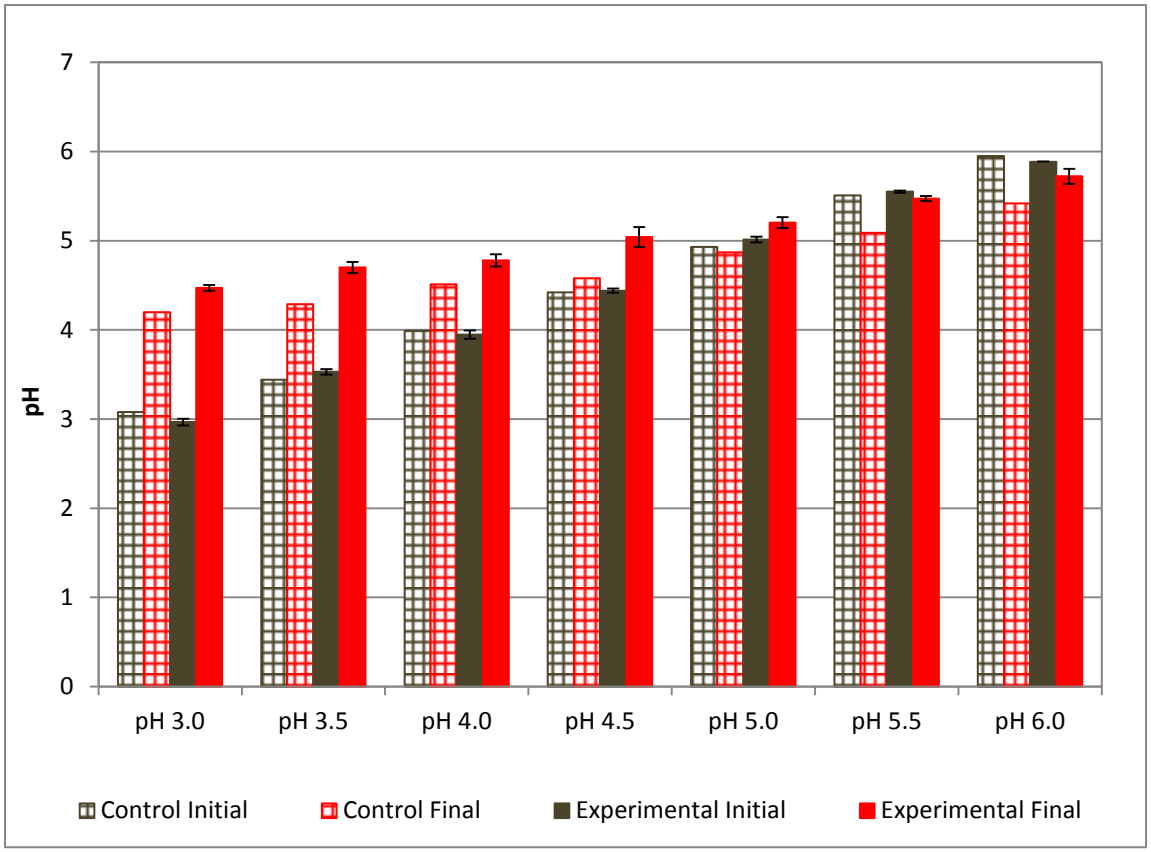

Figure 5.11 pH in aFRB bottles containing sweet potato insignificantly increased in experimental and control bottles at pH 3.0, 3.5, 4.0 and 4.5 and remained the same in control and experimental bottles at pH 5.0, 5.5 and 6.0.

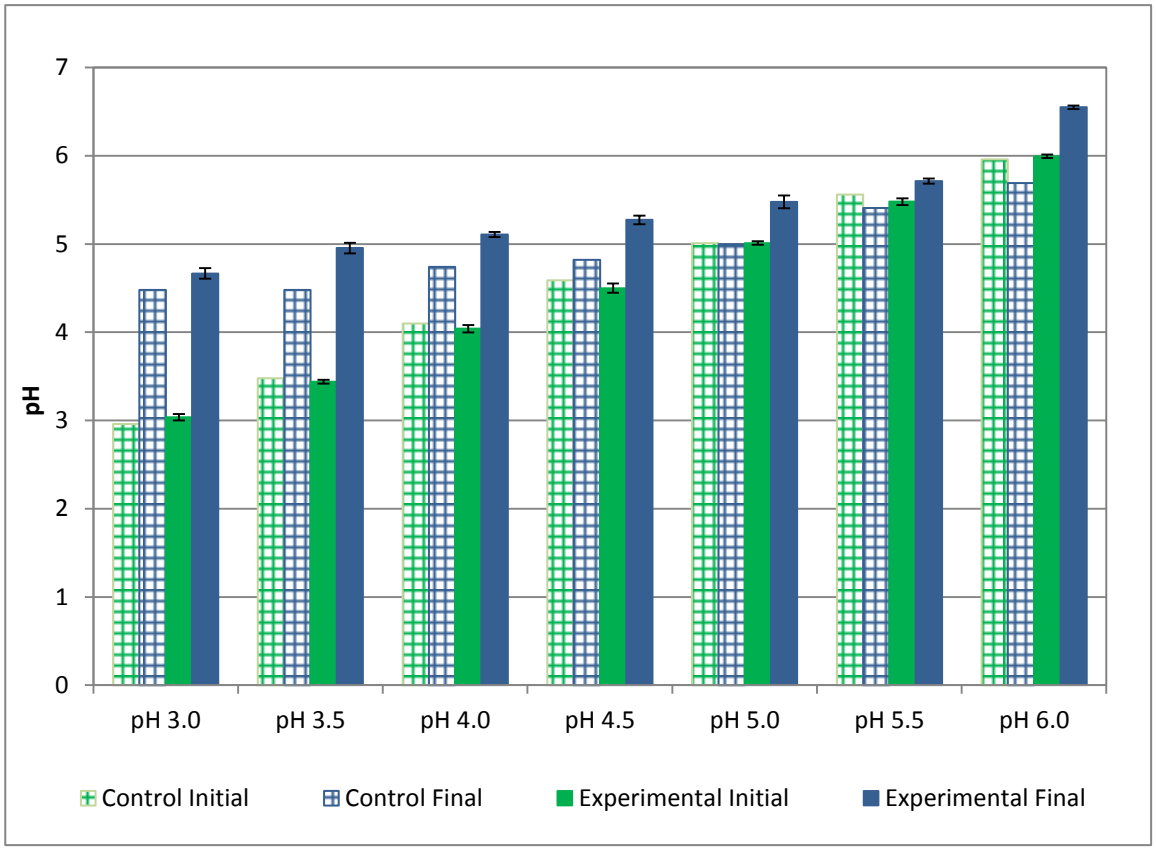

Figure 5.12 pH in aFRB bottles containing horse manure insignificantly increased in experimental and control bottles at pH 3.0, 3.5 and 4.0 and remained the same in control and experimental bottles at pH 4.5, 5.0, 5.5 and 6.0.

