USE OF BIOPOLYMER ENTRAPPED SULFATE REDUCING BACTERIA AND METAL NANOPARTICLES FOR EFFECTIVE AQUEOUS SULFATE REMOVAL

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Seydou Cisse

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Use of biopolymer entrapped sulfate reducing bacteria and metal nanoparticles for effective aqueous sulfate removal

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Seydou Cisse

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Achintya Bezbaruah
Chair

Dr. Dinesh Katti

Dr. Xuefeng Chu

Dr. Donna Jacob

Approved:

08/01/2013	Dr. Eakalak Khan
Date	Department Chair

ABSTRACT

Sulfate reducing bacteria (SRB) isolated from activated sludge were used to investigate sulfate removal from aqueous solution using calcium alginate entrapped SRB in batch studies with ethanol and lactose as the carbon sources. The interferences of pH, temperature, Al^{3+}, Cu^{2+}, and Zn^{2+} on sulfate removal were also investigated. Further, sulfate removal experiments were conducted with co-entrapped SRB and nanoscale zero-valent iron (NZVI) and separately entrapped SRB and NZVI.

Results indicate that EntSRB can effectively remove sulfate from aqueous solution. 88-95% sulfate removal was achieved. Both ethanol and lactose worked well as carbon sources for entrapped bacteria. Interference studies indicated low sulfate removal in the presence of 25-50 mg/L of aluminum and zinc. Low pH ($pH \leq 4$) and low temperature (5°C) decreased sulfate reduction. NZVI appeared to have negative effects on SRB. Loading of 0.05 and 0.1 g of NZVI led to lower SO_4^{2-} removal as compared to experiments without NZVI.

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LIST OF ABBREVIATIONS

AMD –Acid mine drainage

BOD –Biochemical oxygen demand

COD –Chemical oxygen demand

DI –Di-ionized, referring to di-ionized water

Fe^{0} –Zero-valent iron

Fe^{2+} –Ferrous iron

Fe^{3+} –Ferric iron

HSRB –hydrogen utilizing sulfate reducing bacteria

MLSS –Mixed liquor suspended solids

MPN –Most probable number

NZVI –Nanoscale zero-valent iron

PRB –Permeable reactive barriers

rpm –Revolutions per minute

SEM –Scanning electron microscopy

SRB –Sulfate reducing bacteria

SRBB –Sulfate reducing bio bed

SRPB –Sulfate reducing passive biofilters

UAPB –Upflow anaerobic packed bed

UAPBB –Upflow anaerobic packed bed bioreactor

USEPA–United States Environmental Protection Agency

WHO–World Health Organization

ZVI –Zero-valent iron

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1. INTRODUCTION

1.1. Mining and the associate environmental issues

Mining activities have made significant contributions to many nations' socio-economic development and industrial growth. They represent significant economical asset for nations and have contributed immensely to the global economic growth. Major mining products that have contributed to the economic growth of various nations worldwide include manganese (Rivera-Becerril et al., 2013), aluminum, diamond, gold, iron ore, and rare earth elements (Bermudez-Lugo, 2009; Soto-Viruet, 2010; Bermudez-Lugo, 2011), and coal (Zhengfu et al., 2010; Yenilmez et al., 2011). Mining products are used for various purposes directly related to the wellbeing of the world population for energy generation (coal, natural gas, petroleum), production of metals for use in the manufacturing of cars, trains, boats, and airplanes, and in construction materials (limestone, sand, stone, metals), precious stones (diamond) and metals (gold, silver, platinum) in jewelry, rare earths in electronics (lanthanum, scandium, europium, yttrium), and fertilizers for use in agriculture (phosphorus, potassium).

While mining is an important contributor to economic growth, it creates significant environmental problems, including land subsidence, land degradation (where the vegetation is cleared off), and the modification of the topography (Zhengfu et al., 2010; Yenilmez et al., 2011). Metals (trace elements) and other mine wastes such as acid mine drainage can lead to severe pollution of water and soil in the surrounding environment (Zhengfu et al., 2010; Yenilmez et al., 2011; Garcia-Lorenzo et al., 2012). In addition, air, water and soil can be contaminated with high concentrations of toxic elements that can deteriorate the health of surrounding vegetation, wildlife and the population (Wahsha et al., 2012).

The alteration of soil, water, and air quality due to mining activities has negative consequences in human activities such as low productivity in agriculture (loss of soil fertility), destruction of terrestrial and aquatic resources caused by accumulation of toxic elements in animal and plant tissues in those ecosystems, and disturbance of the food chain. All these lead to serious health concerns for human and animals (Romero et al., 2011; Martínez-Sánchez et al., 2012).

1.2. Mining in Guinea

The mining situation in Guinea is specially discussed in this dissertation as it is the intension of this researcher to make this research relevant to his home country Guinea in particular and other countries in general. Guinea is located in the West Africa, on the Atlantic Coast and is surrounded by Guinea-Bissau, Senegal, and Mali to the North, Cote D'Ivoire to the East, Liberia and Sierra Leone to the South, and the Atlantic Ocean to the West. The country is 245,857 Km^2 with 11.176 million people (2013 estimates, CIA) and is divided into four natural regions, viz., Lower Guinea (a narrow coastal belt), Middle Guinea (the pastoral Fouta Djallon highlands), Upper Guinea (the northern savannah area), and Forest Guinea (the southeastern rainforest region). The capital city Conakry is located on the coast. Guinea is a tropical country with a rainy season from June through November. The annual rain fall varies from 1,500 to 3,000 mm with a record high of 4,000 mm in the Coastal Lower Guinea (ONTG, 2013). Temperature varies from 10^oC in Fouta Djallon to 33^oC in Upper Guinea (ONTG, 2013) and average relative humidity is 70% (Climatemps, 2013).

Guinea is known for its significant and diverse natural resources. The mining sector is one of the most significant contributor to Guinean economy (Soto-Viruet, 2011). Mining contributes around 89% of the Government income (Mobbs, 1997) and up to 95% of Guinea's

exports (Bermudez-Lugo, 2013). Major mineral resources of Guinea include aluminum, gold, diamond, iron, and raw materials for cement.

Guinea has the world's largest bauxite deposit and is one of the world's largest bauxite (aluminum ore) producers. In 2010, it was ranked the world fifth largest producer of bauxite (Soto-Viruet, 2011; Bermudez-Lugo, 2013) and 90% of bauxite production in Africa is attributable to Guinea (Yager et al., 2012). Bauxite export accounted for approximately 90% of Guinea total exports. Szczesniak (2003) indicated that "32% of the United States' metallurgical-grade bauxite was supplied by Guinea during 2001 and the country's bauxite production accounted for 15% of world aluminum production. Estimates indicate that total existing reserves are 343.2 million tons of 42.78% pure Al_2O_3 (Bermudez-Lugo, 2009).

Guinea is also known for gold production. Gold in Guinea occurs as veins and alluvial deposits (Szczesniak, 2003) and was mined on artisanal, small, and industrial scales. Société Ashanti de Guinée (SAG) is the largest gold mining company and owns a mining lease in Siguiri. SAG produced 392,000 troy ounces (12,200 kg) of gold in 2008 (Szczesniak, 2003). Other gold mining companies are Société Minière de Dinguiraye (SMD), a Norwegian based company, which operates in Dinguiraye near Siguiri and Semafo Inc. of Canada that mines in Kiniéro near Kouroussa.

Diamond production was reported to be 3,098,490 carats (619.698 kg) in 2008 and the majority that came from small-scale operations (Bermudez-Lugo, 2011). In 2010, the country was ranked the 12th largest (by volume) rough diamond producer (Soto-Viuret, 2011). Guinea's main diamond deposits were located in Kérouane, Kissidougou, and Macenta along the Baoulé, the Diani, and the Milo Rivers (Bermudez-Lugo, 2005). Other diamond occurrences were identified in Forécariah and Kindia (Bermudez-Lugo, 2005). The country's diamond resources

were estimated to be between 25 and 30 million carats (5,000 to 6,000 kg). Diamond is mined from alluvial, eluvial, and kimberlite deposits (Bermudez-Lugo, 2005).

Iron ore production has also started in 2012 (Bellzone Mining, 2013) and is expected to be the largest contributor to Guinea economy in term of income for the Government as well as employment opportunity for people (Rio Tinto, 2013). Bellzone Mining has already started mining iron ore with its first iron ore shipment from its Forécariah mine in December 2012 (Bellzone Mining, 2013). In addition, the Simandou project which is being developed by Rio Tinto Iron Ore with its partners Chalco, the International Finance Corporation (IFC), and Guinean Government, is expected to be the "largest integrated iron ore mine and infrastructure project ever developed in Africa", with an annual iron ore production of 95 million tons with an iron content estimated between 66% and 68% (Rio Tinto, 2013). Figure 1.1 shows current situation of mining projects and operations in Guinea.

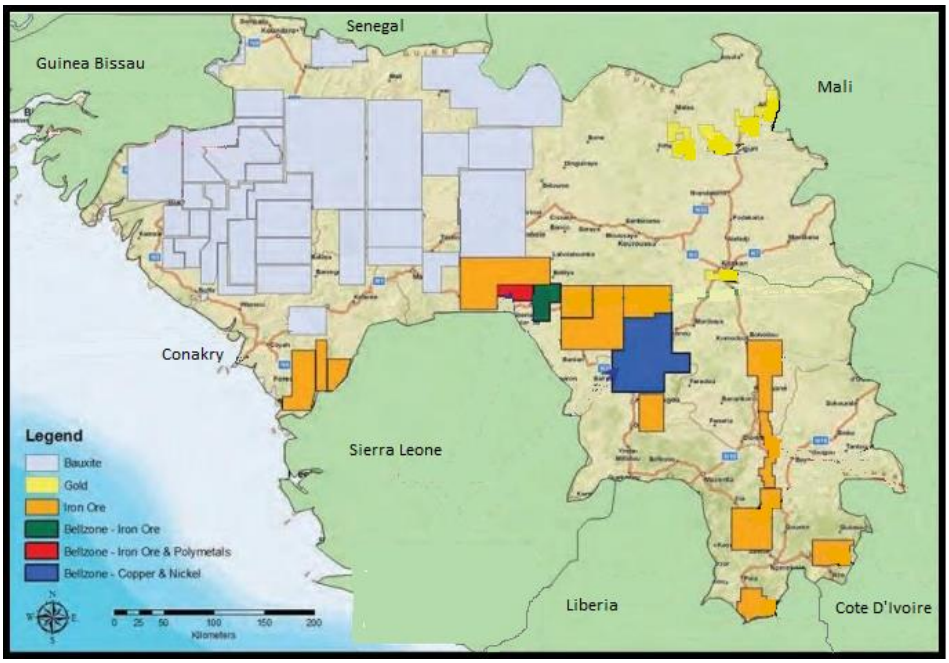

Figure 1.1. Map of mineral resources under exploration in Guinea (adapted from World Press, 2012).

Guinea's large potential for mineral resources is likely to play significant role in the country's economic development for the coming years. However, mining activities are known to create a number of negative environmental impacts that could adversely affect the country's natural landscape, impair the biodiversity including flora and fauna, water resources, domestic livestock, and human health (Zhengfu et al., 2010; Garcia-Lorenzo et al., 2012). Among those environmental impacts that can be associated with mining activities is acid mine drainage (AMD) which is one of the most common and major environmental issue in mining industry (White et al., 2011).

1.3. Acid mine drainage (AMD)

Acid mine drainage (AMD) is the result of oxidation of sulfide minerals (Johnson and Hallberg, 2005; Neculita et al., 2007; Costa et al., 2008; Schmidtova and Baldwin, 2011; Choudhary et al., 2011). The sulfide minerals of importance include pyrite (FeS_2), chalcopyrite ($CuFeS_2$), galena (PbS), and sphalerite (ZnS) (Baker et al., 2003; Johnson et al., 2005; Hogsden et al., 2012). These sulfide minerals are associated with coal, copper, silver, lead, and zinc mining operations as impurities (Baker and Banfield, 2003; Hogsden and Harding, 2012). Mining for metals, coal, and other natural resources exposes those sulfide minerals to oxygen and water, and, thus causes AMD generation. AMD can be extremely acidic in nature with low pH ($pH< 3$, Hogsden and Harding, 2012; Koschorreck and Wendt-Potthoff, 2012) and have high concentrations of sulfate and toxic metals like aluminum, iron, copper, zinc (Hogsden and Harding, 2012), manganese (Johnson and Hallberg, 2005) and other metalloids such as arsenic (Johnson and Hallberg, 2005). Hydrogen sulfide (H_2S), a byproduct of sulfate reduction, is commonly found in soluble form in AMD (Lamers et al., 2002; Martins et al., 2009) and H_2S is

the reason for the unpleasant odor (rotten smell) of AMD. In addition, heat is generated from sulfide oxidation (Baker and Banfield, 2003) and that effects the aquatic environment.

1.4. Existing technologies for AMD treatment

Different technologies are available for AMD remediation in order to achieve acceptable limits for contaminants (Table 1.1). One of the most commonly used technologies is lime treatment in which lime (limestone) is added to the AMD to neutralize its acidity and precipitate out metals. Beside lime treatment there are passive treatments such as adsorptive media and constructed wetlands. The other promising and used method is the biological treatment using sulfate reducing bacteria (SRB). However, these treatment options have some inherent drawbacks and it is felt that there is need for a technology which can remediate AMD in a more effective manner. Further, free cells of SRB are responsible for metal corrosion as they generate H_2S and dissolved H_2S is known to be major cause for corrosion (Wen et al., 2010).

Table 1.1. Available AMD treatment technologies.

Technology	Advantages	Limitations	Major Contaminant Removed	Source
Chemical precipitation	Reduces metals wastes from sludge, produces stable precipitates.	Requires O_2, produces high hydroxide sludge, can only remove divalent metals. Does not remove SO_4^{2-}. Limited effect.	Al 81.98% Mg 99.87% Mn 99.98% Fe 99.99% Sb 89.62% Sr 39.66%	Matlock et al. 2002
Electrolysis (CMDS)	Reduce the volume of AMD sludge, increases the pH, low operating costs.	Pretreatment required, Can only remove some metals. Does not remove SO_4^{2-}.	Cd 97.8% Cu 99.75% Fe 99.97% Pb 96.7% Zn 99.8%	Cui et al. 2012
Constructed wetlands	In-situ treatment, effective metals and sulfate removal achieved, minimal energy required.	Takes time, high initial cost for the construction of the wetland, suitable plants for targeted contaminants, remobilization of contaminants.	Fe ~ 92%	White et al. 2011
Permeable reactive barriers (PRB)	In-situ treatment, low costs, no energy is required.	Limited to groundwater, takes long time.	SO_4^{2-} 43% Al 80% Cu 76% Zn 47%	Gibert et al. 2011
Biological Treatment	High rate of metal and sulfate removal, cost effective, minimal energy consumption, possible metal recovery.	Takes long time, anaerobic conditions required, pH varies between 3-6. The involved bacteria (e.g., sulfate reducing bacteria) are responsible for corrosion.	Fe 98.5% S^{2-} 98.6% SO_4^{2-} 99.2%	Jong & Parry 2006

2. LITERATURE SURVEY

2.1. Acid mine drainage (AMD)

AMD results from oxidation of sulfide minerals (e.g., iron sulfide or pyrite) as a result of their exposure to oxygen and water, leading to the generation of acidic waters characterized by low pH, high concentrations of sulfate, sulfides, and dissolved metals (Johnson and Hallberg, 2005; White et al., 2011). AMD is the most problematic issue in mining industry worldwide affecting freshwater systems. AMD can be generated in both active and abandoned mining sites (Hogsden and Harding, 2012), and is very acidic in nature, contains high concentrations of sulfate, and a number of metals (Johnson and Hallberg, 2005; White et al., 2011).

As stated earlier, AMD is the result of oxidation of sulfide minerals (Johnson and Hallberg, 2005; Neculita et al., 2007; Costa et al., 2008; Choudhary et al. 2011; Schmidtova and Baldwin, 2011). The sulfide minerals of importance include pyrite (FeS_2), chalcopyrite ($CuFeS_2$), galena (PbS), and sphalerite (ZnS) (Baker et al., 2003; Johnson and Hallberg, 2005; Hogsden and Harding, 2012). These sulfide minerals are associated with coal, copper, silver, lead, and zinc mining operations as impurities (Baker and Banfield 2003; Hogsden and Harding 2012). Mining for metals, coal, and other natural resources exposes those sulfide minerals to oxygen and water, and, thus, causes AMD generation. AMD can be extremely acidic in nature with low pH (pH< 4, Hogsden and Harding, 2012; Koschorreck and Wendt-Potthoff, 2012) and have high concentrations of sulfate and metals like aluminum, iron, copper, zinc (Hogsden and Harding, 2012), manganese (Johnson and Hallberg, 2005) and metalloids such as arsenic (Johnson and Hallberg, 2005). Hydrogen sulfide (H_2S), a byproduct of sulfate reduction, is commonly found in soluble form in AMD (Lamers et al. 2002; Martins et al. 2009) and H_2S is the reason for the unpleasant odor (rotten smell) of AMD. In addition, heat is generated from

sulfide oxidation (Baker and Banfield, 2003) and that effects the aquatic environment. Sulfide mineral dissolution and AMD generation rates are governed by a number of factors, including fluid chemistry and flow, bacterial activities, and pH of the fluid (Baker and Banfield, 2003; Neculita et al., 2007). Fluid chemistry and flow control the rate of supply of oxidant and, thus, the rate of pyrite dissolution (Baker et al., 2003). Oxygen (O_2) and ferric iron (Fe^{3+}) are the typical oxidants. The rate of pyrite oxidation by ferric iron is much higher than by oxygen (Neculita et al., 2007). Pyrite oxidation by Fe^{3+} generates 16 moles of H^+ while only 2 moles are generated when pyrite is oxidized by O_2 (Eq. 2.1 & 2.2, Neculita et al., 2007).

$$FeS_{2(S)} + 7/2O_2 + H_2O \rightarrow 2SO_4^{2-} + Fe^{2+} + 2H^+ \quad (2.1)$$

$$FeS_{2(S)} + 14Fe^{3+} + 8H_2O \rightarrow 2SO_4^{2-} + 15Fe^{2+} + 16H^+ \quad (2.2)$$

Pyrite oxidation process involves several reactions. The process starts at neutral pH when pyrite is oxidized in the presence of O_2 to form Fe^{2+} (Eq. 2.1, Neculita et al., 2007). That reaction is followed by the oxidation of Fe^{2+} to Fe^{3+} at low pH (< 4, Eq. 2.3, Neculita et al., 2007).

$$Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O \quad (2.3)$$

The final step of the oxidation reactions is the reduction of ferric iron by pyrite (Eq. 2.2, Baker and Banfield, 2003; Neculita et al., 2007) leading to sulfate generation and acidity. Microorganisms such as acidophilic bacteria (*Acidithiobacillus ferroxidans*, Neculita et al., 2007) and iron-oxidizing bacteria (*Gallionella ferruginea*, Johnson and Hallberg, 2005) can impact the rate of sulfur oxidation by oxidizing pyrites into sulfate. Pyrite oxidation by those

microorganisms is influenced by the solution pH. Baker and Banfield (2003) reported that pyrite oxidation mediated by microorganisms is increased at low pH by five orders of magnitude.

2.2. Metal and other pollutants

Acid mine drainage (AMD) not only contains high levels of sulfate, but also metals and other dissolved pollutants. Among metals and metalloids present in AMD are Fe, Ni, Pb, Zn (Matlock et al., 2002; Gibert et al., 2011; Davies et al., 2011; Cui et al., 2012; Genty et al., 2012), Al, Cu, Mn (Matlock et al., 2002; Davies et al., 2011; Gibert et al., 2011; Cui et al., 2012), As, Sb, Sr (Matlock et al., 2002), and Cd (Cui et al., 2012). These metals can be found at relatively high concentrations in AMD polluted water (Table 2.1). In addition, AMD also contains high concentrations of hydrogen sulfide (H_2S), which causes rotten egg odor, corrosion of metals, and toxicity to plants and invertebrates (Kuo and Shu, 2004).

Table 2.1. Examples of AMD water quality (All concentrations are in mg/L).