5.3 COLUMN EXPERIMENT

Columns remained stagnant for 30 days after construction and saturated with media in the anaerobic glove bag. The columns were visually inspected for black precipitate. Columns containing sweet potato had no visual evidence, where as columns containing horse manure appeared to have black precipitate present.

Media flowed through the setup columns for 10 days prior to testing pH and sulfate concentration. Samples were not able to be taken from the mininert ports, even after being flushed with media, as the sediment in the columns was too packed to retrieve a sample. To ensure the media was flowing through the sediment and not following a preferential pathway, a tracer test with sodium bromide was conducted (Figure 5.13). The columns were running at a hydraulic retention time of 39 hours and after being intially spiked to a conductivity of 2.7 mS, the tracer appeared in all of the columns effluent after 40 hours, indicating preferential flow was not occuring.

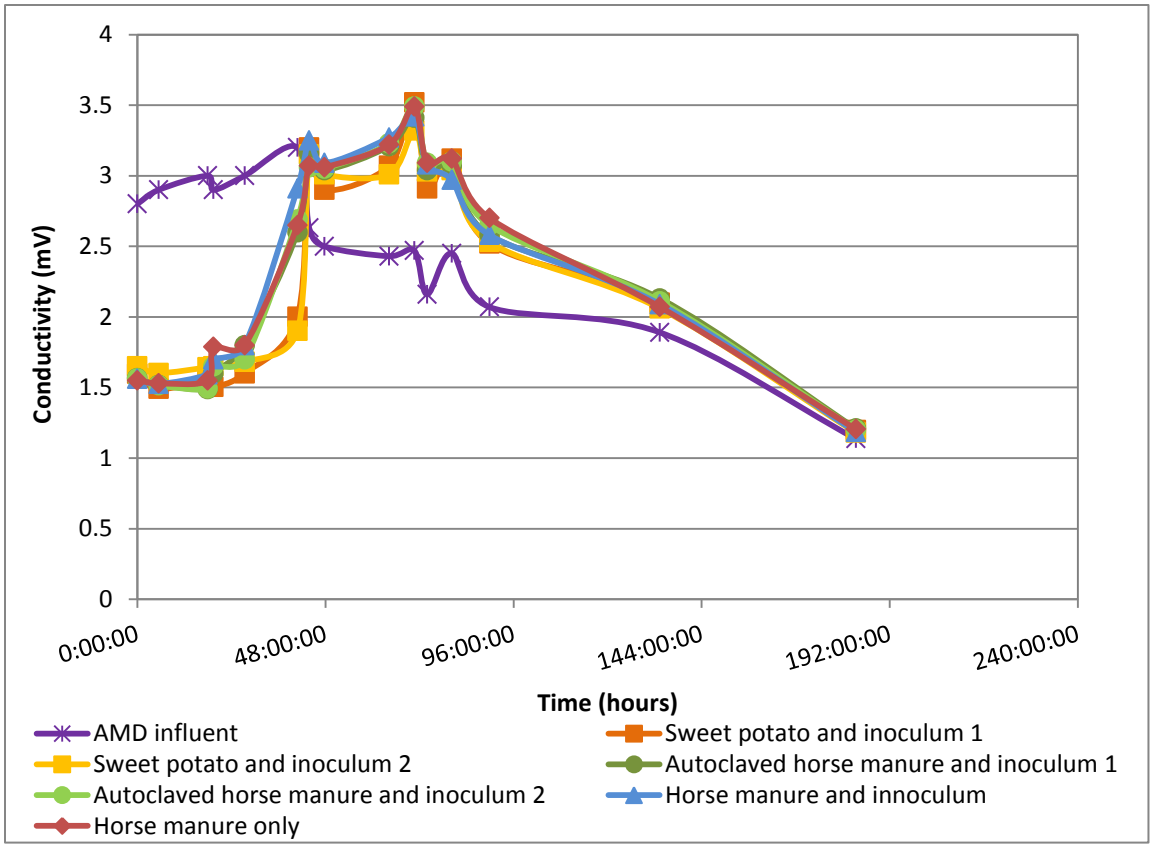

Figure 5.13 Conductivity change over time. Conductivity spiked in columns after 40 hours.

After pH 3.7 media flowed through the columns for 74 days (12 days for column 2), no significant sulfate reduction or pH change was observed and the flow through the columns was terminated to allow stagnation (Figure 5.14, Figure 5.15). Forty three days later there was evidence of black precipitate in the second sweet potato column, the column that had to be remade, and the flow through the columns was turned back on, with the pH of the synthetic AMD increased to between 6.0 and 7.0. Six days later the columns were reinoculated with samples from serum bottles of similar carbon source.

Forty days after the columns were reinoculated, the second column containing sweet potato and inoculum started to show a significant decrease in sulfate concentration ($P<0.0028$), but the low pH present was not indicative of sulfate reduction.

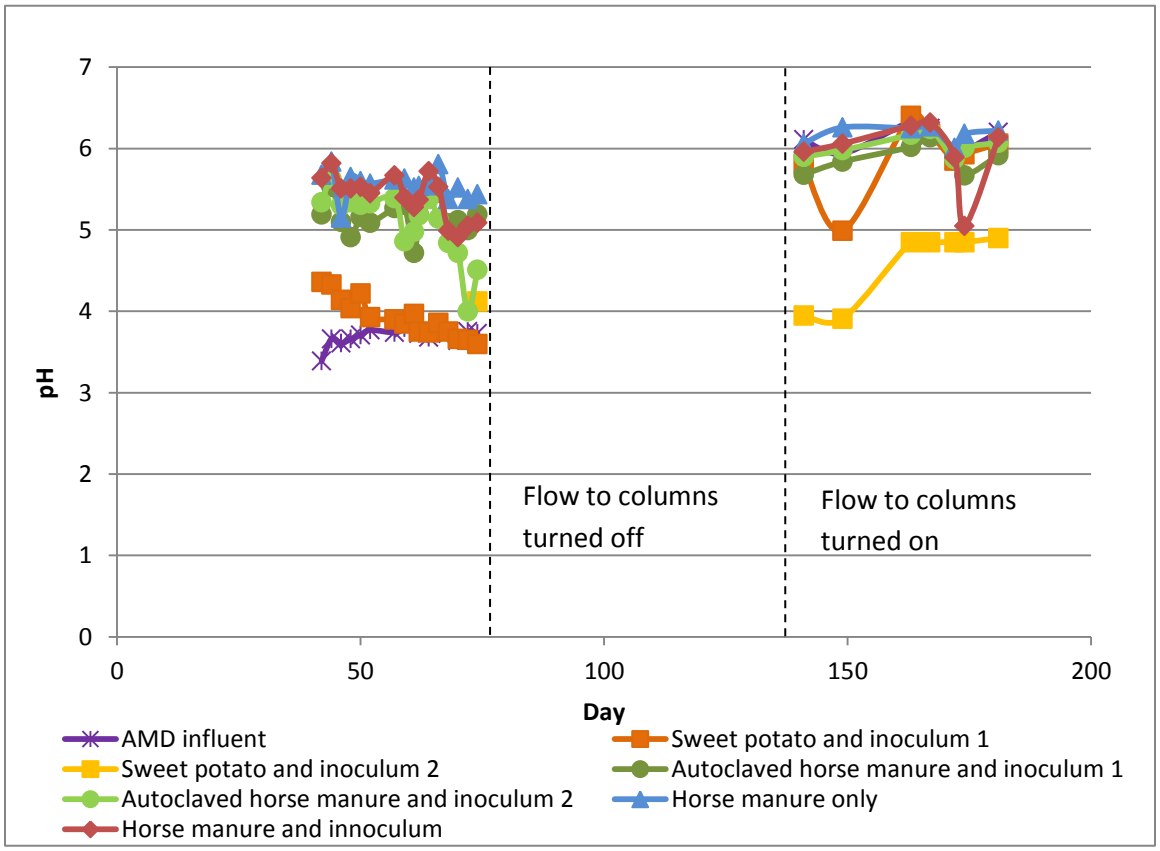

Figure 5.14 Column pH increased only in sweet potato and inoculum column 2.

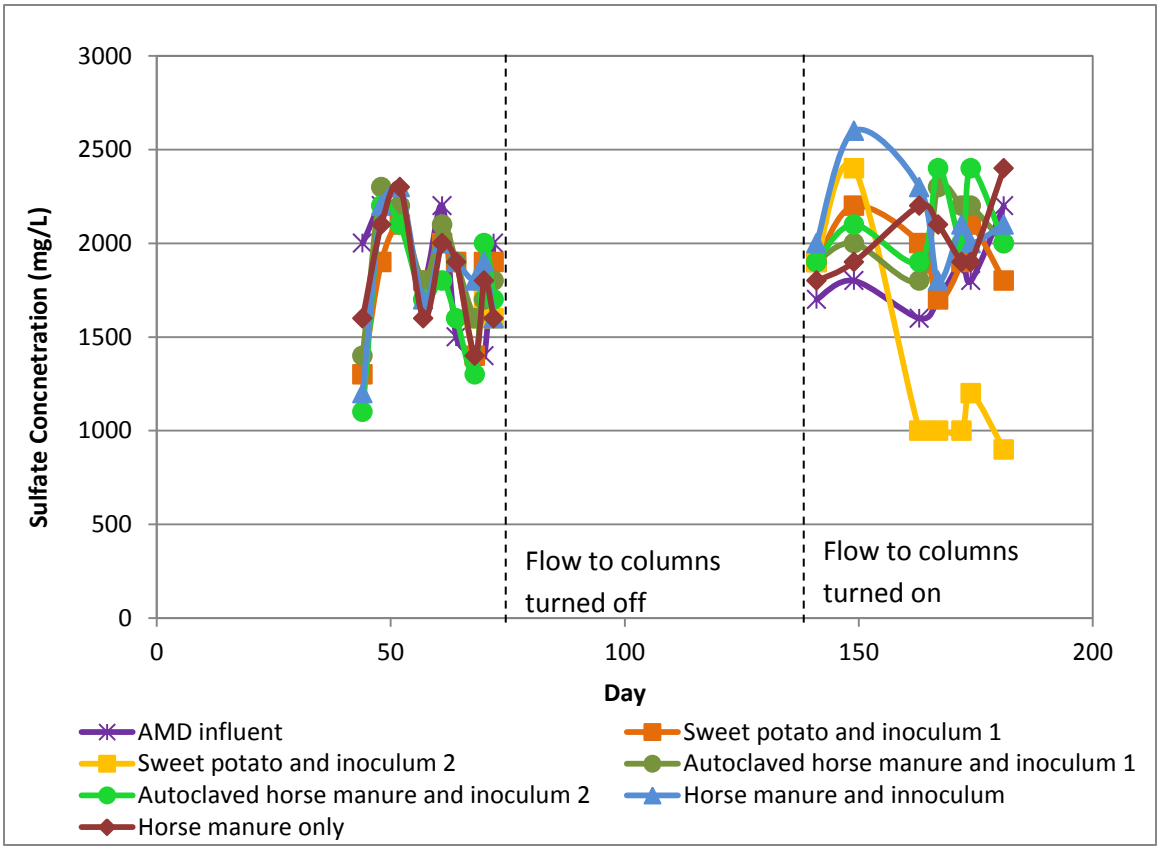

Figure 5.15 Column sulfate concentration decreased in sweet potato and inoculum column 2.

The column effluents were tested for volatile fatty acids, to assess if fermentating bacteria were the dominant bacteria within the column. Columns sweet potato and inoculum one, autoclaved horsemanure and inoculum one and two, horse manure and inoculum, and horse manure only had concentrations of acetic acid, propionic acid and butyric acid similar to the concentrations found in the influent, as seen in Figure 5.16. Sweet potato and inoculum column 2 however had higher levels of acetic acid, propionic acid and butyric acid compared to the influent. A hypothesis for the difference in volatile fatty acid generation is the bacteria in sweet potato and inoculum column 2 did not

experience low pH concentrations as extensively as the other columns had because of being remade and thus the consortia of bacteria survived. Shortly after the sweet potato and inoculum column 2 was setup, all the columns were shut off and a month later had media with a pH of 6.5 to 7.0 flowing through them. The fermenting bacteria and clostridia preferred the near neutral pH, and were able to break down the sweet potato into simpler forms of sugar that can be utilized by the aSRBs to increase the pH, reduce redox potential, and reduce sulfate concentration. However, because fermenting bacteria are present and dominant in the column, the pH did not increase from the aSRBs but the sulfate concentration decreased.