Characteristics	Source
pH 2.65 SO_4^{2-} 2,550 Ca 125; Fe 98; Mg 1.35; K 2.8; Al 0.082; Cu 25.5; Zn 44; Cd 0.196; Pb 0.091	Cui et al. 2012
pH 3.0 SO_4^{2-} 61,326 As 0.0209; Cd 4.9; Cu 11.7; Fe 19.4; Mn 5,040; Pb 8.6; Zn 11,000	Garcia-Lorenzo et al. 2012
pH 4.0 SO_4^{2-} 8,640 Fe 2,972; Mg 30.9; Mn 33.4; Ni 0.1; Pb 0.3; Zn 1.1	Genty et al. 2012
pH 2.7 SO_4^{2-} 1,305 Ca193.6; Mg 25.3; Na 6; Fe 142.8; Mn 2.87; NO_3^--N 0.013	Koschorreck and Wendt-Potthoff 2012
pH 2.9 SO_4^{2-} 659 Al 66; Cu 0.09; Fe 59.5; Zn 1.84; Mg 12.3	Davies et al. 2011
pH 4.0 SO_4^{2-} 1,000 Zn 15; Al 15; Cu 1	Gibert et al. 2011
pH 2.0 SO_4^{2-} 3,100 Fe 500; Cu 50; Zn 110	Martins et al. 2009
pH 2.4 SO_4^{2-} 3,100 Fe 497; Cu 49; Zn 107	Costa & al. 2008
pH 2.3 SO_4^{2-} 11,300 Al 126; As 10.4; Cd 5.0; Cu 61; Fe 154; Hg 3.1; Mn 18; Ni 4.6; Zn 60	Wilkin & McNeil 2003
pH not reported Al 0.483; Sb 1.31; As 0.017; Cu 0.012; Fe 194; Mg 57.4; Mn 4.65; Se 0.022; Sr 3.53	Matlock et al. 2002

2.3. Impacts of AMD on the environment

AMD can seriously affect water quality and surrounding ecosystems. Strong acidity of AMD runoff can lead to poor fertility of soil due to washing out nutrients. Low pH increases solubility of many metals and make them available to the plants, land animals (including

humans), and aquatic organisms and higher life forms (e.g., fish) (USEPA, 1994; Liljeqvist et al., 2010; Singh et al., 2011). AMD can also cause other economic loss by restricting recreational activities on impacted streams and rivers (USEPA, 1994). Rotten egg odor associated with AMD is due to the presence of H_2S (Christia-Lotter et al., 2007; Claudet et al., 2012) and that make the waters unacceptable for recreational and other uses.

Sulfate (SO_4^{2-}) is found in relatively high concentrations in AMD. Varying amounts of sulfate has been reported by researchers. A few representative concentrations of sulfate in AMD include 659 mg/L associated with coal mining (Davies et al., 2011), 3,100 mg/L associated with copper mining (Martins et al., 2009), 8,640 mg/L associated with iron mining (Genty et al., 2012), and 760,000 mg/L associated with copper, gold, silver, zinc, and pyrite mining (Nordstrom et al., 2000). Toxicity of sulfate depends upon its chemical form (Wan et al., 2011; Dar et al., 2006). Sulfate may not be of a serious environmental concern if the sulfate compound is chemically inert and non-toxic, and if the prevailing environmental conditions help in the transformation of the sulfate to a benign form (Dar et al., 2006).

Sulfate reducing bacteria (SRB) have the ability to use sulfate as final electron acceptor in anaerobic degradation of organic substrates leading to the production of dissolved sulfides (HS^- and H_2S, Wen et al., 2010; Lamers et al., 2002). H_2S causes serious environmental problems to most aquatic plant and animal species and can also cause economic loss (Lamers et al., 2002; Kuo and Shu, 2004; Dar et al., 2007; Wen et al., 2010; Wan et al., 2011). H_2S is a highly reactive gas which is toxic to aquatic plants (Lamers et al., 2002), SRB themselves (Nagpal et al., 2000; Wen et al., 2010), and humans (Christia-Lotter et al., 2007; Claudet et al., 2012). In addition, H_2S is also responsible for metal corrosion (Wen et al., 2010), and corrosion

of bridges (USEPA, 1994). Cast iron, copper, aluminum, steel and stainless steel are also affected by H_2S corrosion (Wen et al., 2010).

H_2S is one of the most toxic gases, causing intoxication and death to humans (Christia-Lotter et al., 2007). Due to its liposolubility, H_2S has neurotoxic effect on cerebral system, and affects liver, kidneys, and small intestine (Claudet et al., 2012). Its rotten egg odor is perceivable at a very low concentrations in air (0.1 ppm, Christia-Lotter et al., 2007) and olfactory saturation occurs at in the range of 100 ppm (Christia-Lotter et al., 2007) to 150 ppm (Claudet et al., 2012). Once oxidized by the liver, H_2S induces a breakdown of the electron transport chain and inhibit oxidative metabolism in human body (Christia-Lotter et al., 2007). H_2S accumulates in water bodies resulting in inhibitory effect on SRB thereby impairing sulfate remediation (Nagpal et al., 2000; Wen et al., 2010). A concentration of 250 mg/L of H_2S can retard SRB growth while at 547 mg/L H_2S can completely inhibit the growth of SRB (reported for Desulfovibrio by Nagpal et al., 2000).

Sulfate is also associated with methylmercury (MeHg) production in anaerobic sediments (Kongchum et al., 2006; Gilmour et al., 2011). MeHg is a major environment contaminant and an acute neurotoxin which accumulates in the food chain in aquatic environment (Kongchum et al., 2006; Franco et al., 2009; Olsvik et al., 2011). MeHg causes severe damage to the central nervous system where it alters the ultrastructure and biochemistry of neurons and astrocytes (Franco et al., 2009).

Lamers et al. (2002) indicated that sulfate reduction occurring in freshwater and marine sediments modifies nutrients kinetics as it interferes with phosphate binding to iron, and releases phosphate into water and causing "internal eutrophication". In a wetland mesocosm sulfate acts as electron acceptor during organic substrate decomposition to produce sulfides and bicarbonate

(HCO_3^-), HCO_3^- neutralizes acidity in the sediments, neutral pH increases organic matter decomposition resulting in the release of phosphorus, and sulfides bind to iron and reduce the availability of iron to sequester phosphates and, thus, release phosphorous from the sediment (Geurts et al., 2009; Dierberg et al., 2011). Eutrophication leads to high biomass production (Geurts et al., 2009) and deterioration of the health of the affected water body.

2.4. Treatment of acid mine drainage

AMD treatment options are mainly based on chemical methods (pH neutralization and, precipitation of heavy metals, ionic exchange), physical separation (reverse osmosis), adsorption (biofilters), and biological remediation using sulfate-reducing bacteria.

The most common treatments method of AMD is chemical treatment where limestone (or its derivatives) is added AMD to neutralize the acid. Addition of limestone raises the pH and induces the precipitation of metals as hydroxides and carbonates (Johnson and Hallberg, 2005; Neculita et al., 2007; Costa et al., 2007; Choudhary et al., 2011). These methods are expensive as they require physical infrastructure (e.g., treatment plants) and involve high operation and maintenance (e,g., cost of chemicals) (Choudhary et al., 2011). Chemical treatment also produces large volumes of sludge, which contains high concentrations of metals (Costa et al., 2007). It involves additional efforts and costs to treat the sludge generated during chemical treatment.

To overcome the problem of toxic metal sludge Matlock and his coworkers (2002) used a ligand (1,3-benzenediamidoethanethiol, BDET) to selectively remove up to 90% of toxic metals (Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Se, and Sr) from AMD in a short amount of time (only 20 h). However, it was not effective in removing sulfate and neutralizing the pH of the contaminated water. The technique can only be used in association with other treatment methods (e.g., lime addition) for effective AMD remediation.

Another chemical treatment method for AMD remediation is to add solid-phase phosphate to pyrite mine waste. Phosphates react with Fe^{3+} and form ferric phosphate and precipitate out of solution. Since Fe^{3+} is necessary for the oxidation of pyrites, the complexation of Fe^{3+} with phosphate inhibits the whole process and, thus, prevents AMD generation. This approach can only be a temporary solution because the soluble phosphates can react with pyrites in the presence of hydrogen peroxide (H_2O_2) (Johnson and Hallberg, 2005).

Reverse osmosis technology is another treatment process that has been successfully used in South Africa for AMD treatment (Mulopo et al., 2011). However, it requires pre-treatment and regular maintenance to prevent membrane fouling. This treatment technology is not cost effective.

Physical treatment of AMD is very popular. This treatment uses, among others, sulfate reducing passive biofilters (SRPB). Such biofilters consist of a mixture of wood ashes, sand, and gravel. This technique enables metal removal by precipitation, co-precipitation, and sorption onto the surface of fly ash (Genty et al., 2012). In their study, Genty and his team (Genty et al., 2012) were able to neutralize acidity (pH raised from 3 and 6 up to 10 and 8, respectively) for a limited period of time (80 days). However, pH dropped rapidly and remained below 5 beyond that period until the end of the experiment (150 days). Iron and manganese removal efficiency reached ~100 and 70%, respectively after 108 days while sulfate removal was 51%. The drawback of this treatment system is the need for periodic renewal of the treatment matrices (wood ashes) and maintenance of the solution pH above 7. Due to its low sulfate removal, this system itself is not sufficient to meet regulatory limits and requires a combination of additional technologies. In addition, this technology takes a long time for the desired treatment.

Wetlands can be considered as "the kidneys of the landscape" because of their ability to filter water and to remove contaminants (Mitsch and Gosselink, 2007). For that reason, constructed wetlands are designed for wastewater remediation including AMD treatment. AMD remediation in constructed wetlands is done in two phases. First, metals are uptaken by plants or precipitated in soil sediments as sulfide minerals (White et al., 2011). In the second phase, sulfate reduction by SRB (followed by an increase in pH) occurs under anaerobic conditions where the redox potential is between -100 and -500 mV (Mitsch and Gosselink, 2007). AMD treatment by wetlands was first reported between in 1970s and 1980s in the US when AMD water was treated while passing through a natural wetland of sphagnum bogs (Choudhary and Sheoran, 2011; White et al., 2011). Various plant species have been successfully used in AMD remediation in wetlands including bulrush or cattails (*Typha latifolia*), reed (*Phragmites australis*), and cottongrass (*Eriophorum angustifolium*) (White et al., 2011). Wetlands require a large land area (Amos and Younger, 2003), involve high initial cost for construction of the wetland (White et al., 2011). Remobilization of contaminants is another major issue encountered in constructed wetlands (White et al., 2011).

$$Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2.4)$$

$$4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_3 + 12H^+ \quad (2.5)$$

Permeable reactive barriers (PRBs) are also used for AMD remediation. PRBs use SRB, organic substrate and limestone to treat groundwater impacted by AMD. This technology is suggested as an alternative to pump-and-treat method for groundwater remediation (Gibert et al., 2011). The technique consists of installing an engineered permeable barrier of reactive materials selected for the removal of targeted contaminants. The contaminated groundwater is treated as it

passes through the materials. This method was used by Gibert et al. (2011) for AMD remediation in Aznacollar, Spain. They used limestone, compost (from two different organic substrates), and zero-valent iron (ZVI) as reactive barrier. Metals and sulfate were removed up 80, 76, 47, and 43% for Al, Cu, Zn and sulfate, respectively. Another place where this method was used for AMD treatment is Shilbottle, Northeast England (Johnson and Hallberg, 2005). This technique is limited to groundwater remediation (Gibert et al., 2011). Other issues associated with PRBs include the selection of adequate reactive materials for targeted contaminants removal (Amos and Younger, 2003) as well as maintaining the permeability of the system (Gibert et al., 2011).

2.5. Sulfate reducing bacteria

Sulfate reducing bacteria (SRB) are anaerobic microorganisms that are known to thrive in acidic media. They are found in various ecosystems such as soil and sediment and also in marine environment (Bussmann and Reichardt, 1991; Sim et al., 2012) as well as fresh and wastewaters. Wastewaters where SRB are found include municipal, industrial, and mining wastewaters (Martins et al., 2009). These bacteria use organic carbon as food and energy sources and convert sulfate to sulfide, sulfate being the final electron acceptor. This process is completed in two phases. In the first phase, H_2S is produced by SRB (Eqs. 2.6 and 2.7, Costa et al., 2008). In the second phase, metals are precipitated as insoluble metal sulfides (Foucher et al., 2002). Hydroxides and carbonates precipitates are also observed in the second phase of the process (Jong and Parry, 2006).

$$2H^+ + SO_4^{2-} + 2C_{(org)} \rightarrow 2H_2S + 2CO_2 \quad (2.6)$$

$$M^{2+} + 2H_2S \rightarrow MS + 2H^+ \quad (2.7)$$

Organic substrates are the electron donor in the process. Their composition is the main factor that control SRB activities. These organic substrates should be easy to biodegrade and must be available to maintain bacterial activities (Eq. 2.8, Choudhary et al., 2012). The biological treatment option is advantageous because of the fact that the microorganisms are easy to grow in AMD environment (they typically thrive in that medium), and no major operation and maintenance issues involved (Choudhary and Sheoran, 2011; Choudhary and Sheoran, 2012).

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (2.8)$$

CH_2O in Eq. 2.8 represents the organic carbon sources that are oxidized by SRB to form bicarbonates and reduce sulfates to H_2S. The produced bicarbonates reduce the acidity in the system and facilitate mineral carbonate precipitation. H_2S produced in the process reacts with metals to form insoluble metal sulfides.

2.5.1. Parameters influencing SRB sulfate removal capability

Organic substrates are the electron donors in the process of sulfate reduction. They are used to support SRB activity. Acetic acid, formic acid, lactic acid, malic acid, pyruvic acid, and propionic acid (Cao et al., 2012); manure (goat, buffalo, and cow), woodchips and sawdust (Choudhary and Sheoran, 2012); rice straw (Lu et al., 2011); wine waste (Martins et al., 2011); grass cuttings (Mulopo et al., 2011); leaves from oak, walnut, red maples, cherry, pear and horse chestnuts trees (Viggi et al., 2010); lactate (Bussmann and Reichardt, 1991; El Bayoumy et al., 1999; Jong and Parry, 2006; Costa et al., 2008), acetate, succinate (Bussmann and Reichardt, 1991), ethanol (Nagpal et al., 2000; Costa et al., 2008), lactose (Costa et al., 2008) are some examples used as carbon sources in AMD remediation by SRB. Bussmann and Reichardt (1991)

indicated that besides SRB population density, sulfate reduction rate is dependent on the availability of both electron donors and acceptors at sufficient concentrations. Mulopo et al. (2006) established a relationship between sulfate removal by SRB and the concentration of chemical oxygen demand (COD, an indicator of organic matter present). They achieved a faster sulfate reduction (from 2,150 to 850 mg/L over 2 days) in the presence of high concentration of COD (10,590 mg/L).

AMD is often inorganic in nature and does not contain enough organic substrates for use by microorganisms (Lu et al., 2011). Organic substances work as the electron donors and are necessary to support SRB activity to enable them to reduce sulfate via anaerobic respiration (Eq. 3.1, Choudhary et al., 2012). Neculita et al. (2007) have identified carbon availability as limiting factor for bacterial activity. Cao et al. (2012) in their study showed how different carbon sources influenced SRB's ability to remove sulfate. Therefore, having an efficient and cost effective carbon source is the key for any biological sulfate remediation system.

SRB are versatile microorganisms capable of using varied carbon sources, including simple organic carbon, fatty acids and complex hydrocarbons (Chakraborty et al., 2011). However, SRB prefer biodegradable organic matter with low molecular weight and simple chemical structure (Neculita et al., 2007). Table 2.2 shows example of some organic matters reported in sulfate remediation studies. Ethanol (C_2H_6O) is one the most frequently used organic substrates to support biological sulfate reduction by SRB (Nagpal et al., 2000; Hammack et al., 2006; Neculita et al., 2007; Costa et al., 2008; Martins et al., 2009; Choudhary and Sheoran, 2011; Cao et al., 2012; Choudhary and Sheoran, 2012). This is a simple alcohol containing two carbon atoms linked by a single bond Ethanol is also one of the most cost effective carbon

sources for SRB and can be made by the fermentation of plant cellulose (Costa et al., 2008).

Ethanol oxidation yields carbon dioxide, bicarbonate, and water (Eq. 2.9 Hammack et al., 2006).

$$4C_2H_5OH + 6SO_4^{2-} + 5H^+ \rightarrow 3H_2S + 3HS^- + 4CO_2 + 4HCO_3^- + 8H_2O \quad (2.9)$$

Table 2.2. Different organic substrates used as carbon source to support SRB activities.

Organic substrate	Experiment type and duration	Maximum sulfate removed	Source
Formic acid	Batch study, 6 day	97%	Cao et al. (2012)
Lactic acid		89%	
Malic acid		88%	
Pyruvic acid		71%	
Acetic acid		65%	
Propionic acid		60%	
Manure (goat, buffalo, and cow)	SRBB, 10 days	54%	Choudhary & Sheoran (2012)
Cellulosic wastes (woodchips and sawdust)		25%	
Rice Straw	Batch study, 60 days		Lu et al. (2011)
Wine waste	UAPB, 190 days	90%	Martins et al. (2011)
Grass cuttings and volatile fatty acids	Batch study, 49 days	100%	Mulopo et al. (2011)
Glucose	Batch study, 3 days	70.3%	Singh et al. (2011)
Sucrose		12.45%	
Fructose		31.2%	
Lactose		82.5%	
Tree leaves (oak, walnut, red maples, cherry, pear and horse chestnuts)	Batch test, 22 days Column test, 22 days	83% 50%	Viggi et al. (2010)
Lactate	UAPBB, 75 days	82.5%	Jong & Parry (2006)

SRBB: Sulfate reducing bio bed; UAPB: Upflow anaerobic packed bed; UAPBB: Upflow anaerobic packed bed bioreactor.

Although most research has indicated simple organic carbon is the most suitable for SRB, some studies reported the use of lactose ($C_{12}H_{22}O_{11}$) as an electron donor for bacterial sulfate removal (Costa et al., 2008; Martins et al., 2009). Lactose is a relatively complex carbon derived from galactose and glucose. The positive behavior of SRB in the presence of lactose is attributed to its ability to undergo a fermentation process that creates anaerobic condition favorable for SRB activities (Costa et al., 2008).

SRB are anaerobic bacteria, and, therefore, exposure to oxygen can cause stress to these bacteria. However, some studies have shown their ability to tolerate oxygen at concentrations as high as 1.5 mM (Wan et al., 2011). The presence of SRB in areas like surface sea waters, cyanobacterial mats, and activated sludge indicate SRB's tolerance toward oxygen (Brioukhanov et al., 2010; Wan et al., 2011). SRB are found coexisting with aerobic microorganisms such as cyanobacteria (Brioukhanov et al., 2010). The adaptability of SRB to oxygen is explained by the application of various defense strategies. Lobo et al. (2007) showed that *Desulfovibrio desulfuricans* NCIB 8301 was able to grow at 0.4% oxygen exposure, while weak growth was observed at 0.5% and 2% for two other strains of *D. desulfuricans* and *Desulfobacterium autotrophicum* DSM. When exposed to oxygen, SRB use self-protection enzymes to enable them to survive during the exposure period. This mechanism varies from one group of SRB to the other. Some SRB are capable of generating the relevant enzymes necessary for nullifying the negative effects of the oxygen species (Wan et al., 2011). Other SRB in oxic environments possess terminal membrane oxygen reductases for oxygen consumption (Lobo et al., 2007; Wan et al., 2011).

2.6. Entrapment

Entrapment or immobilization of bacterial cells is a preferred way to contain the microbial population within the desired treatment zone. Entrapment also offers a way to reuse the microbial population and makes it easy to handle them (Pramanik et al., 2009; Karabika et al., 2009; Pramanik et al., 2011; Quan et al., 2011; Wadhawan et al., 2011; Siripattanakul et al., 2010; Ahmad and Kunhi, 2011; Quan et al., 2011). Researchers have indicated that entrapment also protect the cells from stressors (Pramanik et al., 2009; Wadhawan et al., 2010; Siripattanakul et al., 2010; Ahmad and Kunhi, 2011). Moreover, immobilization of bacteria ensures high efficiency in removing contaminants and guarantee a good operational stability (Ahamad and Kunhi, 2011; Quan et al., 2011; Wadhawan et al., 2011).

Natural biopolymers (agar, agarose, sodium alginate, kappa-carrageenan) are widely used for bacterial cells entrapment (Bezbaruah et al., 2009a; Pramanik et al., 2009; Wadhawan et al., 2010; Siripattanakul et al., 2010; Ahmad and Kunhi, 2011; Wadhawan et al., 2011). They are reported to be nontoxic to microorganisms and easily biodegrade.

Among the most reported biopolymer used in entrapment are agar gel (Ahmad and Kunhi, 2011), alginate (Hill and Khan, 2008; Bezbaruah et al., 2009a; Pramanik et al., 2009; Siripattanakul et al., 2010; Ahmad and Kunhi, 2011; Quan et al., 2011), carrageenan (Pramanik et al., 2009; Wadhawan et al., 2010; Quan et al., 2011; Wadhawan et al., 2011), cellulose triacetate (CTA, Kuo and Shu, 2004), and polyvinyl alcohol (PVA, Ahmad and Kunhi, 2011; Wadhawan et al., 2011).

To date only a few studies have investigated the use of entrapped SRB cells for sulfate removal. One of them, conducted by Kuo and Shu (2004), investigated sulfate removal in packed filter by SRB entrapped in cellulose acetate and achieved 93% sulfate removal.

2.7. Alginate as an entrapment matrix

Sodium alginate has been used by many for cell entrapment for environmental applications (Ahmad and Kunhi, 2011; Quan et al., 2011; Hill and Khan, 2008; Siripattanakul et al., 2010; Pramanik et al., 2009; Bezbaruah et al., 2009a). This anionic polysaccharide is mainly extracted from brown algae (brown seaweed) cells where it is a structural component of the cell wall (Chee et al., 2011; Feneradosoa et al., 2010; Gomez et al., 2009; and Murillo-Alvarez and Hernandez-Carmona, 2007). Some telluric bacteria (a few species of the genus Pseudomonas and Azobacter) are also known to excrete acetylated alginates (Feneradosoa et al., 2010). Alginates extracted from seaweeds comprise two monomers, (1,4) linked β-D-mannuronic acid (M) with C1 ring conformation and α-L-guluronic acid (G) with C4 ring conformation (Feneradosoa et al., 2010; Gomez et al., 2009; Murillo-Alvarez and Hernandez-Carmona, 2007)

The most common brown seaweeds used in the production of commercial alginates are *Ascophyllum nodosum*, *Durvillea antartica*, *Ecklonia maxima*, *Laminaria hyperborea*, *Lessonia nigrescens*, and *Macrocystis pyrifera* (Gomez et al., 2009; Dhargalkar and Verlecar, 2009; Feneradosoa et al., 2010; Chee et al., 2011), *Eisenia arborea* and *Sargassum sinicola* (Murillo-Alvarez and Hernandez-Carmona, 2007). These species can produce up to 40% (dry weight) of alginate. Annual food and non-food application of alginates account for about 40,000 tons (Feneradosoa et al., 2010). USA, Japan, China, France, Norway, and Argentina are the most important alginates producers with estimated annual market value of $213 million (Dhargalkar and Verlecar, 2009).