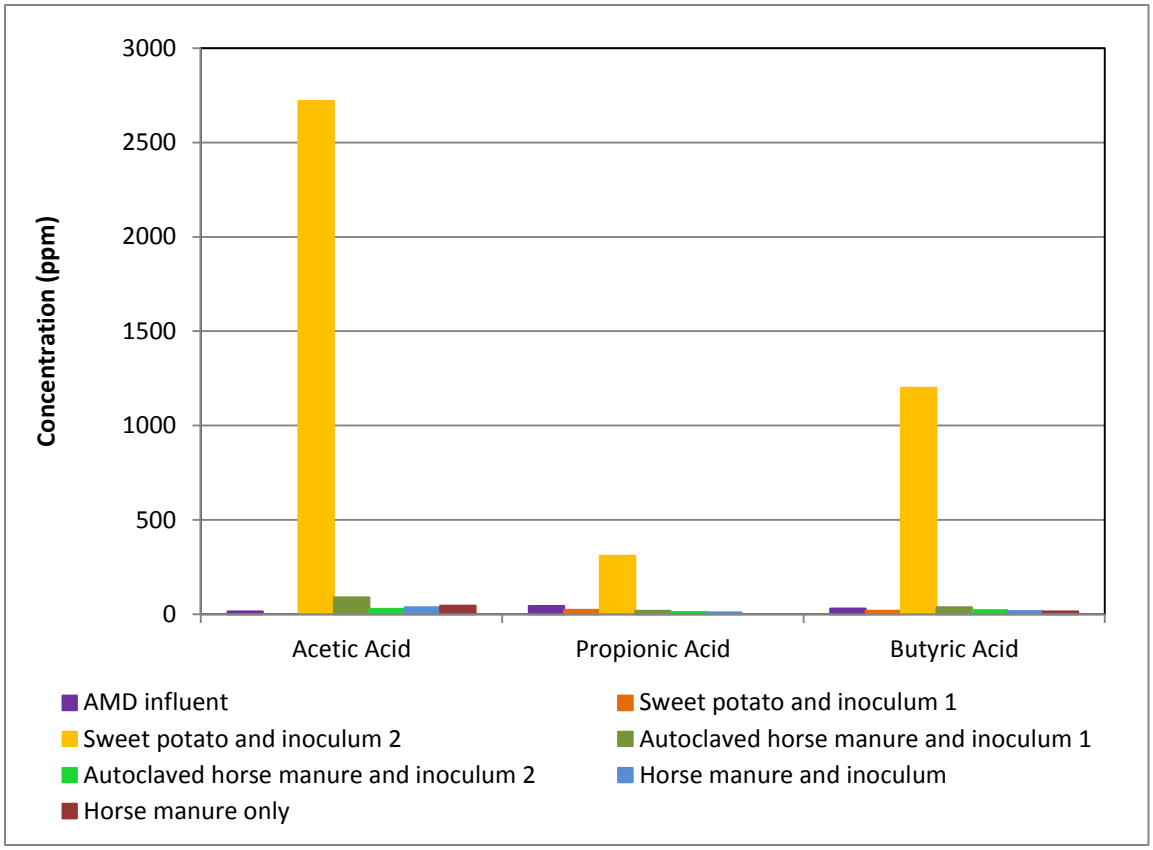

Figure 5.16 Column volatile fatty acid concentration was elevated in sweet potato and inoculum column 2 only.

6 CONCLUSIONS

Overall the project revealed potential for aSRBs to be beneficial in BCR design and implementation. The consortia of bacteria found at Red Lake were able to use sweet potato as a carbon source, breaking it down to simpler carbon sources, allowing aSRBs to reduce the sulfate concentration. The first goal of this study was reached through evaluating aSRBs ability to reduce sulfate under various sulfate concentrations, pH values and carbon sources. The first hypothesis that the consortia of bacteria will utilize the most readily available carbon sources was supported as black precipitate was present in bottles containing sweet potato and horse manure, and not in bottles containing Whatman filter paper number 42, switch grass and cellulose. In addition, the second hypothesis that sulfate reduction and pH increase will occur at all pH ranges was supported in batch studies with sweet potato as a carbon source. However, opposing the hypothesis, sulfate reduction only occurred in bottles containing sweet potato and sulfate reduction and pH increase occurred equally across all pH levels and sulfate concentrations. Sulfate reduction was not found in bottles containing horse manure. The black patches present were possibly present due to the consortia of bacteria previously living in the horse manure utilizing it as a carbon source.

The third hypothesis, that the consortia of bacteria will utilize the specified carbon source to reduce sulfate and increase pH, was not supported. In columns sweet potato and inoculum one, horse manure and inoculum column one and two, horse manure and inoculum column and horse manure only, having synthetic AMD flowing through at an initial pH between 3.5 and 4.0 appeared to attenuate the necessary consortia of bacteria.

In the sweet potato and inoculum column two there was evidence of sulfate reduction but the pH did not increase. This is likely due to not experiencing the lower pH synthetic AMD before the columns were shut off. Once the columns were shut off, it allowed the consortia of bacteria time to break down the complex carbon sources into simpler carbon sources to be utilized by the aSRBs. The decrease in sulfate concentration is evidence of aSRBs present in the column, however because the column was dominated by fermenting bacteria, the pH remained around 4.6.

Overall the specific conclusions are:

- In batch experiments, the consortia of bacteria found at Red Lake are able to utilize sweet potatoes as a carbon source, allowing the aSRBs to increase pH and decrease sulfate concentration
- Horse manure raises pH but the consortia of bacteria are not able to utilize horse manure to decrease sulfate concentration at a low pH
- The consortia of bacteria present at Red Lake, including fermentative bacteria, were able to breakdown sweet potato into simpler carbon sources to be utilized by the aSRB to reduce sulfate, but the fermentative bacteria possibly outcompeted the ability of aSRBs to increase the pH of the AMD

7 RECOMMENDATIONS

For future work, it is suggested the second batch study be completed again, testing sulfate concentration and pH values every three days, giving exact time table of when sulfate reduction and pH increase started to occur. The columns should be remade with synthetic AMD at an initial pH of 6.5. The flow rate through the columns should be maintained at a pulse flow. By running at a pulse flow, when the flow is off the columns will mimic the batch study, allowing the aSRB enough time to utilize the broken down carbon sources and raise the pH. Raising the pH will limit the growth of the inhibitory fermenting bacteria. After running long enough to establish sulfate reduction, the pH can gradually be dropped over time, and maintaining a pulse flow allows the aSRBs sufficient time to utilize the carbon sources and raise the pH.

APPENDIX

Batch Experiment 2, aSRB Media – pH Change

Bottle	pH initial- Day 0	pH final- Day 30
Sweet potato pH 3 exp. 1 high sulfate	2.99	6.5
Sweet potato pH 3 exp. 2 high sulfate	3.09	6.51
Sweet potato pH 3 exp. 3 high sulfate	2.92	6.47
Sweet potato pH 3 control high sulfate	3.01	3.2
Sweet potato pH 3.5 exp. 1 high sulfate	3.51	6.52
Sweet potato pH 3.5 exp. 2 high sulfate	3.48	6.51
Sweet potato pH 3.5 exp. 3 high sulfate	3.49	6.57
Sweet potato pH 3.5 control high sulfate	3.48	3.72
Sweet potato pH 4.0 exp. 1 high sulfate	4.04	6.46
Sweet potato pH 4.0 exp. 2 high sulfate	4.05	6.57
Sweet potato pH 4.0 exp. 3 high sulfate	4.03	6.57
Sweet potato pH 4.0 control high sulfate	4.04	4.35
Sweet potato pH 4.5 exp. 1 high sulfate	4.45	6.63
Sweet potato pH 4.5 exp. 2 high sulfate	4.56	6.66
Sweet potato pH 4.5 exp. 3 high sulfate	4.4	6.66
Sweet potato pH 4.5 control high sulfate	4.57	4.93
Sweet potato pH 5.0 exp. 1 high sulfate	5.06	6.61
Sweet potato pH 5.0 exp. 2 high sulfate	4.88	6.67
Sweet potato pH 5.0 exp. 3 high sulfate	4.94	6.64
Sweet potato pH 5.0 control high sulfate	4.92	5

Sweet potato pH 5.5 exp. 1 high sulfate	5.53	6.8
Sweet potato pH 5.5 exp. 2 high sulfate	5.48	6.77
Sweet potato pH 5.5 exp. 3 high sulfate	5.58	6.73
Sweet potato pH 5.5 control high sulfate	5.52	5.5
Sweet potato pH 6.0 exp. 1 high sulfate	5.95	6.84
Sweet potato pH 6.0 exp. 2 high sulfate	5.96	6.77
Sweet potato pH 6.0 exp. 3 high sulfate	6	6.84
Sweet potato pH 6.0 control high sulfate	6	5.89
Horse manure pH 3 exp. 1 high sulfate	3.01	6.26
Horse manure pH 3 exp. 2 high sulfate	2.96	6.27
Horse manure pH 3 exp. 3 high sulfate	3.11	6.33
Horse manure pH 3 control high sulfate	3.04	4.17
Horse manure pH 3.5 exp. 1 high sulfate	3.52	6.37
Horse manure pH 3.5 exp. 2 high sulfate	3.45	6.28
Horse manure pH 3.5 exp. 3 high sulfate	3.55	6.26
Horse manure pH 3.5 control high sulfate	3.5	5.1
Horse manure pH 4.0 exp. 1 high sulfate	3.98	6.32
Horse manure pH 4.0 exp. 2 high sulfate	4.01	6.3
Horse manure pH 4.0 exp. 3 high sulfate	3.95	6.34
Horse manure pH 4.0 control high sulfate	3.92	5.25
Horse manure pH 4.5 exp. 1 high sulfate	4.48	6.47
Horse manure pH 4.5 exp. 2 high sulfate	4.44	6.4
Horse manure pH 4.5 exp. 3 high sulfate	4.56	6.42
Horse manure pH 4.5 control high	4.56	5.34

sulfate		
Horse manure pH 5.0 exp. 1 high sulfate	4.96	6.52
Horse manure pH 5.0 exp. 2 high sulfate	4.92	6.54
Horse manure pH 5.0 exp. 3 high sulfate	5.1	6.46
Horse manure pH 5.0 control high sulfate	5.03	5.38
Horse manure pH 5.5 exp. 1 high sulfate	5.57	6.41
Horse manure pH 5.5 exp. 2 high sulfate	5.55	6.46
Horse manure pH 5.5 exp. 3 high sulfate	5.47	6.55
Horse manure pH 5.5 control high sulfate	5.46	5.61
Horse manure pH 6.0 exp. 1 high sulfate	5.91	6.61
Horse manure pH 6.0 exp. 2 high sulfate	5.95	6.59
Horse manure pH 6.0 exp. 3 high sulfate	5.94	6.43
Horse manure pH 6.0 control high sulfate	5.95	5.93
Sweet potato pH 3 exp. 1 low sulfate	3.04	6.5
Sweet potato pH 3 exp. 2 low sulfate	3	6.44
Sweet potato pH 3 exp. 3 low sulfate	2.97	6.4
Sweet potato pH 3 control low sulfate	3.04	3.27
Sweet potato pH 3.5 exp. 1 low sulfate	3.56	6.49
Sweet potato pH 3.5 exp. 2 low sulfate	3.53	6.54
Sweet potato pH 3.5 exp. 3 low sulfate	3.5	6.57
Sweet potato pH 3.5 control low sulfate	3.47	3.57
Sweet potato pH 4.0 exp. 1 low sulfate	3.95	6.71
Sweet potato pH 4.0 exp. 2 low sulfate	4.02	6.5
Sweet potato pH 4.0 exp. 3 low sulfate	3.97	6.5
Sweet potato pH 4.0 control low sulfate	4.04	4.24
Sweet potato pH 4.5 exp. 1 low sulfate	4.58	6.51
Sweet potato pH 4.5 exp. 2 low sulfate	4.59	6.47
Sweet potato pH 4.5 exp. 3 low sulfate	4.57	6.55
Sweet potato pH 4.5 control low sulfate	4.58	4.73

Sweet potato pH 5.0 exp. 1 low sulfate	5.09	6.46
Sweet potato pH 5.0 exp. 2 low sulfate	5.08	6.57
Sweet potato pH 5.0 exp. 3 low sulfate	5.02	6.54
Sweet potato pH 5.0 control low sulfate	4.95	4.99
Sweet potato pH 5.5 exp. 1 low sulfate	5.55	6.61
Sweet potato pH 5.5 exp. 2 low sulfate	5.48	6.54
Sweet potato pH 5.5 exp. 3 low sulfate	5.45	6.61
Sweet potato pH 5.5 control low sulfate	5.46	5.42
Sweet potato pH 6.0 exp. 1 low sulfate	6.03	6.5
Sweet potato pH 6.0 exp. 2 low sulfate	5.97	6.47
Sweet potato pH 6.0 exp. 3 low sulfate	5.96	6.49
Sweet potato pH 6.0 control low sulfate	6.07	5.85
Horse manure pH 3 exp. 1 low sulfate	3.11	6.13
Horse manure pH 3 exp. 2 low sulfate	3.03	6.19
Horse manure pH 3 exp. 3 low sulfate	3.06	6.18
Horse manure pH 3 control low sulfate	2.92	5.18
Horse manure pH 3.5 exp. 1 low sulfate	3.49	6.26
Horse manure pH 3.5 exp. 2 low sulfate	3.49	6.25
Horse manure pH 3.5 exp. 3 low sulfate	3.5	6.22
Horse manure pH 3.5 control low sulfate	3.47	5.63
Horse manure pH 4.0 exp. 1 low sulfate	4.07	6.33
Horse manure pH 4.0 exp. 2 low sulfate	4.08	6.35
Horse manure pH 4.0 exp. 3 low sulfate	4.05	6.33
Horse manure pH 4.0 control low sulfate	3.81	5.28
Horse manure pH 4.5 exp. 1 low sulfate	4.6	6.45
Horse manure pH 4.5 exp. 2 low sulfate	4.42	6.33
Horse manure pH 4.5 exp. 3 low sulfate	4.53	6.37
Horse manure pH 4.5 control low sulfate	4.47	5.43

Horse manure pH 5.0 exp. 1 low sulfate	4.99	6.23
Horse manure pH 5.0 exp. 2 low sulfate	5.03	6.37
Horse manure pH 5.0 exp. 3 low sulfate	4.95	6.4
Horse manure pH 5.0 control low sulfate	5.08	5.44
Horse manure pH 5.5 exp. 1 low sulfate	5.58	6.41
Horse manure pH 5.5 exp. 2 low sulfate	5.49	6.43
Horse manure pH 5.5 exp. 3 low sulfate	5.52	6.49
Horse manure pH 5.5 control low sulfate	5.49	5.62
Horse manure pH 6.0 exp. 1 low sulfate	6.07	6.51
Horse manure pH 6.0 exp. 2 low sulfate	6.03	6.55
Horse manure pH 6.0 exp. 3 low sulfate	5.96	6.45
Horse manure pH 6.0 control low sulfate	6.03	5.92