Sodium alginate is a polymer that is soluble in water and produces a highly viscous solution. In the presence of polyvalent cations (e.g., Ca^{2+}), sodium alginate forms a gel (Ca-alginate, Gomez et al. 2009). Ca-alginate has multiple applications in food industries for its

ability to form stabilized suspensions as well as to form gel (thicken solution) (Feneradosoa et al., 2010; Gomez et al., 2009). In addition to food industry, alginates have also been used to support plant growth, heavy metal absorption, pharmacological experiments, and encapsulation (Bezbaruah et al., 2011; Feneradosoa et al., 2010).

2.8. Nanoscale zero-valent iron (NZVI)

Nanoscale zero-valent iron (NZVI) is an established technology for environmental remediation (Almeelbi and Bezbaruah, 2012; Krajangpan et al., 2012; Bezbaruah et al., 2011; Bezbaruah et al., 2009). It has been successfully used to remove contaminants from waters and wastewaters, including chlorinated chemicals, pesticides, heavy metals, and explosives (Bezbaruah et al., 2011) and inorganics (Almeebi and Bezbaruah, 2012; Bezbaruah et al., 2009b).

2.9. Objectives

In the present study the performance of entrapped sulfate reducing bacteria (SRB) in removing aqueous sulfate was investigated. The objectives of this research were to:

1. Investigate sulfate removal by calcium alginate entrapped SRB;
2. Study the interferences of some heavy metals (Al, Cu, and Zn) on the performance of entrapped SRB; and
3. Evaluate the potential for nanoscale zero-valent iron (NZVI) to enhance SRB's performance in removing sulfate.

2.10. Hypotheses

In line with the research objectives, the following hypotheses were formulated:

1. Biopolymer (Ca-alginate) entrapped sulfate reducing bacteria (SRB) will remove sulfate more effectively than free cells;
2. A simple carbon source will boost sulfate removal by SRB; and
3. Nanoscale zero-valent iron will enhance sulfate reduction by SRB as NZVI will act as an electron donor for the reductive reaction.

2.11. Research tasks

Tasks involved in this research consisted of multistep experiments to investigate sulfate removal by a mixed culture of SRB. The effects of other environmental parameters (pH, temperature, interference of Al, Cu, and Zn) on sulfate reduction were also investigated. In addition, the potential for NZVI to enhance sulfate removal by entrapped SRB was investigated. To achieve those tasks, it was necessary to:

1. Isolate and grow a mixed culture of SRB from activated sludge in laboratory conditions;
2. Conduct sulfate removal in a pilot scale column study with free cells of SRB;
3. Select an entrapment matrix for SRB and entrap SRB for sulfate removal;
4. Conduct sulfate removal batch experiments using entrapped SRB;
5. Investigate effects of pH on sulfate removal by entrapped SRB;
6. Investigate effects of temperature on sulfate removal by entrapped SRB;
7. Investigate the interference of Al, Cu, and Zn on sulfate removal by entrapped SRB; and

8. Investigate the potential for NZVI in enhancing sulfate removal by entrapped SRB.

2.12. Organization of this thesis

This thesis is divided into six chapters. Chapter 1 is the introduction which includes background of this research. Chapter 2 covers the literature survey on acid mine drainage (AMD) and available treatment options with special focus on biological treatment using sulfate reducing bacteria (SRB), entrapment matrices, this research objectives, hypotheses, a list of research tasks, and thesis organization. Chapter 3 discusses experiments done with free cells SRB in pilot scale column studies and batch studies done with Ca-alginate entrapped SRB. Interference of pH, temperature, and metals (Al, Cu, and Zn) are also discussed here. Chapter 4 discusses the use of co- and separately entrapped SRB and nanoscale zero-valent iron (NZVI) for sulfate removal. Chapter 5 presents the overall conclusions and Chapter 6 is in the form of recommendations for future work. This thesis also includes additional information as appendices.

3. BIOLOGICAL SULFATE REMOVAL USING SULFATE REDUCING BACTERIA

3.1. Abstract

Calcium alginate (Ca-alginate) was successfully used to entrap sulfate reducing bacteria (SRB) and tests were conducted to evaluate the efficacy of entrapped SRB in removing aqueous sulfate (SO_4^{2-}). Entrapment with Ca-alginate did not impair SRB's ability to remove sulfate. About 95% sulfate was removed by entrapped SRB in batch experiments and 88% was achieved by free SRB cells in pilot scale column experiments. The role of different carbon sources was evaluated and it was found that both ethanol and lactose performed well as carbon source for sulfate reducing bacteria. Two-way ANOVA showed no significant difference ($p < 0.05$, $n = 90$) between ethanol and lactose treatments. Interference studies showed that low pH (≤ 4) and low temperature (5°C) as well as Al and Zn at high concentrations (25 mg/L and 50 mg/L Al and Zn) adversely affected sulfate removal by the entrapped SRB. Cu (0.5, 25, and 50 mg/L) did not show any significant interference in sulfate removal by the entrapped SRB.

3.2. Materials

3.2.1. Equipment

Hach DR 5000 UV-Vis Laboratory Spectrophotometer (Wavelength range from 190 to 1100 nm), two-sided disposable plastic cuvettes (VWR Catalog No. 7591-70), glass tubes (Hach Catalog No. 2517600), and disposable serological pipets (VWR Catalog No.89107-884) were used to determine sulfate concentrations using the Turbidimetric method as indicated by USEPA (Method No. 375.4).

Peristaltic pump (with 0.1 mm ID tubing, 1.5 mL min^{-1} flow rate), centrifuge (Model IEC CL Centrifuge), and Magnetic Stirrer Plate (8"x8" surface, VWR Scientific 360) were also used

in the bead making process. A pH meter (Model Orion 2 Star, Thermo Scientific) was used to determine pH of samples during the course of this research.

VWR Vacuum oven, (VWR International) connected to pure nitrogen tank was used to dry NZVI under nitrogen environment.

Incubator (Model LI5, Shell Lab) and Speci-Mix Test Tube Rockers (18 rpm, Thermo Scientific) were used for temperature studies.

The incubator Incu-Shaker Mini (Benchmark Scientific) was used for bacterial growth as well as for conducting sulfate remediation experiments by co-entrapped NZVI-SRB.

3.2.2. Supplies

Most of the chemicals used in this research were purchased from VWR International. However a few of them were sourced from Sigma Aldrich. All chemicals were used as received from the suppliers unless otherwise specified.

Ammonium chloride (NH_4CL, ACS Grade, BDH), calcium sulfate anhydrous ($CaSO_4$, 99.0%, VWR), ferrous sulfate heptahydrate ($FeSO_4.7H_2O$, ACS AR Grade, 99.5%, VWR), magnesium sulfate ($MgSO_4$, 97+%, Aldrich), potassium dibasic phosphate (K_2HPO_4, ACS Grade, 98.0%, VWR), sodium citrate ($C_6H_5Na_3O_7$, 99.0%, VWR), sodium L- lactate ($C_3H_5NaO_3$, 98.0%, VWR), and yeast extract (VWR) were used to prepare ATCC1249 media for sulfate reducing bacteria (SRB).

Micronutrient solution for SRB was prepared using the following chemicals: Boric acid (H_3BO_3, ACS Reagent Grade, ≥99.0%, Aldrich), cobalt chloride hexahydrate ($CoCl_2.6H_2O$, ACS Reagent Grade, 98.0%, Aldrich), copper chloride dehydrate ($CuCl_2.2H_2O$, ACS Reagent Grade, ≥99.0%, Aldrich), Ferrous chloride tetrahydrate ($FeCl_2.4H_2O$, Puriss. p.a. Grade, ≥99.0%, Aldrich), manganese chloride tetrahydrate ($MnCl_2.4H_2O$, 99.99%, Aldrich), nickel sulfate

hexahydrate ($NiSO_4.6H_2O$, ACS Reagent Grade, 99%, Aldrich), nitrilotriacetic acid ($N(CH_2COOH)_3$, ACS Reagent Grade , ≥99.0%, Sigma), sodium molybdate (Na_2MoO_4, ACS Reagent Grade, ≥98.0%, Aldrich), sodium selenite (Na_2SeO_3, ACS Reagent Grade, 99.0%, Aldrich), sodium tungstate dehydrate ($Na_2WO_4.2H_2O$, ACS Reagent Grade, 99.0%, Aldrich), and zinc chloride ($ZnCl_2$, Reagent Grade, ≥98.0%, Aldrich).

Sodium alginate was purchased from Sigma Aldrich and Calcium chloride ($CaCl_2$, ACS Reagent Grade, BDH) were used to prepare Ca-alginate capsules for SRB entrapment.

Anhydrous sodium sulfate (Na_2SO_4, ACS Grade, 99.0-100.5%, BDH,) was used for the preparation of sulfate solutions used during this research.

Magnesium chloride hexahydrate ($MgCl_2.6H_2O$, ACS Grade, 99.0-102.0%, VWR), sodium acetate ($CH_3COONa.3H_2O$, 99.0-100.5%, VWR), potassium nitrate (KNO_3, 99.0-100.5%, VWR), and acetic acid (CH_3COOH, 1.0 M, Aldrich) were used to prepare buffer solution used along with barium chloride ($BaCl_2$, 99+%, VWR) for sulfate concentrations determination (Turbidimetric method).

Sodium borohydride ($NaBH_4$, ≥98.0%, VWR), sodium hydroxide (NaOH, ACS Reagent Grade, 98%, VWR), ferrous sulfate heptahydrated ($FeSO_4.7H_2O$, ACS AR, 99.5%, VWR), and ethanol (95.0%, BDH Grade, VWR) were used for synthesizing nanoscale zero-valent iron (NZVI) particles.

Aluminum sulfate [$Al_2(SO_4)_3$, ACS Grade, 98.0 -102%, VWR], copper sulfate pentahydrate ($CuSO_4.5H_2O$, ACS Reagent Grade, 98+%, Aldrich), zinc sulfate heptahydrate, crystal ($ZnSO_4.7H_2O$, ACS Reagent Grade, 101.3%, Aldrich) were used as sources of aluminum, copper, and zinc for metal interference studies.

3.3. Methods

3.3.1. Bacteria for pilot scale column study

Sludge samples were collected from Moorhead (Minnesota) municipal wastewater treatment facility and allowed to settle for 24 h under refrigerated conditions in the laboratory. Then 250 mL of settled sludge (containing bacteria) was mixed with 500 mL of glucose nutrient solution. Glucose nutrient solution (6 L) was prepared using (1) 3.0 g of glucose ($C_6H_{12}O_6$), (2) 0.36 g of manganese sulfate ($MnSO_4.H_2O$,), (3) 0.09 g of ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2$,], (4) 1 capsule of BOD nutrient (HACH Company), and (5) DI water to make 6 L solution.

The mixture was incubated in a 1,000 mL beaker under anaerobic conditions for 3 months at room temperature (22± 2^oC) under stirring condition. The nutrient solution was changed every week by draining the supernatant from beaker and adding new deoxygenated nutrient solution. The presence of SRB was indicated by blackening of the solution and black precipitates (Vester and Ingvorsen, 1998). At that point 500 mL of the incubated solution containing SRB was transferred to the column (Section. 3.3.4) for sulfate removal studies.

3.3.2. Bacteria for entrapment

The incubation media (ATCC 1249 for sulfate reducers, Wen et al. 2010) consisted of three separate solutions. Solution 1 contained (1) 1.0 g of $CaSO_4$, (2) 2.0 g of $MgSO_4$, (3) 1.0 g of NH_4Cl, (4) 5.0 g of sodium citrate, and (5) 400 mL of DI water. Solution 2 had (1) 0.5 g of K_2HPO_4, and (2) 200 mL of DI water. Solution 3 was made with (1) 3.5 g of sodium lactate, (2) 1.0 g of yeast extract, and (3) 400 mL of DI water. The pH of each solution was adjusted at 7.5 and the three solutions were mixed together and purged with pure nitrogen for ~20 min to remove dissolved oxygen and, thus, create anaerobic conditions prior to autoclaving them at

120°C for 20 min. No further addition of nitrogen occurred for the rest of the experiments. This was to reflect real life condition where oxygen's presence in AMD is reported (Neculita et al, 2007). The autoclaved ATCC 1249 media (25 mL each) was now mixed with 500 µL of 5% $Fe(NH_4)_2(SO_4)_2$, and 200 µL of the settled activated sludge in multiple 50 mL sterile plastic centrifuge tubes and incubated in the shaker (Incu-Shaker Mini, Benchmark Scientific) at 30°C and 100 rpm for ~ 6 days. The presence of SRB was indicated by complete blackening of the media after 6 days and the rotten egg smell of H_2S (Figure 3.1, Choudhary and Sheoran 2011).

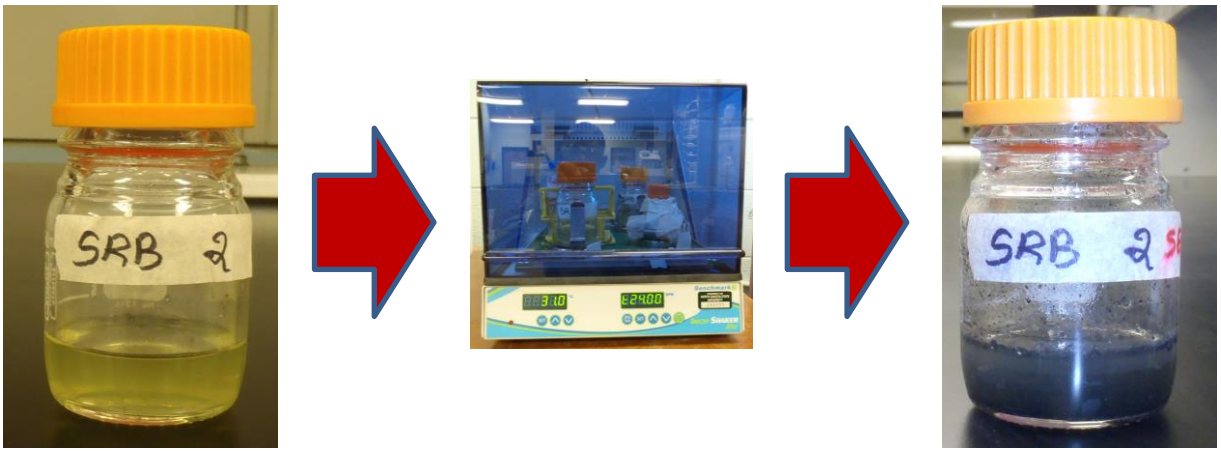

Figure 3.1. Incubation of SRB in ATCC 1249 media. Left: Mixing of growth media with activate sludge. Middle: Incubation of the bacteria culture in Incu-Shaker Mini Incubator. Right: Completion of incubation (indicated by the appearance of black precipitates from SRB metabolism).

The black contents in the centrifuge tubes were centrifuged (Model IEC CL Centrifuge) at 7,000 rpm for 10 min to separate the SRB (separated solids contained the SRB) and 3.0 g of separated wet solid was used for entrapment in Ca-alginate beads (Section 3.2.3). Bacterial growth curve was determined using the optical density (OD) method (Lobo et al. 2007). SRB were incubated in ATCC 1249 media under 30^{o}C and OD was determined by taking light absorbance reading at 600 nm of the incubation hourly in the Hach DR 5000 UV-Vis Laboratory

Spectrophotometer. In addition, SRB count was done using the most-probable number (MPN) technique (ASTM, 2010; Wan et al., 2011; Gilmour et al., 2011; Pedersen, 2012). Eight dilutions (aliquots) were performed (each in triplicates) in ATCC 1249 media and incubated at 30°C for a period of 168 h. The incubated bacterial population was inspected daily for blackening in the aliquots and the result reported as positive (if blackening is observed) or negative (no blackening). The appearance of black precipitates is the indicator SRB growth (Jong and Parry 2006; ASTM, 2010). Scanning electron microscopy (SEM) images of bacteria in the culture media and the cross section of the SRB entrapped beads after use in sulfate removal experiment were taken using JEOL JSM-6490LV scanning electron microscope (JEOL USA, Peabody, MA) operating at 15 kV accelerating voltage.

3.3.3. Entrapment of SRB

Sodium alginate solution (2.0%) was prepared by dissolving 20.0 g of sodium alginate powder in 1,000 mL of DI water. The mixture was warmed in a heater while stirred until complete dissolution of the alginate powder and allowed to cool down in the room temperature (22± 2^{o}C). The incubated microorganisms were centrifuged (after 6 days) at 7,000 rpm for 10 min. The bacterial biomass (mostly SRB) was harvested and weighted. Wet biomass (3.0 g) was added to 300 mL of 2% Na-alginate solution. The mixture was stirred to thoroughly to ensure that SRB is homogenously mixed with Na-alginate. The entrapment was done using method described by Bezbaruah et al. (2009a) by replacing iron nanoparticles with SRB. The mixture of SRB and Na-alginate was dropped into a beaker that contained 2% calcium chloride ($CaCl_2$) solution. A peristaltic pump (VWR, 0.1 mm ID tubing, 1.5 mL min^{-1} flow rate, Figure 3.2) was used to continuously and uniformly drop the bacteria-alginate mixture to the $CaCl_2$ solution. Ca-alginate beads (with SRB entrapped in them) were formed as soon as the Na-alginate solution

(with SRB mixed in there) came in contact with $CaCl_2$ solution. The synthesized beads were allowed to harden in 2% $CaCl_2$ solution for 9 h (Bezbaruah et al., 2009a). The beads were washed several times with deoxygenated DI water before they were used in sulfate removal studies. All procedures were carried out at room temperature (22 ± 2^{o}C).

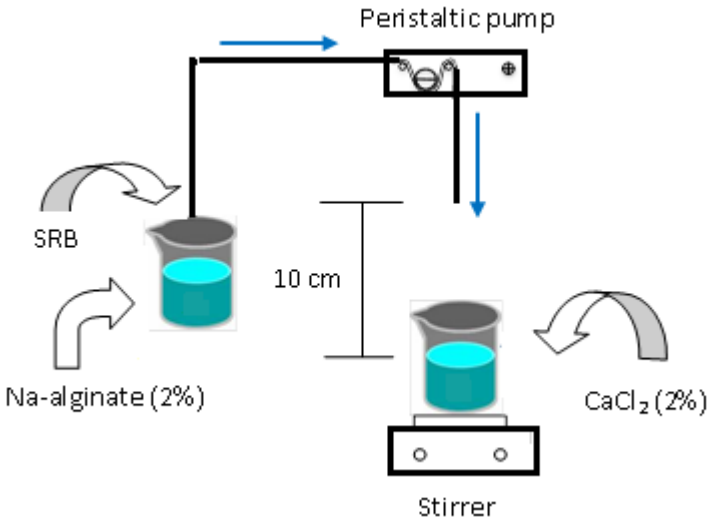

Figure 3.2. Schematic of Ca-alginate bead preparation process. Na-alginate solution (2%) is pumped through a peristaltic pump into $CaCl_2$ solution (2%) under stirring condition.

Blank sodium alginate solution (2%) was prepared using the same process. SRB were not added to blank Ca-alginate. Ca-alginate beads were synthesized by dropping the 2% Na-alginate solution into calcium chloride ($CaCl_2$) solution (2.0%) using a peristaltic pump (VWR, 0.1 mm ID tubing, 1.5 mL min^{-1} flow rate). The freshly synthesized beads were allowed to harden in 2% $CaCl_2$ solution for 9 h prior to washing them with DI water (Bezbaruah et al., 2009a). The beads were washed several times with DI water before they were used in sulfate removal studies.

3.3.4. Pilot scale study set-up

This investigation was conducted to confirm the presence of sulfate reducing bacteria (SRB) in the isolated media in order to proceed sulfate remediation study using SRB entrapped in Ca-alginate beads. Laboratory-scale permeable reactive barriers (PRBs) were constructed using transparent (clear) acrylic plastic pipes (Figure 3.3). The following are the dimensions for the column reactor: 60.0 cm height was and 10.0 cm internal diameter was, with five sampling ports placed along the height of the column at 5.0 cm intervals. The empty bed (EB) volume of the column was 4 L. The column was filled with granite type of gravel of different sizes and silica sand to provide surface area for bacterial growth. The bottom 5.0 cm of the column was filled with small size gravel (2.5 cm) which constituted the first layer from the bottom to the top. The next layer (12.0 cm) on top of that was made of 0.5 to 1.2 cm gravel. The third layer, 10.0 cm high, was a mix of gravel and sand. The last layer was a 10.0 cm height of fine silica. Synthetic fabrics were used to separate the layers of different materials and to protect inlet and sampling points from being clogged by fine sediments. These materials were washed with de-ionized (DI) water and dried in the oven at 205°C for 24 h before using them in the column. A 20 L container containing synthetic acid mine drainage (AMD) solution was connected to the column via a low flow peristaltic pump (VWR, 0.1 mm ID tubing, 1.5 mL min^{-1} flow rate). The influent flow rate was maintained at 0.88 L/d. The reactor was run in an upflow condition. The EB hydraulic detention time was estimated to be 4.75 days. Commercial grade untreated woodchips were used as the carbon source for the SRB in this experiment. A measured amount (200 mg) of woodchips was added to the column. Woodchips are considered as recalcitrant carbon source (Hiibel et al. 2011) that can be used to maintain electron donor for long term sulfate remediation.