Batch Experiment 2, aSRB Media – Sulfate Concentration Change

Bottle	**Sulfate initial- Day 0 (mg/L)**	**Sulfate final- Day 30 (mg/L)**
Sweet potato pH 3 exp. 1 high sulfate	2670	400
Sweet potato pH 3 exp. 2 high sulfate	2670	700
Sweet potato pH 3 exp. 3 high sulfate	2670	600
Sweet potato pH 3 control high sulfate	2670	2900
Sweet potato pH 3.5 exp. 1 high sulfate	2670	100
Sweet potato pH 3.5 exp. 2 high sulfate	2670	400
Sweet potato pH 3.5 exp. 3 high	2670	300

sulfate		
Sweet potato pH 3.5 control high sulfate	2670	3100
Sweet potato pH 4.0 exp. 1 high sulfate	2670	700
Sweet potato pH 4.0 exp. 2 high sulfate	2670	1000
Sweet potato pH 4.0 exp. 3 high sulfate	2670	900
Sweet potato pH 4.0 control high sulfate	2670	3000
Sweet potato pH 4.5 exp. 1 high sulfate	2670	500
Sweet potato pH 4.5 exp. 2 high sulfate	2670	400
Sweet potato pH 4.5 exp. 3 high sulfate	2670	100
Sweet potato pH 4.5 control high sulfate	2670	3000
Sweet potato pH 5.0 exp. 1 high sulfate	2670	600
Sweet potato pH 5.0 exp. 2 high sulfate	2670	400
Sweet potato pH 5.0 exp. 3 high sulfate	2670	800
Sweet potato pH 5.0 control high sulfate	2670	2700
Sweet potato pH 5.5 exp. 1 high sulfate	2670	200
Sweet potato pH 5.5 exp. 2 high sulfate	2670	400
Sweet potato pH 5.5 exp. 3 high sulfate	2670	900
Sweet potato pH 5.5 control high sulfate	2670	2700
Sweet potato pH 6.0 exp. 1 high sulfate	2670	300
Sweet potato pH 6.0 exp. 2 high sulfate	2670	500
Sweet potato pH 6.0 exp. 3 high sulfate	2670	300
Sweet potato pH 6.0 control high sulfate	2670	3100
Horse manure pH 3 exp. 1 high sulfate	2670	3400

Horse manure pH 3 exp. 2 high sulfate	2670	3000
Horse manure pH 3 exp. 3 high sulfate	2670	2900
Horse manure pH 3 control high sulfate	2670	3100
Horse manure pH 3.5 exp. 1 high sulfate	2670	4100
Horse manure pH 3.5 exp. 2 high sulfate	2670	3500
Horse manure pH 3.5 exp. 3 high sulfate	2670	2800
Horse manure pH 3.5 control high sulfate	2670	3000
Horse manure pH 4.0 exp. 1 high sulfate	2670	3300
Horse manure pH 4.0 exp. 2 high sulfate	2670	3300
Horse manure pH 4.0 exp. 3 high sulfate	2670	3100
Horse manure pH 4.0 control high sulfate	2670	3200
Horse manure pH 4.5 exp. 1 high sulfate	2670	3100
Horse manure pH 4.5 exp. 2 high sulfate	2670	3000
Horse manure pH 4.5 exp. 3 high sulfate	2670	3200
Horse manure pH 4.5 control high sulfate	2670	3100
Horse manure pH 5.0 exp. 1 high sulfate	2670	2900
Horse manure pH 5.0 exp. 2 high sulfate	2670	3400
Horse manure pH 5.0 exp. 3 high sulfate	2670	3300
Horse manure pH 5.0 control high sulfate	2670	3100
Horse manure pH 5.5 exp. 1 high sulfate	2670	3900
Horse manure pH 5.5 exp. 2 high sulfate	2670	2900
Horse manure pH 5.5 exp. 3 high sulfate	2670	3100
Horse manure pH 5.5 control high sulfate	2670	3200
Horse manure pH 6.0 exp. 1 high	2670	2700

sulfate		
Horse manure pH 6.0 exp. 2 high sulfate	2670	2900
Horse manure pH 6.0 exp. 3 high sulfate	2670	3100
Horse manure pH 6.0 control high sulfate	2670	3000
Sweet potato pH 3 exp. 1 low sulfate	1530	200
Sweet potato pH 3 exp. 2 low sulfate	1530	100
Sweet potato pH 3 exp. 3 low sulfate	1530	0
Sweet potato pH 3 control low sulfate	1530	1900
Sweet potato pH 3.5 exp. 1 low sulfate	1530	200
Sweet potato pH 3.5 exp. 2 low sulfate	1530	100
Sweet potato pH 3.5 exp. 3 low sulfate	1530	100
Sweet potato pH 3.5 control low sulfate	1530	1800
Sweet potato pH 4.0 exp. 1 low sulfate	1530	300
Sweet potato pH 4.0 exp. 2 low sulfate	1530	200
Sweet potato pH 4.0 exp. 3 low sulfate	1530	200
Sweet potato pH 4.0 control low sulfate	1530	1800
Sweet potato pH 4.5 exp. 1 low sulfate	1530	100
Sweet potato pH 4.5 exp. 2 low sulfate	1530	100
Sweet potato pH 4.5 exp. 3 low sulfate	1530	0
Sweet potato pH 4.5 control low sulfate	1530	1600
Sweet potato pH 5.0 exp. 1 low sulfate	1530	100
Sweet potato pH 5.0 exp. 2 low sulfate	1530	0
Sweet potato pH 5.0 exp. 3 low sulfate	1530	0
Sweet potato pH 5.0 control low sulfate	1530	1900
Sweet potato pH 5.5 exp. 1 low sulfate	1530	100
Sweet potato pH 5.5 exp. 2 low sulfate	1530	100
Sweet potato pH 5.5 exp. 3 low sulfate	1530	100
Sweet potato pH 5.5 control low sulfate	1530	1500
Sweet potato pH 6.0 exp. 1 low sulfate	1530	0
Sweet potato pH 6.0 exp. 2 low sulfate	1530	100
Sweet potato pH 6.0 exp. 3 low sulfate	1530	300
Sweet potato pH 6.0 control low sulfate	1530	1700
Horse manure pH 3 exp. 1 low sulfate	1530	2200
Horse manure pH 3 exp. 2 low sulfate	1530	1700

Horse manure pH 3 exp. 3 low sulfate	1530	1800
Horse manure pH 3 control low sulfate	1530	1700
Horse manure pH 3.5 exp. 1 low sulfate	1530	2100
Horse manure pH 3.5 exp. 2 low sulfate	1530	2100
Horse manure pH 3.5 exp. 3 low sulfate	1530	2000
Horse manure pH 3.5 control low sulfate	1530	2100
Horse manure pH 4.0 exp. 1 low sulfate	1530	2100
Horse manure pH 4.0 exp. 2 low sulfate	1530	2200
Horse manure pH 4.0 exp. 3 low sulfate	1530	1900
Horse manure pH 4.0 control low sulfate	1530	1600
Horse manure pH 4.5 exp. 1 low sulfate	1530	2200
Horse manure pH 4.5 exp. 2 low sulfate	1530	2100
Horse manure pH 4.5 exp. 3 low sulfate	1530	2500
Horse manure pH 4.5 control low sulfate	1530	2200
Horse manure pH 5.0 exp. 1 low sulfate	1530	2500
Horse manure pH 5.0 exp. 2 low sulfate	1530	2100
Horse manure pH 5.0 exp. 3 low sulfate	1530	2400
Horse manure pH 5.0 control low sulfate	1530	2000
Horse manure pH 5.5 exp. 1 low sulfate	1530	2300
Horse manure pH 5.5 exp. 2 low sulfate	1530	2400
Horse manure pH 5.5 exp. 3 low sulfate	1530	2200
Horse manure pH 5.5 control low sulfate	1530	1900
Horse manure pH 6.0 exp. 1 low sulfate	1530	1900

Horse manure pH 6.0 exp. 2 low sulfate	1530	1800
Horse manure pH 6.0 exp. 3 low sulfate	1530	1800
Horse manure pH 6.0 control low sulfate	1530	1700

Batch Experiment 2, aFRB Media – pH Change

Bottle	**pH initial- Day 0**	**pH final- Day 30**
Sweet potato pH 3 exp. 1	2.95	4.48
Sweet potato pH 3 exp. 2	3.04	4.52
Sweet potato pH 3 exp. 3	2.91	4.41
Sweet potato pH 3 control	3.08	4.2
Sweet potato pH 3.5 exp. 1	3.59	4.82
Sweet potato pH 3.5 exp. 2	3.5	4.67
Sweet potato pH 3.5 exp. 3	3.5	4.61
Sweet potato pH 3.5 control	3.44	4.29
Sweet potato pH 4.0 exp. 1	3.89	4.65
Sweet potato pH 4.0 exp. 2	4.04	4.81
Sweet potato pH 4.0 exp. 3	3.91	4.88
Sweet potato pH 4.0 control	3.99	4.51
Sweet potato pH 4.5 exp. 1	4.48	4.98
Sweet potato pH 4.5 exp. 2	4.44	5.26
Sweet potato pH 4.5 exp. 3	4.4	4.89
Sweet potato pH 4.5 control	4.42	4.58
Sweet potato pH 5.0 exp. 1	4.98	5.32
Sweet potato pH 5.0 exp. 2	5.08	5.17
Sweet potato pH 5.0 exp. 3	4.98	5.12
Sweet potato pH 5.0 control	4.93	4.87
Sweet potato pH 5.5 exp. 1	5.55	5.45
Sweet potato pH 5.5 exp. 2	5.57	5.44
Sweet potato pH 5.5 exp. 3	5.53	5.53
Sweet potato pH 5.5 control	5.51	5.09
Sweet potato pH 6.0 exp. 1	5.89	5.62
Sweet potato pH 6.0 exp. 2	5.87	5.66
Sweet potato pH 6.0 exp. 3	5.89	5.89
Sweet potato pH 6.0 control	5.95	5.42
Horse manure pH 3 exp. 1	3.09	4.78

Horse manure pH 3 exp. 2	3.05	4.58
Horse manure pH 3 exp. 3	2.97	4.64
Horse manure pH 3 control	2.96	4.48
Horse manure pH 3.5 exp. 1	3.47	4.87
Horse manure pH 3.5 exp. 2	3.4	4.92
Horse manure pH 3.5 exp. 3	3.45	5.07
Horse manure pH 3.5 control	3.48	4.48
Horse manure pH 4.0 exp. 1	4.09	5.13
Horse manure pH 4.0 exp. 2	3.96	5.05
Horse manure pH 4.0 exp. 3	4.07	5.14
Horse manure pH 4.0 control	4.1	4.74
Horse manure pH 4.5 exp. 1	4.53	5.37
Horse manure pH 4.5 exp. 2	4.4	5.21
Horse manure pH 4.5 exp. 3	4.57	5.24
Horse manure pH 4.5 control	4.59	4.82
Horse manure pH 5.0 exp. 1	4.99	5.33
Horse manure pH 5.0 exp. 2	5.05	5.56
Horse manure pH 5.0 exp. 3	4.99	5.54
Horse manure pH 5.0 control	5.01	5
Horse manure pH 5.5 exp. 1	5.41	5.66
Horse manure pH 5.5 exp. 2	5.54	5.76
Horse manure pH 5.5 exp. 3	5.49	5.72
Horse manure pH 5.5 control	5.56	5.41
Horse manure pH 6.0 exp. 1	6.03	6.51
Horse manure pH 6.0 exp. 2	5.99	6.57
Horse manure pH 6.0 exp. 3	5.96	6.57
Horse manure pH 6.0 control	5.96	5.69

Column Study - pH Change.

Day	AMD influent	Sweet potato and inoculum 1	Sweet potato and inoculum 2	Auto-claved horse manure and inoculum 1	Auto-claved horse manure and inoculum 2	Horse manure and inoculum	Horse manure only
40	3.87	5.06		4.98	5.4	5.87	5.94
42	3.39	4.36		5.19	5.34	5.64	5.68
44	3.66	4.33		5.53	5.63	5.82	5.84
46	3.61	4.14		5.1	5.3	5.51	5.15

48	3.66	4.04		4.91	5.34	5.51	5.65
50	3.71	4.22		5.16	5.31	5.53	5.6
52	3.77	3.93		5.09	5.33	5.45	5.57
57	3.74	3.9		5.27	5.39	5.67	5.62
59	3.8	3.85		5.31	4.86	5.4	5.63
61	3.93	3.97		4.72	4.98	5.28	5.52
62	3.73	3.75		5.19	5.18	5.37	5.54
64	3.68	3.74		5.52	5.38	5.72	5.55
66	3.77	3.86		5.46	5.14	5.53	5.81
68	3.73	3.75		5.08	4.84	4.99	5.38
70	3.64	3.66		5.12	4.72	4.91	5.52
72	3.75	3.65		5	4	5.05	5.38
74	3.73	3.6	4.12	5.19	4.51	5.09	5.44
141	6.11	5.81	3.95	5.68	5.9	5.96	6.06
149	5.94	4.99	3.91	5.84	5.98	6.06	6.26
163	6.33	6.4	4.85	6.02	6.17	6.28	6.25
167	6.25	6.18	4.85	6.14	6.24	6.32	6.27
172	5.85	5.85	4.85	5.92	5.87	5.89	6.01
174	5.96	5.93	4.85	5.67	6.01	5.05	6.18
181	6.2	6.06	4.9	5.92	6.07	6.14	6.22

Column Study - Sulfate Change.