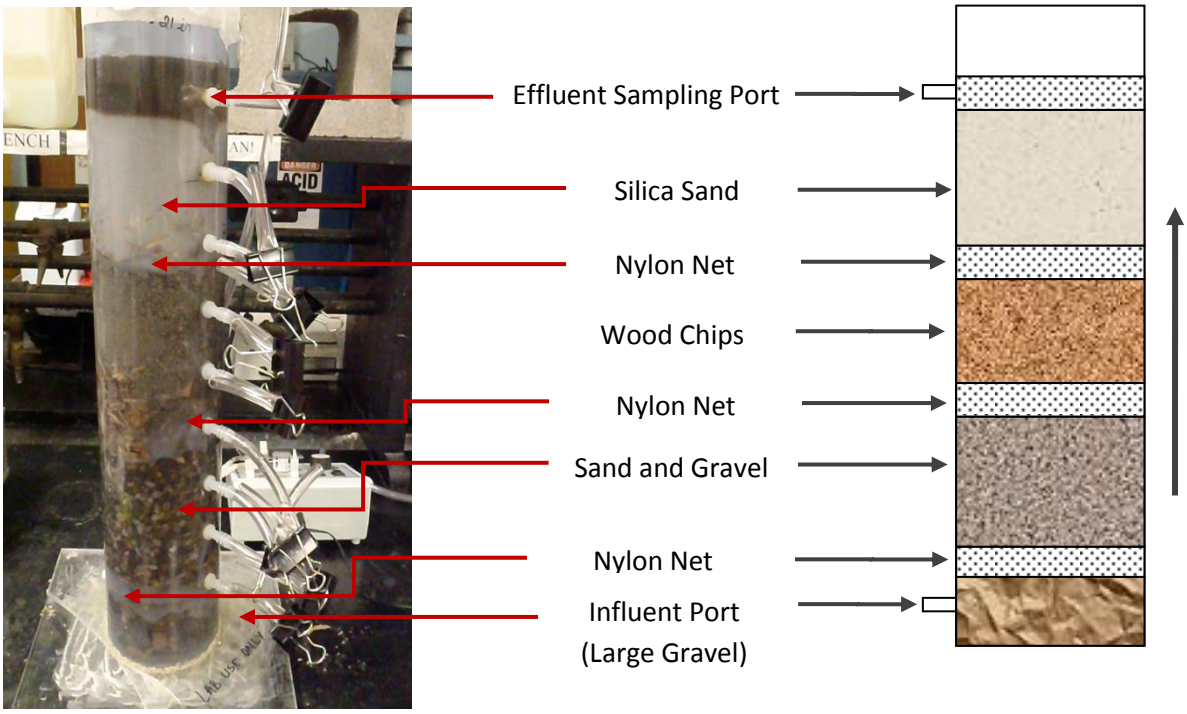

Figure 3.3. Experimental set-up of the column studies. Left: Picture of the reactor. Right: Schematic of the permeable reactive barriers (PRB) set-up. The column was operated in an upflow mode. Granite type gravels and silica sand were used to provide surface area for bacterial growth. Woodchips were the organic matter and synthetic net was used to separate layer of different materials.

Simulated AMD solution (20 L) was prepared (Davies et al., 2011) using $Al_2(SO_4)_3.18H_2O$ (25 g), $CaSO_4$ (2.7 g) $Fe(NH_4)_2(SO_4)_2.6H_2O$ (12.335 g), K_2SO_4 (0.105 g), $MgSO_4$ (1.25 g), and $ZnSO_4$ (0.089 g). This yielded 500 mg/L of SO_4^{2-}. Isolated SRB solution (500 mL) was added to the column prior to start pumping the experimental AMD through it.

The experiment was conducted for a total of 9 days and samples were collected sequentially from the top outlet of the column on day 0, 6, and 9. This experiment was not replicated and SRB's presence in the column was monitored by the blackening of the column and the odor of H_2S generated by sulfate reduction. Sulfate concentrations were determined by Turbidimetric method based on USEPA test method No. 375.4 (USEPA, 1978).

3.3.5. Batch experiments for sulfate removal by entrapped SRB

Batch studies were conducted to determine the extent of sulfate removal (treatability) by the entrapped SRB beads. Four experiments were done to investigate the influence of various factors on SRB's ability to treat sulfate: 1) different carbon sources, 2) pH, 3) temperature, and 4) interference of aluminum, copper, and zinc. The batch experiments were conducted with different initial sulfate concentrations of 250 and 500 mg/L. These concentrations were selected for they are the maximum contaminant levels for sulfate in drinking water (WHO, 2004) and irrigation waters (Costa et al., 2008). Further, sulfate reduction below these concentrations is not always achieved by most of the treatment technologies. The experiments started with initial sulfate concentration of 250 mg/L conducted over a 7-day period and samples collected on day 0, 1, 2, 3, 5, and 7. Sulfate concentration was then increased to 500 mg/L and the experiments conducted over a 15-day period to allow sulfate removal up to a steady state. Here, samples were collected on day 0, 3, 6, 9, 12, and 15 and analyzed for sulfate concentration. All sulfate removal batch experiments with entrapped SRB were carried out using 40 mL amber glass vials as test reactors and control reactors (Figure 3.4). The measured initial pH in the batch experiments was ~5 for all experiments (except the interference study with pH, where the initial pH was adjusted to 2, 4, and 7.5).

Sulfate solution was prepared using Na_2SO_4, carbon source (750 mg/L for ethanol or 400 mg/L for lactose) and micronutrient solutions (500 µL) were added to the sulfate solution as appropriate for each experiment (see each experiment section for more details). SRB entrapped beads and blank beads were washed with DI water and the beads where counted and placed inside the 40 mL amber glass vials (1 batch per reactor) for reactors and controls.

Figure 3.4. Test reactors and control reactors used in batch experiments. There were enough reactors for three replicates in each set of experiment.

Each reactor was then filled to the top with the prepared sulfate solution and closed hermetically. Sulfate solution (30 mL) mixed with carbon source and micronutrient solution was added to the reactors and controls. Test reactors and control reactors were placed in the shaker at room temperature (22± 2^{o}C). Enough reactors and controls were run simultaneously and three test reactors and three control reactors (both sacrificial in nature) were taken out and sampled for sulfate concentration (see each experiment section for more details). For most of the experiments, reactors were placed in a custom made end-over-end rotator at 28 rpm. Temperature studies were carried out in BOD Refrigerated Incubator (Shel Lab) with a Speci-Mix Test Tube Rockers (Thermo Scientific) 18 rpm.

3.4. Impact of carbon source on SRB's ability to treat sulfate

The objective of this investigation was to understand the impact of a readily biodegradable carbon (ethanol) versus a more complex carbon source (lactose) on SRB activity. The batch experiments were conducted with initial sulfate concentration of 250 and 500 mg/L. The experiment investigations were completed using five different scenarios, viz., (1) Sulfate

solution only as Blank, (2) Ca-alginate beads in sulfate solution as Control, (3) Entrapped SRB beads without carbon source as Treatment 1, (4) Entrapped SRB beads with ethanol as Treatment 2, and (5) Entrapped SRB beads with lactose as Treatment 3. Each set of experiments was conducted over a period of 7 and 15 days for 250 and 500 mg/L of initial sulfate concentration, respectively. Altogether, a total of 180 vials (90 for each concentration) were used in these experiments for a total of 110 days.

Ethanol (95%, BDH Grade) and lactose monohydrate (4-0-β-Galactopyranosyl-D-glucose) were supplied by VWR International and used as received from the supplier. Ethanol (750 mg/L) and lactose powder (400 mg/L) were added into two separate sulfate solutions, yielding 0,033 Mol of each carbon source. Each solution was homogenized by mixing them using the magnetic stirrer. Batch studies were carried out in the presence and absence of carbon sources for sulfate removal by SRB entrapped in Ca-alginate beads.

3.5. Interference studies

3.5.1. Batch experiment to study the role of pH in sulfate removal by SRB

This experiment was designed to assess the influence of initial pH on the ability of entrapped SRB in removing sulfate. The control for this experiment was the experiment conducted at initial pH 5.1 as measured after preparation of the sulfate solution. Three pH values (2.0, 4.0, and 7.5) were chosen as initial pH for three different sets of 15-day batch experiments conducted simultaneously at room temperature (22± 2^{o}C). Sulfate solution (500 mg/L) was prepared and the initial pH was adjusted to the needed value by adding hydrochloric acid (HCl, 0.1N) or sodium hydroxide (NaOH, 0.1N) solution. The reactors (18 for each pH experiment) were placed in the end-over-end rotator 28 rpm. Ethanol (750 mg/L) was used as the carbon source in these experiments. Ethanol was selected for the rest of this research since the difference

between ethanol and lactose treatments was not significant (Section 3.8.4). Statistical analysis (Two-way ANOVA) was conducted to compare treatment means.

3.5.2. Interference of temperature

The influence of the temperature on the ability of entrapped SRB beads in removing sulfate was examined in this set of experiments. Temperatures chosen for this experiment were 5, 15, and 30°C. This investigation was conducted progressively (one temperature experiment at the time) in BOD Refrigerated Incubator (Model Li5, Shel Lab). To ensure homogeneous mixing, the reactors were placed in the Speci-Mix Test Tube Rockers 18 rpm rotator and placed inside the incubator. Ethanol (750 mg/L) was used as the carbon source in these experiments. The control experiment was conducted at room temperature 22± 2^{o}C. Each experiment was conducted in triplicates and Two-way ANOVA performed for statistical analysis to compare the results.

3.5.3. Interference of aluminum, copper, and zinc

Interference of aluminum (Al), copper (Cu), and zinc (Zn) on sulfate removal was investigated in this set of experiments. The objective of these studies was to understand how these metals impact sulfate removal by entrapped SRB. Three different concentrations (0.5, 25, and 50 mg/L) of Al, Cu, and Zn were used to investigated their interferences with sulfate removal by entrapped SRB. The three metals were selected because they are typically present in AMD (see Table 1.1) and they are reported to be toxic to SRB (Kieu et al., 2011; Martins et al., 2012; Wang et al., 2012). The concentrations were selected based on their toxicity limits reported in the literature (2-10 mg/L for Cu, and 13-40 mg/L for Zn, Martins et al. 2009) and their reported range in AMD (see Table 1.1 in Chapter 1). The experiments were conducted for 15 days and data compared to the experiment data conducted in the absence of metals. Ethanol

(750 mg/L) was used as the carbon source. Two-way ANOVA performed for statistical analysis to compare the results.

3.6. Analytical method

Sulfate concentration was determined following Turbidimetric method as described in EPA test methods for sulfate (USEPA test Method No. 375.4, 1978). Samples were collected and mixed with a buffer solution and barium chloride using a magnetic stirrer prior to reading them in Hach DR 5000 UV-Vis Laboratory Spectrophotometer at 450 nm. For experiments involving (1) co-entrapped SRB-NZVI (CoSRB-NZVI) and (2) separate entrapped SRB-NZVI (SepSRB-NZVI), samples were centrifuged at 7,000 rpm for 10 min to allow NZVI particles to settle down and sulfate reading was taken in the UV-Vis Spectrophotometer at 510 nm.

Scanning electron microscopy (JEOL JSM-6490LV scanning electron microscope (JEOL USA, Peabody, MA) operating at 15 kV accelerating voltage) images were taken to visualize SRB in the growth culture and the interior of the SRB entrapped beads after they were used in the sulfate remediation experiments. The images were taken for (1) Bare Ca-alginate beads (no SRB) used in DI water (Control), (2) Bare Ca-alginate beads (no SRB) used in sulfate solution, (3) Entrapped SRB beads used in sulfate remediation, (4) SRB culture after 6 days of incubation in ATCC 1249 media.

3.7. Quality control

In each experiment, there were enough vials to perform three replications and average values along with standard deviations are reported. Blanks and controls were run for each set of experiments. Sulfate concentrations in each set of experiments were analyzed with a new calibration curve determined with a standard solution using 10, 20, 30, and 40 mg/L of SO_4^{2-}. A

total of 540 sacrificial vials (vials eliminated after sampling) were used in this part of the investigations for a total of 180 days.

3.8. Statistical analyses

Statistical analyses were conducted using Two-way analysis of variance (ANOVA) to compare the treatments means of the data sets. In addition, Tukey pairwise comparison tests were conducted to detect significance of difference between treatments. All statistical analyses were performed with Minitab 16 software (Minitab, USA).

3.9. Results and discussions

3.9.1. Bacterial growth characteristics

A growth curve for SRB (Figure 3.5) was developed using optical density (OD) method (600 nm, Lobo et al., 2007) in the Hach DR 5000 UV-VIS Laboratory Spectrophotometer. This curve showed a lag phase in the first 12 h of incubation followed by an exponential growth phase going from 12 to 84 h of the incubation. This was followed by a stationary phase. Mukhopadhyay et al. (2006) and Borglin et al. (2009) reported similar trend in the growth of *Desulfovibrio vulgaris*. Both reported a 3-phase growth curve with a lag phase of 15 h, followed by an exponential growth phase from 15 to 40 h, then a stationary phase (40 to 70 h).

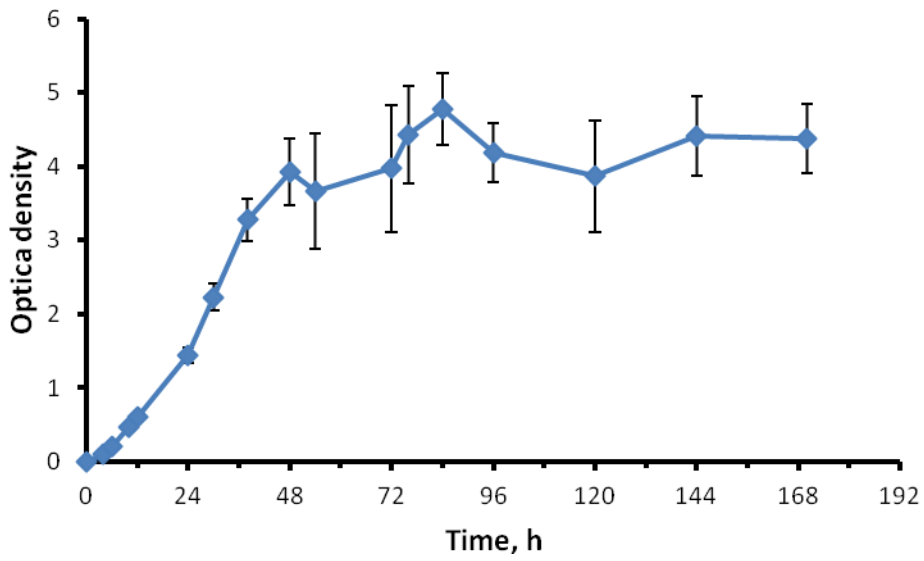

Figure 3.5. The SRB growth curve (based on optical density method) indicates an exponential growth phase till ~84 h and a stationary phase beyond that (till 170 h).

The most-probable number (MPN) method data (Table 3.1) indicate a long lag phase of 5 days followed by an acceleration phase on day 6 and an exponential growth phase from day 6 to day 7 where all the aliquots turned black indicating an MPN of 1.1×10^7 CFU/mL. These values are also reported on Figure 3.6.

Table 3.1. Enumeration of SRB in ATCC 1249 media using the most-probable number (MPN) technique.

Detection Method	**MPN/mL**				
	Day 3	Day 4	Day 5	Day 6	Day 7
Blackening	2.3×10^1	9.3×10^3	1.1×10^5	1.1×10^6	1.1×10^7

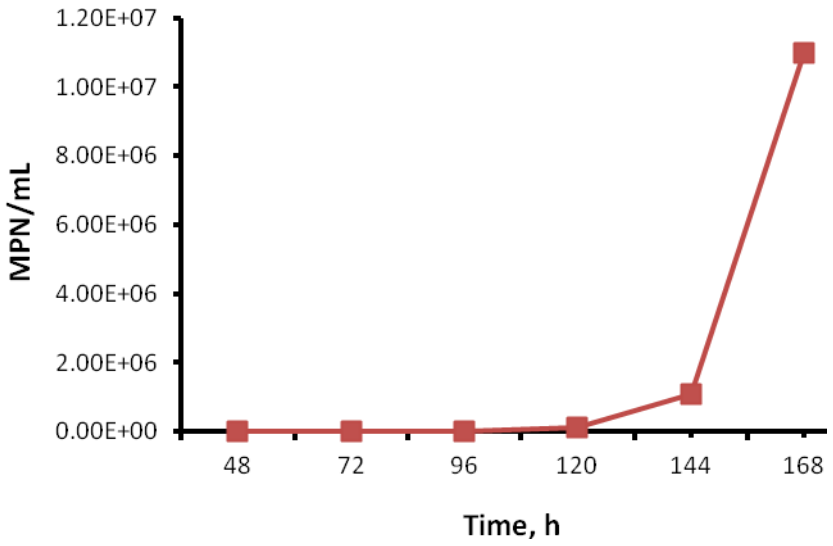

Figure 3.6. The SRB growth curve (based on most-probable number method) indicates a slow growth phase till ~120 h followed by an exponential growth phase (till 168 h).

Martins et al. (2009) reported 1.8 x 10^7 CFU/mL after 5 days of incubation for SRB but Bussmann and Reichardt (1991) reported a much lower density of mix culture SRB (7 x 10^4 CFU/mL after 8 weeks of incubation) and a lag period of 2 to 3 weeks. Vester and Ingvorsen (1998) also reported a much lower values with SRB incubated on synthetic media (4.3 x 10^4 CFU/mL), and this value remained constant for 34 days of incubation.

The growth experiment was not continued beyond 168/170 hours as it was conducted as a verification of results obtained by others (Bussmann and Reichardt, 1991; Vester and Ingvorsen, 1998; Mukhopadhyay et al., 2006; Xin et al., 2008; Borglin et al., 2009; Martins et al., 2009; Ramamoorthy et al., 2009; Wan et al., 2010; Gilmour et al., 2011; Pedersen, 2012). The growth curves obtained by the two methods (OD and MPN methods) showed different results. While Figure 3.5 (OD method) shows an onset of stationary phase after 72 h, Figure 3.6 (MPN method) indicates that exponential growth continued up to 168 h (7 days). The inconsistency observed in the different phases of SRB growth curve is quite common in the literature. This can be

explained by the diversity of culture media used for SRB incubation (Vester and Ingvorsen, 1998). In addition, the incubation temperature conditions are different in various reported research. Incubation temperatures reported so far include 21^{o}C (Martins et al., 2009), 30^{o}C (Wan et al., 2010), 36^{o}C (Xin et al., 2008). In the present research bacteria from the growth phase (6 days) were collected for further experiments (including entrapment, Section 3.2.3).

Scanning electron microscopy (SEM) images show groups of SRB in the culture media. Two distinct forms are visible in the culture media, (1) an elongated form (Figure 3.7), and (2) a spherical form (Figure 3.8). The elongated form (~ 2 µm long and 1 µm wide) was the most dominant. Similar elongated SRB were reported by other investigations (Al-Zuhair et al., 2008; Wan et al., 2010; Akai and Md Anawar, 2013). The spherical shaped SRB were similar to human red blood cells. They are smaller in size with approximately 1 µm in diameter. Spherical shapes of SRB, including the genera *Desulfococcus*, are described as isolated or paired spherical cells (1.4- 2.3 µm) and reported in mixed culture of SRB (Castillo et al. 2012).

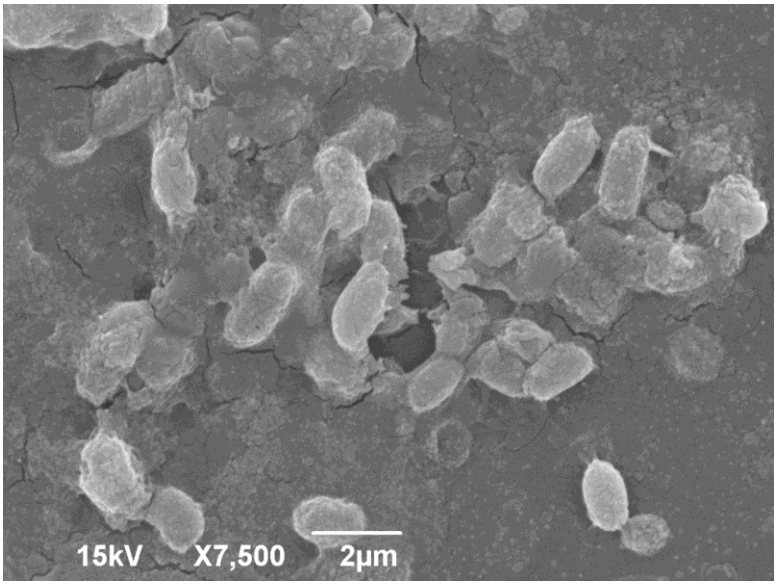

Figure 3.7. SEM image showing SRB after incubation for 6 days in ATCC 1249 media. The elongated SRB were ~2 µm long and 1 µm wide.

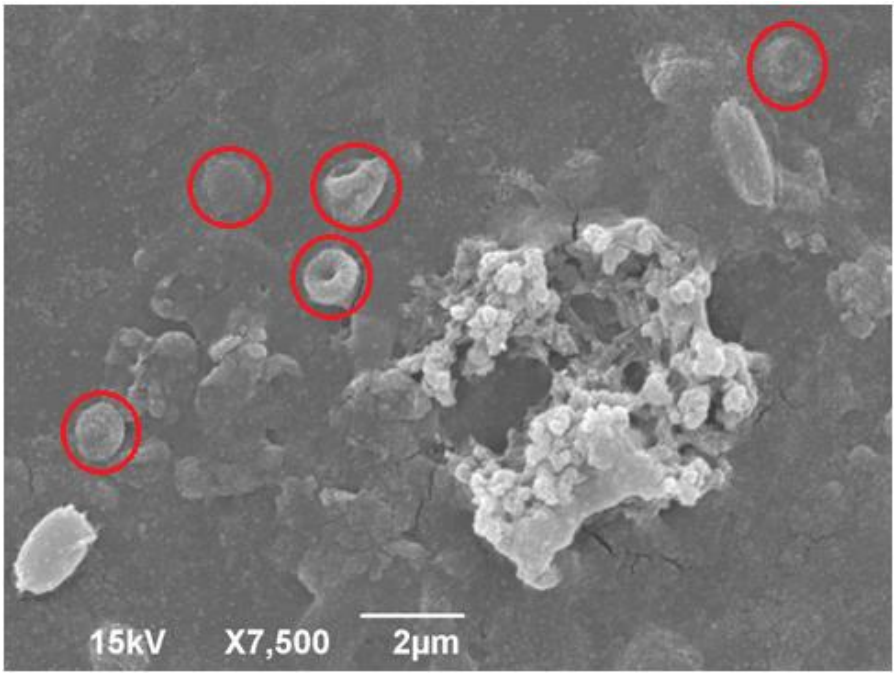

Figure 3.8. SEM image showing SRB after incubation for 6 days in ATCC 1249 media. Spherical shaped SRB (diameter ~ 1 µm) are highlighted with red circles.

3.9.2. Sulfate removal by free cells of SRB in pilot column study

The column studies were conducted to validate that the SRB isolated from activated sludge were able to degrade sulfate, corroborating reports by others (Kuo and Shu, 2004; Gibert et al., 2011; Mulopo et al., 2011). In the column study, 88% of sulfate was removed after 9 days of the experiment (Figure 3.9). When the experiment was launched, progressively, materials inside the column turned black indicating the presence and metabolism of SRB. There was also the characteristic smell of rotten egg of H_2S which further indicated that the anaerobic sulfate reduction reaction was taking place.

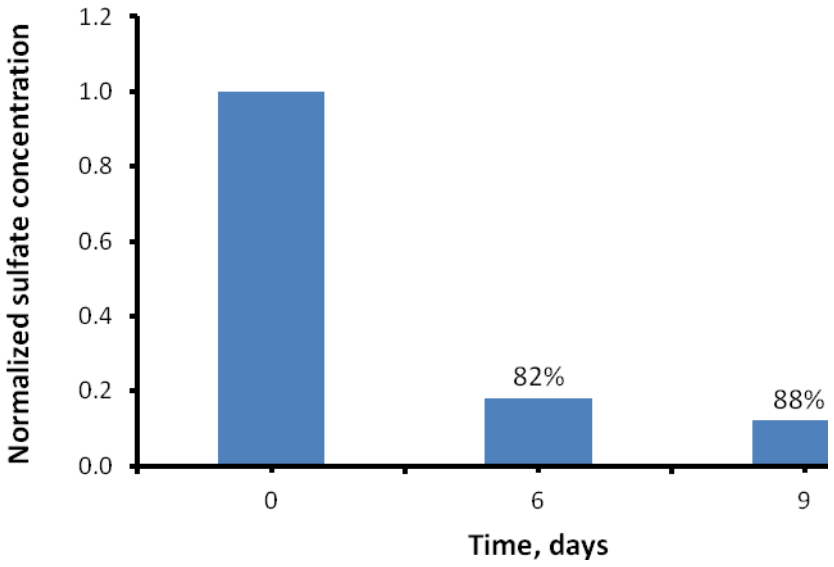

Figure 3.9. Percentage of SO_4^{2-} removed over time by free cells of SRB in pilot scale column experiment from initial SO_4^{2-} concentration of 500 mg/L. 82% SO_4^{2-} removed at day 6 and 88% SO_4^{2-} removed at day 9. This experiment was not replicated.

Martins et al. (2011) reported similar observations in sulfate removal in an up-flow anaerobic packed bed (UAPB) system. They (Martins et al., 2011) obtained 90% sulfate removal after 120 days. However, from Day 120 to the end of their experiment (Day 160), sulfate removal decreased to 50%. Mulopo et al. (2011) observed 100% sulfate removal in 9 days of in the first round of a batch study. In the second round of their experiment maximum sulfate removal did not exceed 83% for the remaining 40 days of their investigation. Kuo and Shu (2004), using SRB cells immobilized in cellulose triacetate in an up flow anaerobic filter, found that sulfate removal rapidly reached more than 90% prior to decreasing to 60%. Choudhary and Sheoran (2011) obtained 36% sulfate reduction in 10 days in the first round of a column bioreactor with goat manure as carbon source. In the second round, only 18% sulfate removal was achieved. The 88% removal of sulfate in the present column studies is indicative of successful growth of an SRB culture.

3.9.3. Characteristics of blank and entrapped SRB beads

The freshly synthesized calcium beads appeared to be transparent, soft, and fragile (Figure 3.10a). The fragility of the beads was qualitatively examined by squeezing between two fingers. The beads were spherical in shape and had an average bead diameter of 5± 2.2 mm (N = 58). They were allowed to harden for an additional 9 h (Bezbaruah et al., 2009a) in 2% $CaCl_2$ solution then washed with DI water. When Na-alginate was dropped into $CaCl_2$ solution, beads were formed due to cross-linking of alginate with divalent calcium (Ca^{2+}). When kept in $CaCl_2$ solution for a long time (9 h), any remaining Na^+ were replaced by Ca^{2+} in the beads (cross linking reaction) and, as they harden, they turned into white, opaque . SEM image (Figure 3.10b) indicates solid matrix formation throughout the bead. Immersion in $CaCl_2$ solution is known to harden the beads and ensure adequate porosity in the alginate matrix (Bezbaruah et al., 2009a). Bezbaruah et al. (2009a) observed no mass transfer resistance in alginate beads for nitrate and there was no problem for the flow of solute into the beads.

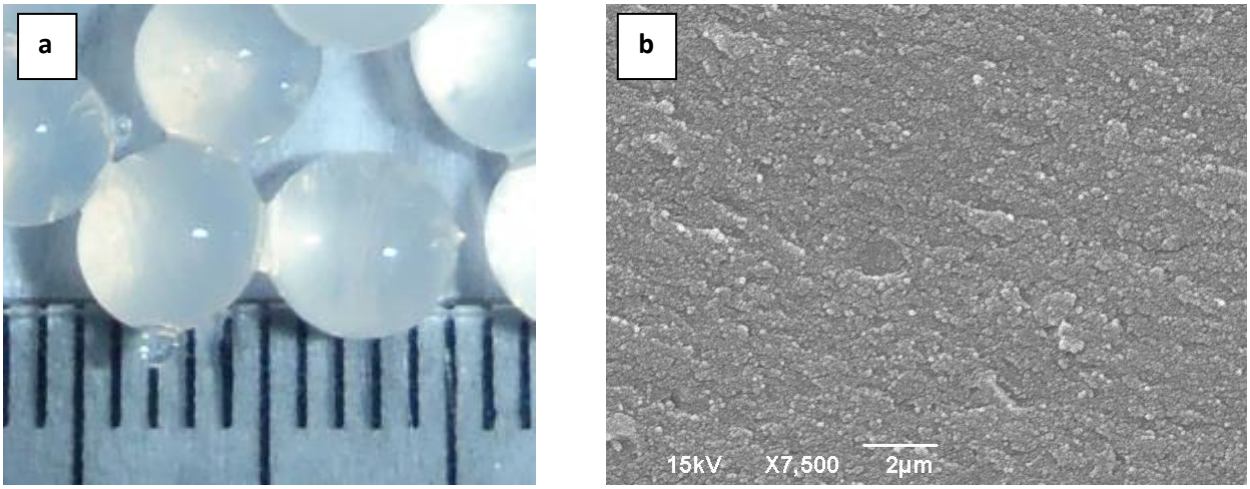

Figure 3.10. (**a**) Synthesized Ca-alginate beads. The average bead diameter was 5± 2.2 mm (n = 58). The reference scale in the figure is a centimeter ruler with each small graduation indicating 1 mm (**b**) SEM image of a cross section through a blank Ca-alginate bead.

Entrapment of bacterial cells to immobilize them within the contaminated media is a preferred approach for biological remediation in order to limit adverse environmental effects of free cells. Based on work done for other microorganisms (and contaminants) it is obvious that immobilization of bacterial cells makes the handling of the microbial population very easy (Karabika et al., 2009; Pramanik et al., 2011; Quan et al., 2011; Wadhawan et al., 2011). It also helps in maintaining the cells within the treatment zone (Pramanik et al., 2009; Siripattanakul et al., 2010; Ahmad and Kunhi, 2011; Quan et al., 2011), and protecting cells from stressors (Pramanik et al., 2009; Siripattanakul et al., 2010; Wadhawan et al. 2010; Ahmad and Kunhi, 2011;). Moreover, immobilization of bacteria ensures high efficiency of contaminant removal and guarantee a good operational stability (Ahamad and Kunhi, 2011; Quan et al., 2011; Wadhawan et al., 2011). Entrapped SRB beads (Figure 3.11) were similar to bare Ca-alginate beads in size (5± 22 mm, n = 58) and shape (spherical). However the beads were black in color because of the presence of SRB.

Figure 3.11. Entrapped SRB beads. The average bead size was 5±2.2 mm (n = 58) and they were spherical in shape. The reference scale in the figure is a centimeter ruler with each small graduation indicating 1 mm.

SEM image of a cross section of an entrapped SRB bead after it was used for sulfate removal (Figure 3.12) shows SRB embedded inside the alginate matrix. Some SRB are seen in groups while most of them remained as isolated cells. The spherical form of SRB was the most dominant inside the beads. Also, they were relatively smaller (< 0.5 µm) than the ones observed in ATCC growth media. The SRB observed in the ATCC growth media were mostly of elongated form. Looking at the structure of the bead (see Figure 3.10b), it appears that space limitation played a major role in reducing the bacterial size.

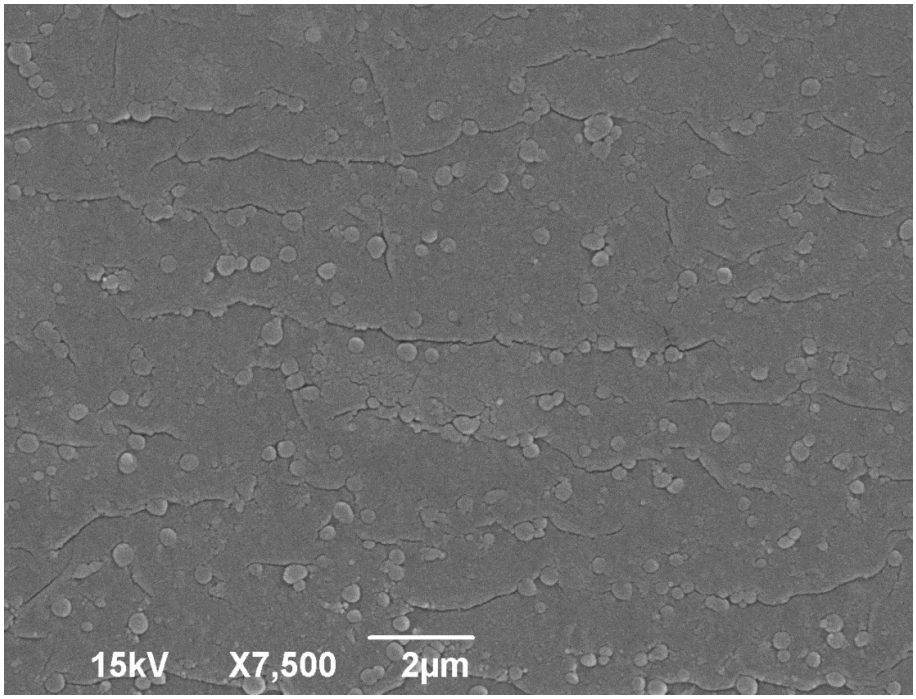

Figure 3.12. SEM images showing SRB scattered and embedded inside the alginate matrix. Bacteria observed here were <1 µm in diameter (smaller than that observed in the incubated media). It is likely that the size reduction is due to space limitation inside the bead.

3.9.4. Sulfate removal by entrapped SRB (EntSRB) with different carbon sources

The batch experiments were conducted with initial sulfate concentration of 250 and 500 mg/L. Entrapped SRB beads were used in five different scenarios, viz., (1) Sulfate solution only as Blank, (2) Ca-alginate beads (no bacteria, no carbon) in sulfate solution as Control, (3)

Entrapped SRB beads without carbon source as Treatment 1, (4) Entrapped SRB beads with ethanol as Treatment 2, and (5) Entrapped SRB beads with lactose as Treatment 3. Entrapped SRB supplied with ethanol were able to achieve 91% of sulfate reduction in 7 days which was the duration of this experiment, while those supplied with lactose achieved 76% sulfate removal during the same period. At the same time, the No Carbon treatment (the entrapped SRB that were not supplied with carbon) removed only 57% of sulfate, the control (only Ca-alginate beads with no SRB and carbon source) removed 22% sulfate, and 4% sulfate degradation was observed in blank solution (sulfate solution only, Figure 3.13).

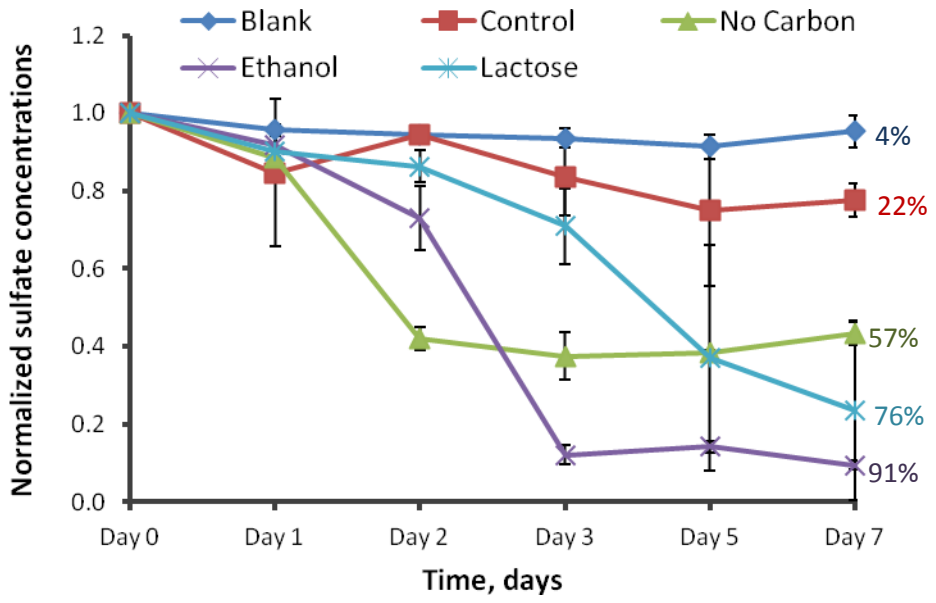

Figure 3.13. Percentage of sulfate concentration removed from 250 mg/L of initial sulfate solution medium over time, representing sulfate removal by Ca-alginate beads (control, no bacteria, 22%), or SRBs entrapped beads (no carbon, 57%), SRBs entrapped beads (with lactose, 76%), and SRBs entrapped beads (with ethanol, 91%). Only 4% sulfate degradation was observed in the blank solution.

When the experiment was repeated with initial sulfate concentration of 500 mg/L and carried over a period of 15 days (Figure 3.14), the maximum sulfate removal (95%) was achieved by entrapped SRB beads supplied with ethanol. The sulfate removal was 88% for the

SRB beads supplied with lactose, 51% for the No Carbon treatment, and 34% for the Ca-alginate beads only. Only 5% sulfate degradation was observed in the blank solution.

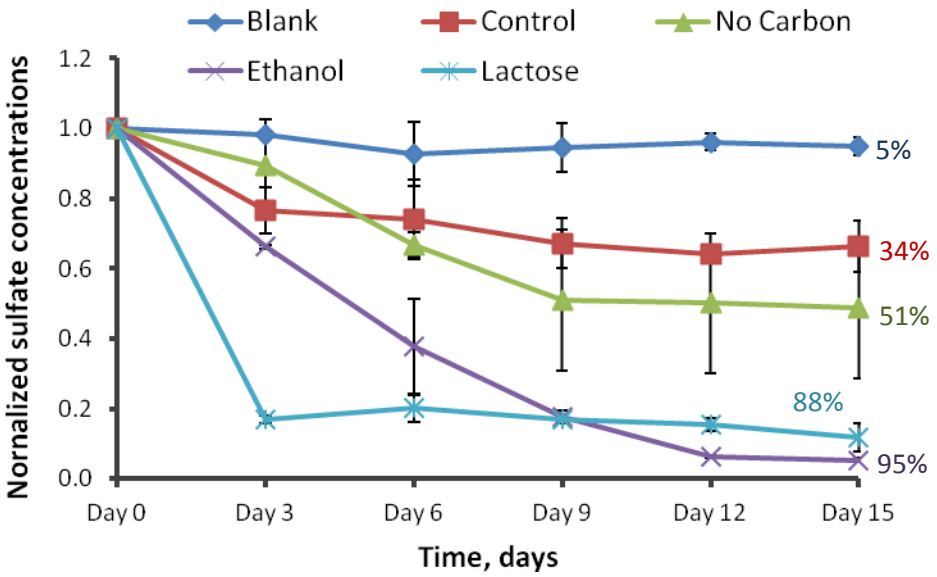

Figure 3.14. Percentage of sulfate concentration removed from 500 mg/L of initial sulfate solution medium over time, representing sulfate degradation in blank (sulfate solution only, 5%), by Ca-alginate beads (control, no bacteria, 34%), or SRBs entrapped beads (no carbon, 51%), SRBs entrapped beads (with lactose, 88%), and SRBs entrapped beads (with ethanol, 95%).

A similar trend has been reported by Hill and Khan (2008), Siripattanakul et al. (2008) and Siripattanakul et al. (2010) for nitrate removal using entrapped bacteria. Siripattanakul et al. (2010) reported limited nitrate removal (20 to 50%) in the absence of methanol (carbon source). When methanol was supplied to the system, nitrate removal was 90 to 99%. Hill and Khan (2008) also reported the use of methanol as the carbon sources in the denitrification phase. An average ammonia removal (47%) was obtained independent of carbon source during 8 h. Siripattanakul et al. (2010) in nitrate removal experiment using entrapped cells denitrifying bacteria reported 90-99% nitrate removal. Sulfate removal achieved by entrapped SRB in the absence of carbon source (51 and 57% with initial sulfate concentrations of 250 and 500 mg/L,

respectively) indicates that SRB did not use (or only slightly used) Ca-alginate as a carbon source for their metabolism. Sulfate reduction requires carbon sources which serve as electron donor for sulfate reduction. In that process, SRB act as electron transfer agent from carbon source to sulfate. It is likely that SRB used part of their remaining energy to perform sulfate reduction up to the observed values with reduced activity and metabolism (vegetative phase).

Effective sulfate removal was achieved with both experiments conducted (91 and 95%, with initial sulfate concentration of 250 and 500 mg/L, respectively). It appeared that entrapment of SRB in Ca-alginate beads did not affect their ability to reduce sulfate. These data can be compared to data reported by others in the literature. Kuo and Shu (2004) achieved 93% sulfate removal with entrapped SRB in cellulose triacetate supplied with glucose and Hsu et al. (2010), using SRB immobilized in polyvinyl alcohol (PVA) reported 99% of sulfate removal, Pramanik et al. (2011) observed about 86% and 88% DOC removal by alginate entrapped cells) and polyvinyl alcohol (PVA) entrapped cells, respectively, ~after 3 weeks of experiments.

Two-way ANOVA test was conducted to determine if there was any difference between the five treatments (including blank). The test results indicated that there is a difference between the treatments with and without a carbon source present ($p < 0.05$, $n = 90$). The data were further analyzed using Tukey's pairwise comparison test to check the significance of the difference among the treatments. The results showed that there were significant differences between treatments with and without carbon sources. However, no significant difference was detected between the two carbon sources, suggesting both ethanol and lactose can be effectively used by SRB as a carbon source. This suggest that a relatively complex carbon source like lactose can diffuse through Ca-alginate beads and provides energy and electron for sulfate reduction by SRB entrapped in Ca-alginate beads.