Day	AMD influent (mg/L)	Sweet potato and inoculum 1 (mg/L)	Sweet potato and inoculum 2 (mg/L)	Auto-claved horse manure and inoculum 1 (mg/L)	Auto-claved horse manure and inoculum 2 (mg/L)	Horse manure and inoculum (mg/L)	Horse manure only (mg/L)
44	2000	1300		1400	1100	1200	1600
48	2200	1900		2300	2200	2200	2100
52	2100	2100		2200	2100	2300	2300
57	1800	1800		1800	1700	1700	1600
61	2200	2000		2100	1800	2000	2000
64	1500	1900		1900	1600	1900	1900
68	1400	1400	1600	1600	1300	1800	1400
70	1400	1900	1700	1700	2000	1900	1800
72	2000	1900	1600	1800	1700	1600	1600
141	1700	1900	1900	1900	1900	2000	1800
149	1800	2200	2400	2000	2100	2600	1900

163	1600	2000	1000	1800	1900	2300	2200
167	1700	1700	1000	2300	2400	1800	2100
172	1900	1900	1000	2200	2000	2100	1900
174	1800	2100	1200	2200	2400	2000	1900
181	2200	1800	900	2000	2000	2100	2400

Column Study – Tracer Test.

Time (hours)	AMD influent (mV)	Sweet potato and inoculum 1 (mV)	Sweet potato and inoculum 2 (mV)	Auto-claved horse manure and inoculum 1 (mV)	Auto-claved horse manure and inoculum 2 (mV)	Horse manure and inoculum (mV)	Horse manure only (mV)
0:00:00	2.8	1.597	1.649	1.566	1.563	1.56	1.55
5:28:00	2.9	1.49	1.602	1.518	1.515	1.525	1.53
17:57:00	3	1.546	1.643	1.489	1.493	1.593	1.549
19:24:00	2.9	1.504	1.65	1.6	1.65	1.7	1.788
27:24:00	3	1.6	1.68	1.8	1.7	1.8	1.8
40:51:00	3.2	2	1.9	2.6	2.69	2.91	2.65
43:51:00	2.63	3.2	3.15	3.16	3.06	3.25	3.07
47:46:00	2.5	2.9	3.01	3.04	3.05	3.09	3.06
64:15:00	2.43	3.07	3.01	3.21	3.23	3.27	3.22
70:41:00	2.47	3.52	3.32	3.41	3.49	3.42	3.49
73:56:00	2.16	2.91	3.03	3.04	3.09	3.08	3.09
80:13:00	2.45	3.12	3.04	3.07	3.1	2.97	3.12
89:51:00	2.07	2.52	2.53	2.58	2.65	2.58	2.7
133:21:00	1.89	2.1	2.06	2.13	2.11	2.09	2.07
183:21:00	1.136	1.199	1.18	1.209	1.201	1.184	1.204

BIBLIOGRAPHY

1. Neculita C-M, Zagury GJ, Bussière B. 2007. Passive Treatment of Acid Mine Drainage in Bioreactors using Sulfate-Reducing Bacteria. J Environ Qual 36(1): 1-16.

2. Association WWG. 1998. Cleaning-up Abandoned Mines: A Western Partnership.

3. Group RR. 2008. Acid Mine Drainage and Effects on Fish Health and Ecology: A Review. Anchorage:U.S. Fish and Wildlife Service.

4. Behum PT, Lefticariu L, Bender KS, Segid YT, Burns AS, Pugh CW. 2011. Remediation of coal-mine drainage by a sulfate-reducing bioreactor: A case study from the Illinois coal basin, USA. Applied Geochemistry 26, Supplement(0): S162-S166.

5. Health H. year. Human Health. Available: http://www.epa.gov/superfund/lead/health.htm [accessed October 27, 2011 2011].

6. Lovley DR, Phillips EJP. 1986. Organic Matter Mineralization with Reduction of Ferric Iron in Anaerobic Sediments. Applied and Environmental Microbiology 51(4): 683-689.

7. Metals H. year. Heavy Metals. Available: http://www.lenntech.com/processes/heavy/heavy-metals/heavy-metals.htm [accessed November 2, 2011 2011].

8. EPA. 1983. Design Manual Neutralization of Acid Mine Drainage. 247.

9. Gazea B, Adam K, Kontopoulos A. 1996. A review of passive systems for the treatment of acid mine drainage. Minerals Engineering 9(1): 23-42.

10. Kaksonen AH, Plumb JJ, Robertson WJ, Riekkola-Vanhanen M, Franzmann PD, Puhakka JA. 2006. The performance, kinetics and microbiology of sulfidogenic fluidized-bed treatment of acidic metal- and sulfate-containing wastewater. Hydrometallurgy 83(1-4): 204-213.

11. Ňancucheo I, Johnson DB. 2012. Selective removal of transition metals from acidic mine waters by novel consortia of acidophilic sulfidogenic bacteria. Microbial Biotechnology 5(1): 34-44.

12. ITRC. 2013. Biochemical Reactors for Mining-Influenced Water. Washington:The Interstate Technology & Regulatory Council.

13. Pereyra LP, Hiibel SR, Pruden A, Reardon KF. 2008. Comparison of microbial community composition and activity in sulfate-reducing batch systems remediating mine drainage. Biotechnology and Bioengineering 101(4): 702-713.

14. Díaz E. 2004. Bacterial degradation of aromatic pollutants: A paradigm of metabolic versatility. International Microbiology 7(3): 173-180.

15. Logan MV, Reardon KF, Figueroa LA, McLain JET, Ahmann DM. 2005. Microbial community activities during establishment, performance, and decline of bench-scale passive treatment systems for mine drainage. Water Research 39(18): 4537-4551.

16. Kolmert Å, Johnson DB. 2001. Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. Journal of Chemical Technology & Biotechnology 76(8): 836-843.

17. Koschorreck M, Wendt-Potthoff K, Geller W. 2003. Microbial sulfate reduction at low pH in sediments of an acidic lake in Argentina. Environmental Science and Technology 37(6): 1159-1162.

18. Alazard D, Joseph M, Battaglia-Brunet F, Cayol J-L, Ollivier B. 2010.Desulfosporosinus acidiphilus; sp. nov.: a moderately acidophilic sulfate-reducing bacterium isolated from acid mining drainage sediments. Extremophiles 14(3): 305-312.

19. Drury WJ. 1999. Treatment of acid mine drainage with anaerobic solid-substrate reactors. Water Environment Research 71(6): 1244.

20. Elliott P, Ragusa S, Catcheside D. 1998. Growth of sulfate-reducing bacteria under acidic conditions in an upflow anaerobic bioreactor as a treatment system for acid mine drainage. Water Research 32(12): 3724-3730.

21. Lyew D, Sheppard J. 1999. Sizing considerations for gravel beds treating acid mine drainage by sulfate reduction. Journal of Environmental Quality 28(3): 1025-1030.

22. Jong T, Parry DL. 2006. Microbial sulfate reduction under sequentially acidic conditions in an upflow anaerobic packed bed bioreactor. Water Research 40(13): 2561-2571.

23. Bernardez LA, de Andrade Lima LRP, Ramos CLS, Almeida PF. 2012. A Kinetic Analysis of Microbial Sulfate Reduction in an Upflow Packed-Bed Anaerobic Bioreactor. Mine Water Environ 31(1): 62-68.

24. Choudhary RP, Sheoran AS. 2012. Performance of single substrate in sulphate reducing bioreactor for the treatment of acid mine drainage. Minerals Engineering 39(0): 29-35.

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