3.9.5. Influence of pH on sulfate removal by entrapped SRB

Results of pH study in this research (Figure 3.15) indicate lower sulfate removal (Two way ANOVA, $p < 0.05$, $n = 72$) with more acidic pH and over time. Entrapped SRB achieved sulfate removal of 14 at pH 2.0 and 41% at pH 4.0. While a very high (91%) sulfate removal was achieved at pH 7.5. At pH 7.5 the maximum sulfate reduction was observed in the first 6 days of the experiment. The initial concentration of sulfate used was 500 mg/L (in the presence of 750 mg/L of ethanol) and was reduced to 303 mg/L in 3 days (i.e., sulfate removal rate of 65.69 mg/L/d for the first 3 days). The sulfate concentration further reduced to 85 mg/L in 6 days (i.e., 72 mg/L/d from day 3 to day 6). After the 6th day the reduction continued but at a slower rate (0.94 mg/L/d).

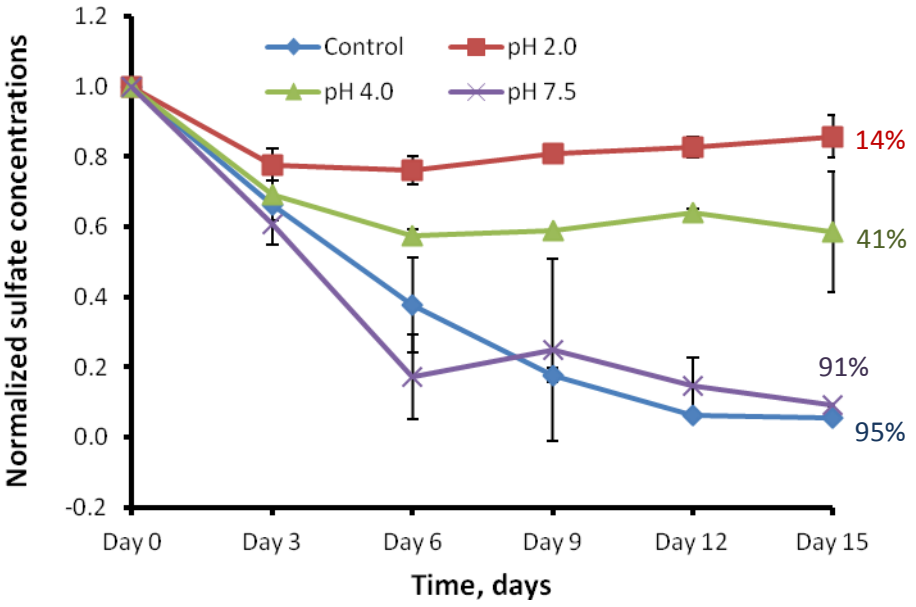

Figure 3.15. Percentage of sulfate concentration removed from 500 mg/L of initial sulfate solution medium over time, representing sulfate removal by Ca-alginate beads at pH 2 (14%), pH 4 (41%), and pH 7.5 (91%). At pH 5.1 (Control) 95% removal was achieved.

In the experiments conducted at pH 2.0, most of the beads were no longer black after 3 days; they turned white transparent (Figure 3.16). This may be an indicator that SRB succumbed due to high acidity. Tukey's pairwise comparison tests indicated significant differences between treatments at pH range 5-7 and those at pH below 4.

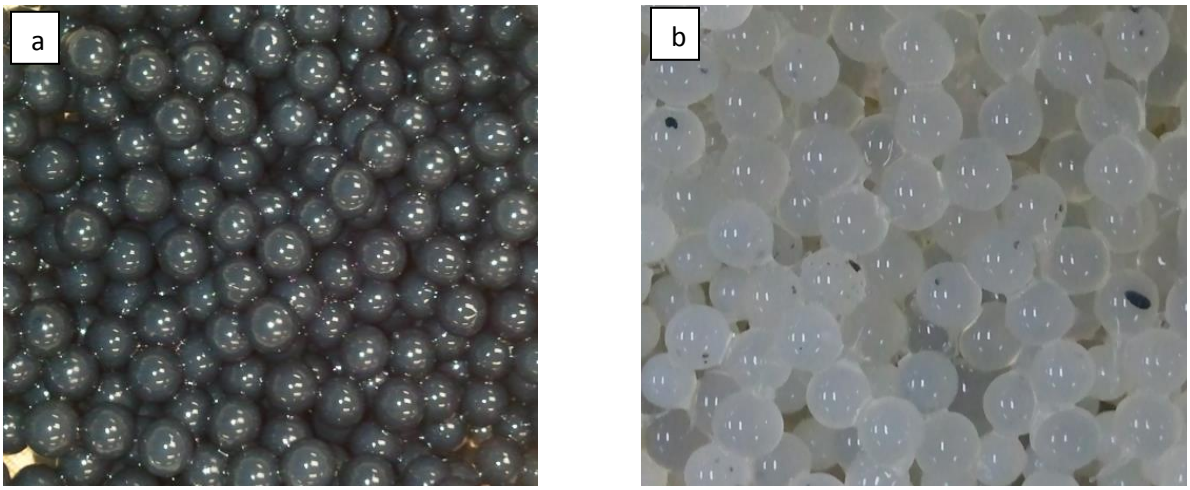

Figure 3.16. Entrapped SRB beads used in pH study. The black color (a) indicated the presence of SRB inside the beads while the disappearance of the blank color (b) indicated the absence (or death) of SRB.

The influence of pH on SRB's ability to remove sulfate from water have been demonstrated by Lu et al. (2011), Costa et al. (2008), and Jong and Parry (2006). Lu and his team (Lu et al. 2011) observed 42% and 31% decrease in sulfate reduction when the initial pH changed from 7.0 to 2.0 and 3.0, respectively. Costa and his team (Costa et al., 2008) did not observe any bacterial activity in their experiments at pH ~2. Jong and Parry (2006) observed 80% sulfate removal at pH 4.0 in an upflow anaerobic packed bed bioreactor (UAPBB) while at pH 3.5, sulfate removal was inhibited due to massive death of the SRB population.

3.9.6. Influence of temperature on sulfate removal by entrapped SRB

Temperature study results (Figure 3.17) indicate lower sulfate removal at 5°C (34%) as compared to 15^{o}C, room temperature (22± 2^{o}C), and 30^{o}C. At 15°C, sulfate removal was slow but 90% removal was achieved at the end of the experiment (15 days). It is thought that entrapped SRB were able to acclimatize to that temperature over time and achieve high sulfate reduction. Sulfate reduction is a highly exothermic reaction (Baker and Banfield, 2003). It is possible that at 15^{o}C, heat generated by sulfate reduction contributed 1) to rapid conversion of carbon source into chemical oxygen demand (COD) and makes them available to SRB and 2) to temperature adjustment to the optimal need of the SRB in the reactor and enabled them to remove sulfate up to 90% by the end of the experiment. Maximum sulfate removal at 30°C was 78% although 70% of the initial sulfate concentration was reduced in 3 days with a sulfate reduction rate of 104.56 mg/L/day). The possible explanation is that sulfate reduction reaction contributed to increasing the heat to the point that it stressed SRB and lowered their performance. However, the best sulfate removal achieved in this study was observed at 22^{o}C. Two-way ANOVA ($p< 0.05$, $n= 72$) was used to analyzed the difference between treatments. The results showed no significant difference between treatments at 15, 22, and 30^{o}C. However, treatment at 5^{o}C was significantly different than the rest.

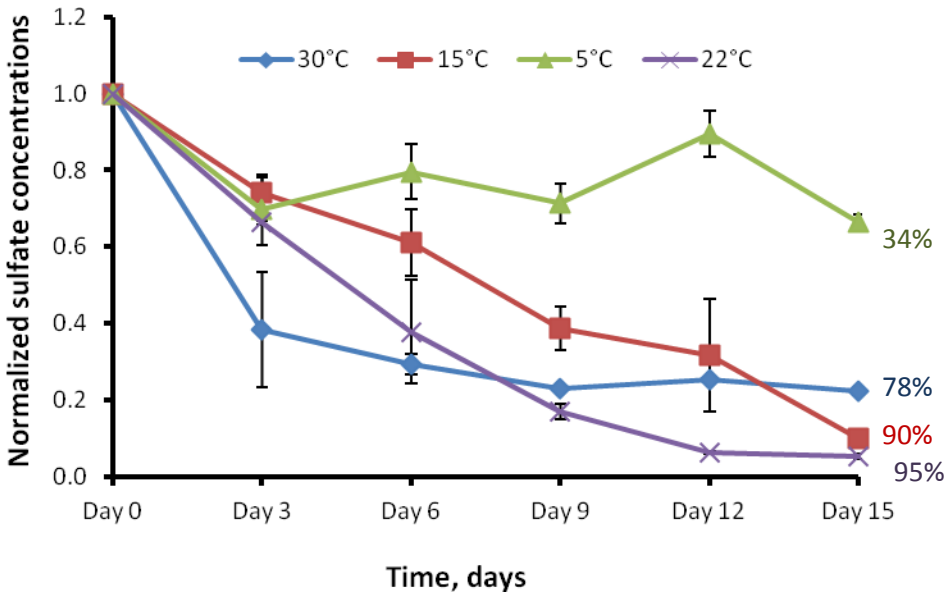

Figure 3.17. Percentage of sulfate concentration removed from 500 mg/L of initial sulfate solution medium over time, representing sulfate removal by SRB entrapped beads at 5°C (34%), 15°C (90%), and 30°C (78%). Sulfate removal at the control temperature (22°C) was 95%.

SRB's response to temperature was investigated by Matsui et al. (2013), Tabuchi et al. (2010), and A-Zuhair et al. (2008). In their work, Matsui and his team (Matsui et al., 2013) conducted studies with SRB in marine sediment and acetate as the carbon source and concluded that minor change in temperature can greatly alter carbon flow affecting SRB performance. Three ranges of temperature were used in their investigations (low: 3°C, Medium: 8 and13°C, and High: 18, 23, and 28°C). At 3°C, no acetate utilization was observed for 197 days and SRB were detected at the end of the experiment. At 8°C and 13°C, the stationary growth phase was considerably longer (80 days at 8°C and 60 days at 13°C) than that observed at higher temperature (18/23/28°C, a brief stationary period). Tabuchi et al. (2010) conducted investigations similar to that of Matsui's team and showed a strong correlation between sulfate reduction rate and changes in temperature. They found that low concentrations of sulfide were produced at lower temperatures (7 and 13°C). At 22°C, sulfide production increased rapidly with

simultaneous dissolved organic carbon decrease. At 29^{o}C, active sulfide production was observed until the complete depletion of sulfate. A-Zuhair et al. (2008) observed a faster sulfate reduction at 35^{o}C as compared to 20 and 50^{o}C. While sulfate reduction observed at 20^{o}C was slightly higher than that at 50^{o}C, no significant difference existed between results obtained at the two temperatures.

3.9.7. Interference of Al, Cu, and Zn on sulfate removal by entrapped SRB

Metal interference investigated in this research indicate strong interferences ($p < 0.05$, $n = 72$) of high concentrations (25-50 mg/L) Al and Zn on sulfate (initial sulfate concentration 500 mg/L in the presence of 1 mg/L ethanol) removal by entrapped SRB. In the presence of 0.5 mg/L Al (Figure 3.18) and Zn ions (Figure 3.19), sulfate reduction occurred effectively and reached 77% and 90%, respectively. When Al was increased to 25 mg/L, 36% sulfate was removed while only 14% removal was observed in the presence of 25 mg/L Zn.

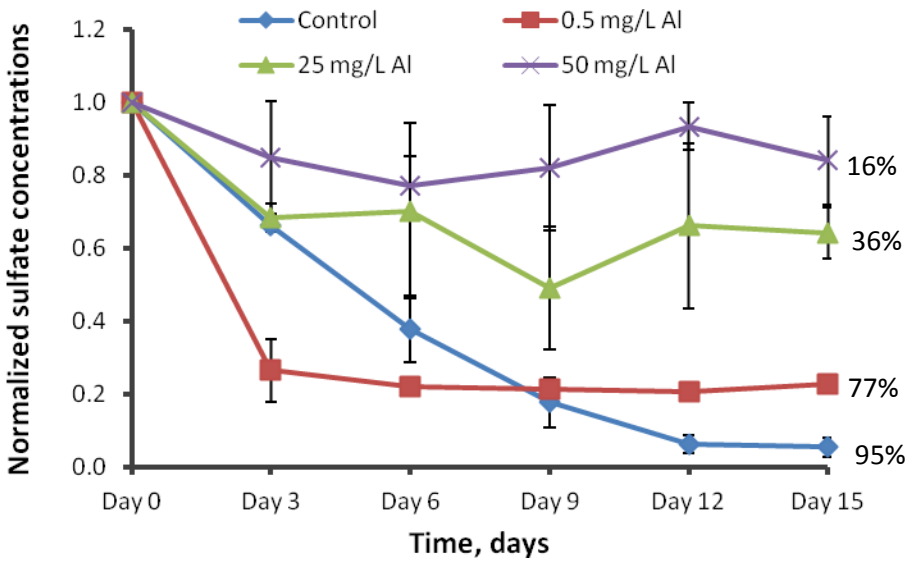

Figure 3.18. Percentage of sulfate concentration removed from 500 mg/L of initial sulfate solution in the presence of Al 0.5 mg/L (77%), 25 mg/L (36%), and 50 mg/L (16%). Sulfate removal in the control experiment (no Al) was 95%.

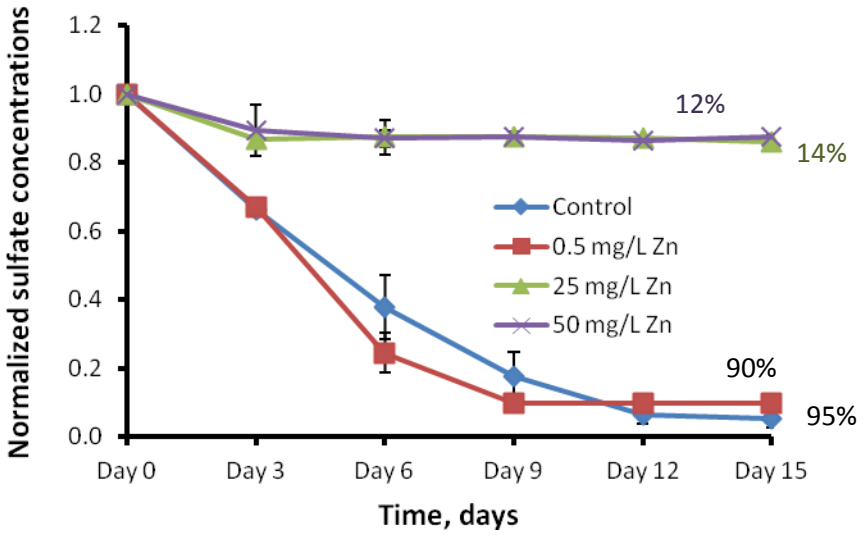

Figure 3.19. Percentage of sulfate concentration removed from 500 mg/L of initial sulfate solution in the presence of Zn 0.5 mg/L (90%), 25 mg/L (14%), and 50 mg/L (12%). Sulfate removal in the control experiment (no Zn) was 95%.

With Al and Zn concentration of 50 mg/L, only 16% and 12% SO_4^{2-} was removed, respectively. Since Al precipitation as $Al(OH)_3$ occurs at pH around 10.5 (Jong and Parry, 2003), while Zn precipitates around neutral pH (4 to 7, Neculita et al., 2007). It is likely that Al could not precipitate in these experiments (pH ranged from 5.1 to 6.8) and remained in soluble form in solution and was toxic to the SRB (discoloration and rapid decomposition of beads were observed). Toxic concentration of Zn range is 13-40 mg/L (Martins et al. 2009). It is possible that entrapment did not protect SRB from Zn toxicity. No major interference was observed in the presence of Cu. About 90%, 87%, and 87% sulfate removal was observed in the presence of 0.5, 25, and 50 mg/L of Cu (Figure 3.20).

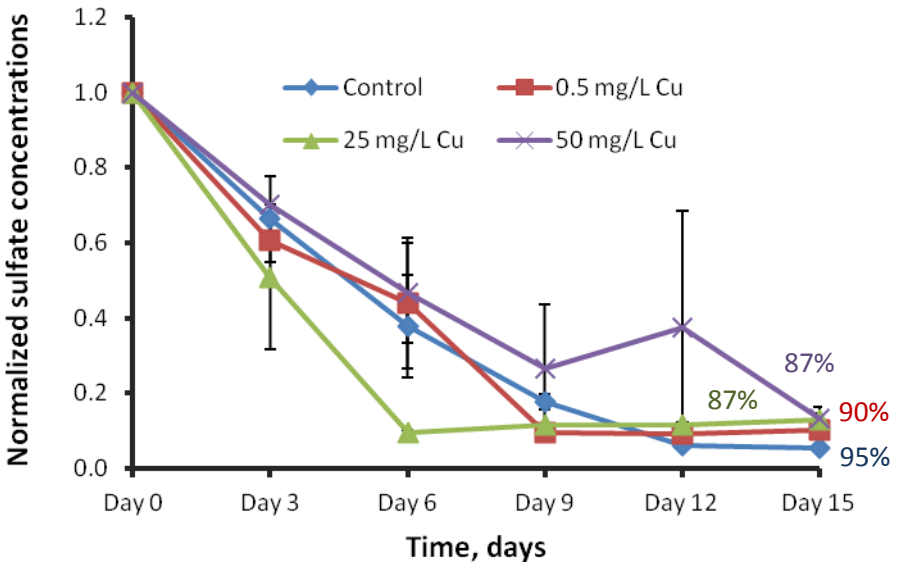

Figure 3.20. Percentage of sulfate concentration removed from 500 mg/L of initial sulfate solution in the presence of Cu 0.5 mg/L (90%), 25 mg/L (87%), and 50 mg/L (87%). Sulfate removal in the control experiment (no Cu) was 95%.

Copper is precipitated as CuS at neutral pH (4 to 7, Neculita et al., 2007). Jalali and Baldwin (1999) demonstrated that SRB were able to grow in the presence of Cu and copper concentration simultaneously reduced from 150 mg/L to 0.1 mg/L in 5 days. Metals toxicity to SRB has been reported by Martins et al. (2012), Wang et al. (2012), and Kieu et al. (2011). The toxic effect of metals is explained by the displacement and substitution of ions on cell walls and functional groups of molecules (enzymes) resulting in their denaturation and inactivation as well disruption of cell integrity and damage to the structure of DNA (Martins et al., 2012; Kieu et al., 2011). Aluminum is among the most abundant metals on earth (Fischer et al. 2002). However, its biological function is not yet reported and it is known to be toxic to many organisms (Hard et al., 2010; Martins et al., 2012; Wang et al., 2012). Al binding to ATP is reported to be 10^{7} times stronger than that of magnesium but its ligand exchange capacity is about 10^{-5} times slower than that of magnesium causing inhibition to magnesium dependent enzymes and ligands (Fischer et

al., 2002). Zinc is also reported to be toxic to SRB with an estimated toxic concentration of 13 - 40 mg/L of zinc. Copper toxicity on bacteria was observed at 2 mg/L to 10 mg/L (Martins et al., 2009). Martin and his team (Martins et al., 2009) reported that sulfate reduction was inhibited in the presence of Cu and Zn at two different AMD affected sites. However, in the third site of their study, sulfate was completely removed after 110 days even in the presence of Zn and Cu, and they (Martins et al., 2009) observed that 80 mg/L Cu were completely precipitated after 15 days of experiments while zinc was reduced from 150 mg/L to 2.0 mg/L within 18 days. Cu was removed first followed by Zn removal. Martins and his team explained this by the solubility product of Cu and Zn. Solubility product (at 25^{o}C) is $10^{-35.1}$ and $10^{-24.5}$ for CuS and ZnS, respectively (Martins et al., 2009; Jameson et al., 2010). Therefore, Cu precipitation requires only low concentrations of sulfide.

3.10. Conclusions

Sulfate removal was investigated in this set of experiments using free cells of sulfate reducing bacteria (SRB) in columns, and entrapped SRB cells in batch experiments. SRB cultures were successfully isolated from activated sludge collected from a municipal wastewater treatment facility. In column studies, 88% SO_4^{2-} removal was achieved by free cells of SRB within 9 days. Entrapped SRB in Ca-alginate beads were successful in removing SO_4^{2-} in batch studies. Removal of SO_4^{2-} by entrapped SRB (with initial SO_4^{2-} concentration of 250 mg/L) in the presence of ethanol as the carbon source was 91 and for lactose was 76%. In the no carbon (entrapped SRB but no carbon) treatment, removal of SO_4^{2-} was 57% and for the control (Ca-alginate beads only) was 22%. A similar trend was observed when initial SO_4^{2-} concentration was 500 mg/L. About 95, 88, 51, and 34% SO_4^{2-} were removed, respectively, in ethanol, lactose, control, and blank treatments. These results show that entrapment of SRB can be a promising

technique for sulfate remediation below regulatory limits of 250 mg/L for drinking water (WHO, 2004) and 575 mg/L for irrigation water (Costa et al., 2008). Such system can be proposed in the form of permeable reactive barriers (PRB) or treatment filter in industries, including mining industry for sulfate remediation.

Interference studies showed that low pH adversely affects SO_4^{2-} removal by entrapped SRB. Only14 and 41% SO_4^{2-} removal was achieved at pH 2.0 and 4.0, respectively but 91% SO_4^{2-} was removed at pH 7.5. Moreover, massive death of SRB was observed at pH 2.0. Low temperature also negatively affected SO_4^{2-} reduction by entrapped SRB with only 34% SO_4^{2-} removal at 5^{o}C as compared to 90 and 78% at 15^{o}C and 30^{o}C, respectively. Among the three metals tested, Al and Zn interfered with sulfate removal and reduced the removal efficiency to 36 and 14% when 25 mg/L of Al or Zn, respectively, was present. Only 16 and 12% SO_4^{2-} were removed with 50 mg/L Al or Zn, respectively. Cu on the other hand did not show major interferences and 90, 87, and 87% SO_4^{2-} was removed in the presence of 0.5, 25, and 50 mg/L Cu, respectively. It is interesting to observe that SO_4^{2-} reduction was achieved in all cases with exception for the experiments at pH 2.0 and with 50 mg/L Al and Zn where the nominal SO_4^{2-} removal was most likely due to physical absorption by Ca-alginate beads as observed in the control experiment conducted with blank Ca-alginate beads (22% SO_4^{2-} removal was achieved). These results suggest that entrapment in Ca-alginate does not protect SRB from stresses caused by low pH (≤ 4), low temperature (5^{o}C), and high concentrations (25-50 mg/L) of toxic metals (Al and Zn).

Further investigations are needed to improve entrapped SRB's ability for sulfate removal and metals under extreme conditions (of AMD) and to understand the mechanisms involved.

4. SULFATE REMOVAL BY SULFATE REDUCING BACTERIA IN THE PRESENCE OF NANOSCALE ZERO-VALENT IRON

4.1. Abstract

Nanoscale Zero-valent iron (NZVI) was used to test the enhancement of sulfate removal by entrapped sulfate reducing bacteria (SRB). NZVI particles (0.05, 0.1, and 0.2 g) were co-entrapped in Ca-alginate with 3.0 g (wet weight) SRB. Sulfate removal batch studies were conducted with SRB co-entrapped with NZVI (CoSRB-NZVI) and the results were compared with sulfate removal data from batch studies conducted with entrapped SRB (EntSRB) and SRB and NZVI entrapped separately (SepSRB-NZVI). The results show that all three concentrations of NZVI in CoSRB-NZVI have negative effects on SRB. Loading of 0.2 g of NZVI in CoSRB-NZVI caused massive death to SRB population during the synthesis process itself. Sulfate removal experiment could not be conducted with these beads. About 11 and 12% lower sulfate removal by CoSRB-NZVI (with 0.05 and 0.1 g NZVI) system was observed as compared to the control (EntSRB). When NZVI and SRB were entrapped in separate beads (SepSRB-NZVI) and used in the same reactor, sulfate removal rate improved (34.53 mg/L/d) between Day 3 and Day 12 as compared to 14.34 mg/L/d and 16.76 mg/L/d for 0.05 g CoSRB-NZVI and 0.1 g CoSRB-NZVI, respectively. However, final sulfate removal was the same, 83%, 83%, and 84% for SepSRB-NZVI, 0.05 g CoSRB-NZVI, and 0.1 g CoSRB-NZVI, respectively. These sulfate removal percentages (83-84%) did not reach the removal efficiency obtained with EntSRB (95%).

4.2. Supplies for nanoscale zero-valent iron

Sodium borohydride ($NaBH_4$, ≥98.0%, VWR), sodium hydroxide (NaOH, ACS Reagent Grade, 98%, VWR), ferrous sulfate heptahydrated ($FeSO_4.7H_2O$, ACS AR, 99.5%, VWR), and

ethanol (95.0%, BDH Grade, VWR) were used for synthesizing nanoscale zero-valent iron (NZVI) particles.

4.3. Methods

4.3.1. NZVI synthesis

NZVI was synthesized following the method reported by others (Liu and Lowry, 2006; Bezbaruah et al., 2009a; Bezbaruah et al., 2011; Krajangpan et al., 2012; Ameelbi and Bezbaruah, 2012) The method is based on reduction of ferrous iron by sodium borohydride (Eq. 4.1). Solution A (30.0% of ethanol solution) was prepared by diluting 30.0 mL of ethanol (95.0%) in 70.0 mL of DI water. Solution B (5 N of sodium hydroxide) was prepared by dissolving 20.0 g of NaOH in 100 mL of DI water. Solution C (2.1 M of sodium borohydride) was prepared by adding 7.9443 g of $NaBH_4$ and 100 mL of DI water.

$$2Fe^{2+} + BH_4^- + 3H_2O \rightarrow 2Fe^0\downarrow + H_2BO_3^- + 4H^+ + 2H_2 \quad (4.1)$$

Iron sulfate ($FeSO_4.7H_2O$, 10.0 g) was mixed with Solution A while stirring it until complete dissolution of the iron sulfate. The pH of the mixture was adjusted to 6.1 by adding Solution B drop wise. As Solution B was added, the mixed solution turned black. Then Solution C was added slowly to the black solution using a burette, and nanoscale zero-valent iron (NZVI) particles were formed and they precipitated out at this stage. The resultant precipitates were centrifuged (7,000 rpm) and washed with ethanol and deoxygenated DI water prior to drying the particles in the vacuum oven under nitrogen environment for 48 h. The dry particles were weighed and entrapped in Ca-alginate (Figure 4.1).

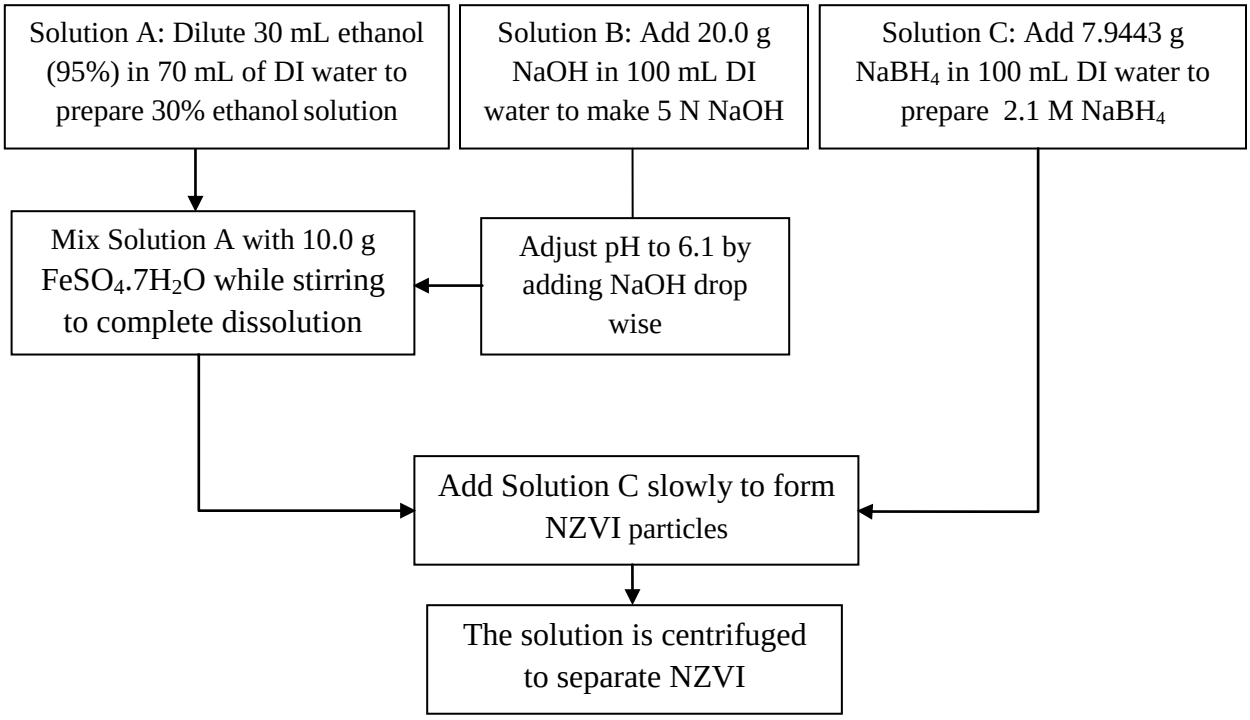

Figure 4.1. Schematic of NZVI preparation process. NZVI was prepared following the reduction of ferrous iron by sodium borohydride at pH 6.1.

4.3.2. Experimental set-up

Sulfate removal was carried out in the presence of nanoscale zero-valent iron (NZVI). The aim of these experiments was to use NZVI to enhance sulfate reduction by entrapped SRB. NZVI acts as electron donor for sulfate reduction in the presence of hydrogen utilizing sulfate reducing bacteria. Bacterial cells and NZVI was entrapped together (CoSRB-NZVI) in Ca-alginate beads. All sulfate removal batch experiments were carried out using 60 mL transparent (clear) glass vials or reactors (Figure 4.2). A batch of CoNZVI-SRB beads were used in the reactors with 50.0 mL of sulfate solution (500 mg/L SO_4^{2-}). Carbon source (750 mg/L ethanol) and 500 µL of trace elements were also added. The reactors were placed in the Incu-Shaker mini at 30°C and 100 rpm.

Figure 4.2. SepSRB-NZVI test reactor (Left: Actual reactor; Right: Schematic). NZVI particles (0.05 g) were entrapped in white colored beads and SRB were entrapped in the black colored beads.

The experiments were performed using the following process: SRB were incubated in ATCC 1249 media for 6 days and the incubated media was centrifuged at 7,000 rpm for 10 min to allow the biomass to settle. Once settled, 3.0 g of the wet weight of the biomass collected and thoroughly mixed with NZVI (0.05, 0.1, and 0.2 g), and 300 mL of Na-alginate. The mixture was dropped into 2% $CaCl_2$ solution to synthesize CoSRB-NZVI beads, and the beads were allowed to harden in 2% $CaCl_2$ solution for 9 h (Bezbaruah et al., 2009a). The hardened beads were washed with DI water and used in the 40 mL amber glass vials (1 batch per reactor). Sulfate solution was prepared using Na_2SO_4, and the carbon source and traces elements solution were added to it. This solution was added to the reactors and the experiments were conducted simultaneously with CoSRB-NZVI. The SepSRB-NZVI experiments were conducted after analyze of the CoSRB-NZVI results and aimed to verify those results. The reactors were then placed in the shaker and sacrificial reactors were taken at predetermined interval (day 0, 3, 6, 9,

12, and 15) and samples were collected for sulfate concentration determination. The initial pH of the sulfate solution was 5.1 and increased to 6.8 at the end of the experiment on day 15.

4.3.3. Co-entrapment of SRB and NZVI (CoSRB-NZVI)

Three different loadings of NVZI (0.05, 0.1, and 0.2 g) were mixed with SRB (3.0 g wet weight) and 300 mL of Na-alginate (2.0%) to synthesize co-entrapped SRB-NZVI (CoSRB-NZVI) beads using the same process described in Chapter 3 (Section 3.2.3). The synthesized beads were allowed to harden in calcium chloride solution for 9 h (Bezbaruah et al. 2009a) prior to washing them with copious amount of deoxygenated DI water. A total of 54 sacrificial reactors were used here.

4.3.4. Separate entrapment of SRB and NZVI (SepSRB-NZVI)

NZVI (0.05 g) and SRB (3.0 g of wet biomass) were also entrapped separately in 2% Ca-alginate beads. The beads were kept in $CaCl_2$ solution for 9 h (Bezbaruah et al. 2009a) then washed with copious amount of deoxygenated DI water. They were then used in the same reactors (mixed together) for sulfate removal studies. Sample size was 36 in this experiment.

4.4. Results and discussions

4.4.1. Characterization of NZVI

The synthesized NZVI appeared as black fine powder (Figure 4.3). Almeelbi and Bezbaruah (2012) reported that NZVI particles were spherical in shape with particles size between 10 to 30 nm. The average size of their particles was 16.24± 4.05 nm and the specific surface area was reported as 25 m^2/g. Fe^0 was mostly predominant in the synthesized particles (Almeelbi and Bezbaruah, 2012). A fine layer of oxide shell was formed around the particles and that protected the particles from rapid oxidation (Ryu et al. 2011; Krajangpan et al. 2012).

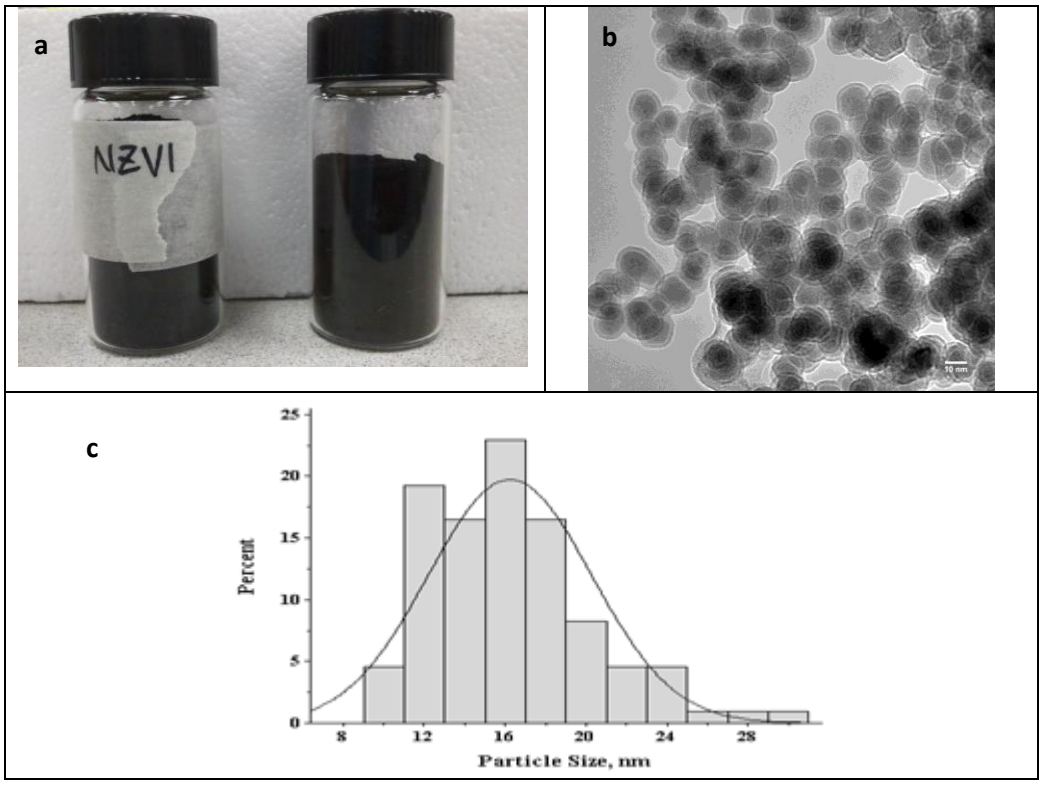

Figure 4.3. (a) Dried NZVI particles, **(b)** Transmission electron microcopy image of synthesized NZVI particles, **(c)**: Particles size distribution of synthesized NZVI (after Almeelbi and Bezbaruah, 2012).

4.4.2. Co-entrapped SRB-NZVI (CoSRB-NZVI) beads

Co entrapped SRB-NZVI beads were successfully prepared in the laboratory. They were spherical in shape, black in color, soft, and fragile. After 9 h residency in 2% $CaCl_2$ solution, they become hard with an average bead size of 4± 1.5 mm (n = 60). No particular modification was observed in the shape of 0.05 g CoSRB-NZV beads (Figure 4.4). However, 0.1 g CoSRB-NZVI beads were spherical in shape and, had a tail attached to each bead (Figure 4.5). The possible explanation for the appearance of the tail can be linked to the interaction between the weight inside the bead (SRB suspended cells and NZVI particles) and the viscosity of the alginate solution. A rapid change in the bead color was observed with NZVI loading of 0.2 g during the synthesis process. The beads became translucent with white to reddish color

indicating oxidation of the NZVI particles and absence of black colored SRB cells (Figure 4.6b). It is likely that NZVI caused a serious damage to SRB cells in the beads due to a combination of NZVI toxicity and stress due to exposure to oxygen (Neculita et al. 2007).

Figure 4.4. Freshly **s**ynthesized CoSRB-NZVI beads. The beads were black in color and spherical in shape with average diameter of 4± 1.5 mm (n = 60).

Figure 4.5. Synthesized CoNZVI-SRB beads after 9 h residency in 2% $CaCl_2$. The average bead size was 4± 1.5 mm (n = 60). A tail is visible on each bead. The scale of reference here is a centimeter scale with each small unit representing 1 mm. The beads have a tail; it is more likely that this resulted from the interaction between the weight inside the beads and the thickness of the alginate solution.

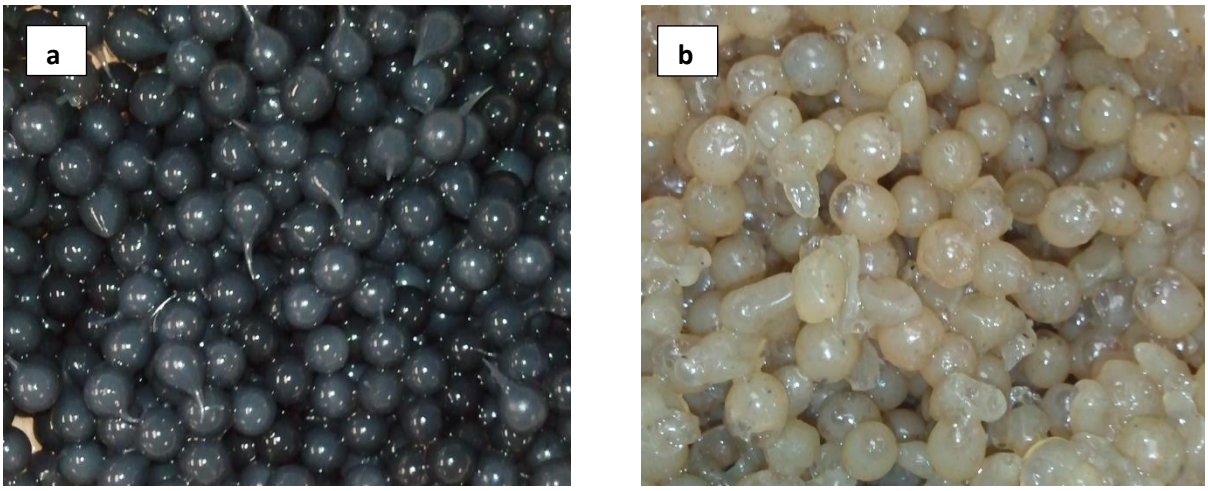

Figure 4.6. (a) Freshly **s**ynthesized 0.1 g co-entrapped SRB-NZVI beads with a tail attached to them. **(b)** Oxidized 0.2 g CoSRB-NZVI beads after synthesis.

4.4.3. Sulfate removal by CoSRB-NZVI

In co-entrapment experiments, three different loadings of NZVI (0.05, 0.1, and 0.2 g) were used. Ethanol (750 mg/L) was used as the carbon source. However, only results from 0.05 g and 0.1 g of CoSRB-NZVI are reported here as 0.2 g CoSRB-NZVI apparently killed all SRB (see Section 4.4.2). With 0.05 g CoSBR-NZVI, the maximum sulfate removal achieved in 15 days was 83%. For 0.1 g CoSBR-NZVI, 84% sulfate removal was achieved during the same period (Figure 4.7).

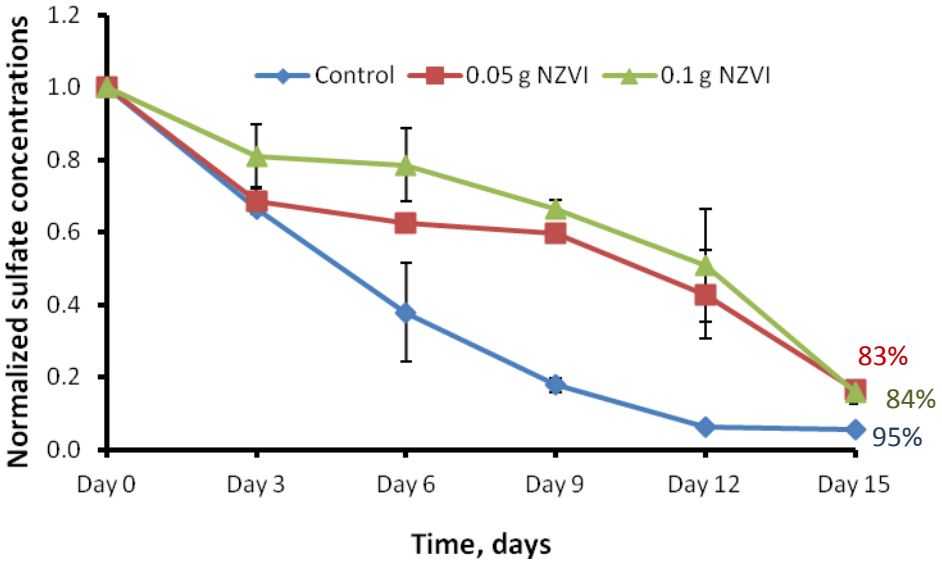

Figure 4.7. Sulfate removal over time by co-entrapped SRB-NZVI beads (CoSRB-NZVI). 83 and 84% SO_4^{2-} removal were achieved in the presence of 0.05 and 0.1 g CoSRB-NZVI, respectively. The control here is SRB entrapped in Ca-alginate (EntSRB) with 95% SO_4^{2-} removal.

It was hypothesized (Section 2.6, Chapter 2) that NZVI will enhance sulfate removal when entrapped with SRB. However, sulfate removal by CoSRB-NZVI was slightly lower than percentage sulfate removal by entrapped SRB (EntSRB, no NZVI). While EntSRB removed 95% of sulfate in 15 days, CoSRB-NZVI removed 83 and 84%, respectively when 0.05 and 0.1 g NZVI was used. One-way ANOVA indicated a difference between the CoSRB-NZVI and EntSRB treatments ($p = 0.035$, $n = 54$). Furthermore, Tukey's pairwise comparison test (95% Confidence Level) showed a significant difference between EntSRB and 0.1 g CoSRB-NZVI. The difference between 0.05 g CoSRB-NZVI and EntSRB treatments was not significant. These results indicate that NZVI have some negative effects on SRB when they are entrapped together and a slow sulfate removal was observed. This was confirmed when NZVI loading was increased

at 0.2 g and that led to massive destruction of the bacterial population during the entrapment process itself.

4.4.4. Sulfate removal by SepSRB-NZVI

The main objective of this experiment was to evaluate the potential for NZVI to improve sulfate reduction by entrapped SRB. Since co-entrapment did not improve sulfate reduction by entrapped SRB. NZVI particles seem to have negative effects on SRB in co-entrapment. It was thought that NZVI will improve sulfate reduction when entrapped separately with sulfate reducing bacteria (SRB). A loading of 0.05 g NZVI particles were entrapped separately and used with entrapped SRB in the batch reactors for sulfate reduction experiments. Sulfate reduction (Figure 4.8) was found to be similar to values observed with CoSRB-NVZI beads at the end of the 15-days experiment (83, 83, and 84% sulfate removal for SepSRB-NZVI, 0.05 g CoSRB-NZVI, and 0.1 g CoSRB-NZVI, respectively).

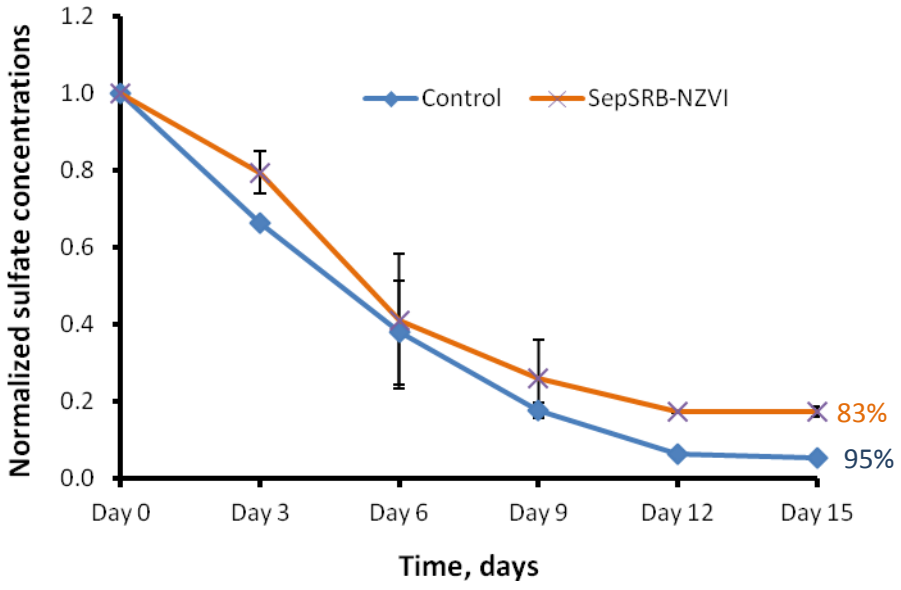

Figure 4.8. Sulfate removal overtime by separately entrapped SRB-NZVI beads. 0.05 g SepSRB-NZVI loading was used. Overall SO_4^{2-} removal by SepSRB-NZVI was 83%. The control here is only SRB entrapped in Ca-alginate (EntSRB) which removed 95% SO_4^{2-}.

However, it is important to note that sulfate reduction rate was accelerated between Day 3 and Day 12 (34.53 mg/L/d) for SepSRB-NZVI) as compared to CoSRB-NZVI (14.34 mg/L/d and 16.76 mg/L/d for 0.05 g CoSRB-NZVI and 0.1 g CoSRB-NZVI, respectively). Sulfate removal achieved in both co-entrapment and separate entrapment was below that of EntSRB (95%, control in Figure 4.9).

These results are in contrast with data obtained by Xin et al. (2008) where they observed an acceleration of sulfate removal (13-22% more) by SRB in the presence of zero-valent iron (ZVI). Moreover, they observed 77% increase in sulfate removal ratio and 93% increase of the reaction rate in the presence of ZVI (pH 7.0 and 25^{o}C). However, when the temperature was increased to 36^{o}C and pH maintained at 7.0, the addition of ZVI did not improve SRB's performance. At 15^{o}C (pH = 7.0), no sulfate removal was observed in the system. The addition of over 2 g/L of ZVI induced a slow sulfate removal after 136 h lag time. When ZVI was increased at 8 g/L, 95% sulfate was removed in 50 h. In fact, Fe^{o} provides hydrogen which acts as electron donor for autotrophic SRB. The work by Xin et al. (2008) used SRB in the presence of non-nano zero-valent iron (not NZVI). This, however, is possible as the possible toxicity of NZVI might have affected the SRB in this research.

Addition of NZVI did not improve sulfate removal by co-entrapped SRB-NZVI (CoSRB-NZVI) possibly because of the toxic effects of NZVI to SRB. NZVI toxicity on bacteria has been reported by others. Lee et al. (2008) investigated *E. coli* inactivation by NZVI and conclude that this inactivation is dose dependent. They observed 2.6 and 3.6 log reduction (inactivation) of *E. coli* population after 60 min exposure to 90 mg/L NZVI under aerobic saturated conditions. In deoxygenated conditions, 3.4 log reduction of the bacterial population was observed after 10 min in the presence of only 9 mg/L NZVI. Li et al. (2010) also observed 2.2 and 5.2 log reduction in

viable *E. coli* cells exposed to 100 mg/L of NZVI in aerobic conditions) after 10 min and 1 h, respectively. Bactericidal effect of NZVI particles is attributed to small particle size, high reactive surface area, and the generation of reactive oxygen species (ROS) during oxidation of Fe^{o} (Eqs. 4.2 and 4.3 Jiang et al., 2013; Li et al. 2010). NZVI produced by borohydride reduction of dissolved iron contains up to 98 wt% Fe^{o} (compared to 20-28 wt% for NZVI obtained by iron oxide reduction by H_2 gas) (Li et al. 2010) making this type of NZVI particles (produced by borohydride reduction) more toxic to bacteria as they generate more ROS. Auffan et al (2008) observed cytotoxicity of NZVI on *E. coli* at concentrations of 70 mg/L. They also observed morphological changes in the shape of E. coli due to the presence of NZVI in the cell walls. Lee and his team observed black spots inside *E. coli* cell wall and within the cytoplasm which indicated that the NZVI particles passed through the cells causing disruption of cell membranes.

$$Fe^{2+} + H_2O_2 + H+ \rightarrow Fe^{3+} + HO\bullet + H_2O \quad (4.2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^{+} \quad (4.3)$$

With separate entrapment (SepSRB-NZVI), the results were quite similar, although sulfate removal was accelerated in between Days 3 and 12 as opposed to the observations with CoSRB-NZVI. However, it is important to note that in all cases, effective sulfate removal was achieved. NZVI particles have high reduction potential (Bai et al., 2012) and have been successfully used to remove inorganics, heavy metals and metalloids from contaminated water and soil. Yan et al. (2012) reported that arsenic was reduced due to the formation of an intermetallic Fe-As phase after 22 h of As exposure to 5 g/L NZVI. Almeelbi and Bezbaruah (2012) observed a rapid phosphate (initial concentration up to 10 PO_4^{3-}-P/L) removal in the

presence of 400 mg/L NZVI. They achieved 88-95% of phosphate removal within the first 10 min of the beginning of the experiment. Kanel et al. (2007) used NZVI to remove As (III) from water and achieved 100% removal of 200, 500, and 1,000 µg/L of initial arsenic in 9, 8, and 4 days, respectively. There are also reports of Cd, Cr, and Pb (Huang et al., 2013), Cu, Pb, and Zn (Roy and Bhattacharya, 2012), and Se (Zelmanov and Semiat, 2013) removal by NZVI. Based on the existing literature it is safe to say that SepSRB-NZVI system can possibly be used for the removal toxic metals present in AMD.

4.5. Conclusions

The results from this set experiments indicated that nanoscale zero-valent iron (NZVI) adversely affected sulfate removal by sulfate reducing bacteria (SRB) when SRB was co-entrapped with NZVI (CoSRB-NZVI). While only SRB entrapped in Ca-alginate (EntSRB) removed 95% of sulfate in 15 days, CoSRB-NZVI removed 83 and 84%, respectively when 0.05 and 0.1 g NZVI was used. The difference in sulfate removal by EntSRB and CoSRB-NZVI with 0.1 g NZVI was statistically significant indicating the NZVI adversely affected the SRB. Again the adverse effect of NZVI on SRB was visually apparent when 0.2 g NZVI was co-entrapped with SRB; the CoSRB-NZVI beads were translucent with no characteristic black color of SRB. It is possible that the high amount of NZVI (0.2 g) killed all the SRB present in the CoSRB-NZVI beads. When SRB and NZVI were entrapped separately (SepSRB-NZVI) and used for sulfate reduction in the same reactor, the percent sulfate removal was found to be similar to that achieved with CoSRB-NZVI system. While 83% sulfate removal was achieved by SepSRB-NZVI, 83%, and 84% sulfate removal was achieved by CoSRB-NZVI with 0.05 g and 0.1 g NZVI, respectively. However, a higher sulfate reduction rate was observed between Day 3 and Day 12 (34.53 mg/L/d) for SepSRB-NZVI) as compared to CoSRB-NZVI (14.34 mg/L/d and

16.76 mg/L/d for 0.05 g CoSRB-NZVI and 0.1 g CoSRB-NZVI, respectively) indicating that NZVI, when not entrapped together, might have some positive impact on SRB. However, further studies will be needed to validate this observation and understand the mechanism for the observed accelerated sulfate removal rate.

5. OVERALL CONCLUSIONS

Biological sulfate removal was investigated in this research using calcium (Ca) alginate entrapped sulfate reducing bacteria (SRB). SRB cultures used in this experiment were isolated from activated sludge collected from the city of Moorhead (Minnesota) wastewater treatment facility. The bacterial culture was successfully incubated using ATCC 1249 media.

Ca-alginate was successfully used as the entrapment media for SRB. Starting with an initial sulfate concentration of 500 mg/L, 95% sulfate removal was achieved in 15 days by entrapped SRB when supplied with ethanol as the carbon source. The sulfate removal was 88% with the SRB beads supplied with lactose as the carbon source as compared to 51% sulfate removal for the control where no carbon was supplied. Two-way ANOVA analysis indicated that there is a difference between the treatments with and without a carbon source present ($p < 0.05$, $n = 90$). It was confirmed via Tukey's pairwise comparison test that this difference is significant between sulfate treatments by SRB with a carbon source supplied and that without a carbon source. However, no significant difference was detected between the treatment with the two carbon sources which suggests that both ethanol and lactose can be effectively used by SRB as carbon sources. The results from this research suggest that entrapped SRB can be considered as a promising sulfate abatement technology. In a typical acid mine drainage (AMD) or an industrial wastewater organic matter is not present in sufficient quantities to support bacterial activities and it would be necessary supply an external carbon source. It is felt that ethanol is a good carbon source given the fact that it is a simple straight chain organic compound (and, hence, easy for biodegradation) and its production is relatively easy as most plant-based biomass can be fermented to yield ethanol.

Interference studies conducted with 500 mg/L initial sulfate concentration indicated that high concentrations of aluminum (Al) and zinc (Zn) interfered with sulfate removal by entrapped SRB while copper (Cu) had no apparent impact. Only 16% and 12% of sulfate were removed in the presence of 50 mg/L Al and Zn, respectively. When the metal ion concentrations were decreased to 25 mg/L, the sulfate removal improved to 36% in the presence of Al but there was no major improvement (only 14% removal) with Zn present in the solution. However, in the presence of 0.5 mg/L Al and Zn ions sulfate reduction occurred effectively and reached 77% and 90%, respectively. No major interference was observed in the presence of Cu. About 90, 87, and 87% sulfate removal was observed in the presence of 0.5, 25, and 50 mg/L of Cu, respectively. It is felt that toxic metal removal from AMD will be necessary before entrapped SRB can be used for sulfate removal.

This study also investigated the influence of environmental parameters such as pH and temperature on sulfate removal by entrapped SRB. The experiment showed that sulfate reduction decreased with the reduction in pH of the sulfate solution (i.e., increased acidic conditions). Sulfate (500 mg/L initial concentration) reduction achieved at pH 7.5 (91%) was 2 times higher than that achieved at pH 4.0 (41%) and 6.4 times higher than that of pH 2.0 (14%). The typical pH value of AMD is known to be < 4 (Table 1.1) and it will, therefore, be necessary to adjust the pH more towards neutral before entrapped SRB can be used for sulfate removal from AMD. Such a pH adjustment will also help in the reduction in the concentration of toxic metals as metals are less soluble in higher pH (than in acid pH of AMD).

Low temperature also affected sulfate removal by entrapped SRB. At 5°C, only 34% of the initial sulfate was removed. SRB played nominal role in this removal as most of the sulfate was sorbed by the entrapment matrix (Ca-alginate). SRB activity was initially slow at 15°C, and

then apparently after some self-adaptations, they were able to remove sulfate (90% removal) better than they did at 30°C (78%) over a 15-day period. Sulfate removal by SRB is known to be an exothermic reaction and it is possible that the heat generated in the reaction was entrapped within the Ca-alginate beads for long enough time for the microorganisms to use them to their advantage. It is significant that the entrapped SRB worked efficiently at 15°C and that puts entrapped SRB matrix as a possible candidate for sulfate removal from in typical surface flow AMD waters.

Nanoscale zero-valent iron (NZVI), being a known electron donor, was expected to enhance biological sulfate reduction by entrapped SRB. However, NZVI appeared to have negative effects on SRB when entrapped together in the same bead. Loading of 0.05 and 0.1 g of NZVI led to lower sulfate removal (83 and 84%, respectively) as compared to that obtained with only entrapped SRB (no NZVI, 95% sulfate removal). Moreover, 0.2 g of NZVI totally destroyed the bacteria population during the entrapment process itself. When NZVI and SRB were entrapped separately in different beads, sulfate reduction rate increased (34.53 mg/L/d) from day 3 to day 12. Maximum sulfate removal achieved (83% removal) was similar to that of co-entrapment of SRB and NZVI (83 and 84% for 0.05g and 0.1 g CoSRB-NZVI, respectively). These percentages were lower than that of entrapped SRB only. The results from this research indicate no major advantage of using NZVI for sulfate removal.

Results from this study indicate that entrapment of sulfate reducing bacteria (SRB) in Ca-alginate is a promising technique for aqueous sulfate removal. Entrapped SRB were able to reduce sulfate concentration through anaerobic respiration, indicating that entrapment in calcium alginate beads does not impact SRB's ability to remediation sulfate. This study can be extended to investigate multiple re-use of entrapped SRB for sulfate removal. It is expected that re-using

entrapped SRB will help achieving better sulfate removal. This can be performed in a multistep system where a pre-treatment will be required to increase the pH of targeted sulfate contaminated water to pH around 7 prior to conduct sulfate reduction by SRB entrapped in Ca-alginate beads placed in an engineered treatment system (treatment filter or permeable reactive barrier, PRB). The lifespan of the remediation can be reduced when the pH is near neutral (70% of initial sulfate concentration was removed in the first 3 to 6 days of these experiments). With further research, the technique can be developed as a cost-effective abatement option to remediate sulfate present in acid mine drainage (AMD) and industrial wastewaters.

6. FUTURE WORK

The current research on sulfate removal using entrapped SRB was carried out by conducting only batch experiments. It will be important to conduct continuous flow studies for an extended period of time to understand the system kinetics. It will also be prudent to use actual acid mine drainage water to simulate real world conditions.

It was also known that a carbon source is necessary as the electron donor for sulfate reduction by SRB. Effective removal of sulfate by entrapped SRB suggests organic substrate (ethanol and lactose) could easily diffuse through the beads. It will be interesting to investigate other organic carbon sources including more commonly available carbon sources like animal manure, wood chips, grass clippings, compost, and municipal wastewater.

It would contribute the body of knowledge if total organic carbon (TOC) is monitored during sulfate reduction. TOC can be very easily used as a design parameter in the engineering design of SRB systems.

Entrapped SRB behaved differently than free cells at 15°C. Very high removal of sulfate was achieved with entrapped SRB at 15°C as compared to 4°C and 30°C. However, this research could not investigate the mechanism involved in the process even though it was thought that entrapment of heat (from exothermic sulfate reduction reaction) might have facilitated the sulfate removal process. Further investigations are needed in this area.

The present experiments were conducted for a limited period of time, and it felt that conducting sulfate remediation experiments for a longer period to investigate biodegradation of calcium alginate used in the beads would be very useful to evaluate environmental compatibility of this entrapment technology. Such long duration experiments will also throw light on preference of SRB for Ca-alginate as a carbon source.

Nanoscale zero-valent iron (NZVI) was used to improve sulfate reduction by SRB. However, this expectation was not realized. In contrast, NZVI appeared to be toxic to SRB in co-entrapped beads. When NZVI and SRB were entrapped in separate beads but used together, the result was similar to that obtained with only entrapped SRB (without NZVI). Nevertheless, this SRB-NZVI relationship needs to be investigated further by varying the NZVI loading. NZVI is known to remove heavy metal very effectively. So, it would be wise to investgate whether NZVI can be used alongside entrapped SRB to removal metal from AMD.

Further, only Ca-alginate was tried as the entrapment matrix, and it would interesting to investigate other biopolymers for entrapment applications. More specifically, it would benefit communities around mining sites if locally available raw materials can be used for biopolymer synthesis for use in entrapment.

REFERENCES

Ahmad, P.Y.A. and Kunhi, A.A.M. (2011). Enhanced degradation of phenol by Pseudomonas sp. CP4 entrapped in agar and calcium alginate beads in batch and continuous processes. *Biodegradation*, 22: 253-265

Ahmed, Md. K., Al-Mamun, Md. H., Parvin, E., Akter, M. S., and Khan, M.S. (2011). Arsenic induces toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (Oreochromis mossambicus). *Experimental and Toxicologic Pathology*, xxx: xxx-xxx

Akai, J. and Anawar, Md. H. (2013). Mineralogical approach in elucidation of contamination mechanism for toxic trace elements in the environment: Special reference to arsenic contamination in groundwater. *Physics and Chemistry of the Earth*, 58-60: 2-12

Almeelbi, T. and Bezbaruah, A.N. (2012). Aqueous phosphate removal using nanoscale zero-valent iron. *J Nanopart Res*, 14: 900

Amos, P.W. and Younger, P.L. (2003). Substrate characterization for a subsurface reactive barrier to treat colliery spoil leachate. *Water Research*, 37: 28-33

Al-Zuhair, S., El-Naas, M.H., and Al-Hassani, H. (2008). Sulfate inhibition effect on sulfate reducing bacteria. *J Biochem Tech*, 1(2): 39-44

Ariga, K., Ishihara, S., Abe, H., Li, M., and Hill, J.P. (2012). Materials nanoarchitectonics for environmental remediation and sensing. *J. Mater. Chem.*, 22: 2369-2377

ASTM (2010). Standard Test Method for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits. *Annual book of ASTM Standards*, Section 11, Volume 11.02, 523-525 pp

Baker, B.J. and Banfield, J.F. (2003). Microbial communities in acid mine drainage. *FEMS Microbiology Ecology*, 44: 139-152

Bao, Q., Zhang, D., Lv, D., and Wang, P. (2012). Effects of two main metabolites of sulfate-reducing bacteria on the corrosion of Q235 steels in 3.5 wt.% NaCl media. *Corrosion Science*, 65: 405-413

Bellzone Mining (2013). Bellzone Mining ships first iron ore from Forecariah. Available at http://www.proactiveinvestors.co.uk/companies/news/52001/bellzone-mining-ships-first-iron-ore-from-forecariah-52001.html. Accessed July 2013

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

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

Bermúdez-Lugo, O. (2013). The Mineral Industry of Guinea. *U.S. Geological Survey*, 21.1-21.5

Bermúdez-Lugo, O. (2011). The Mineral Industry of Guinea. *U.S. Geological Survey*, 20.1-20.3

Bermúdez-Lugo, O. (2007). The Mineral Industry of Guinea. *U.S. Geological Survey*, 21.1-21.3

Besaury, L, Ouddane, B., Pavissich, J.P., Dubrulle-Brunaud, C., Gonzalez, B., and Quillet, L. (2012). Impact of copper on the abundance and diversity of sulfate reducing prokaryotes in two chilean marine sediments. *Marine Pollution Bulletin*, 64: 2135-2145

Bezbaruah, A.N., Krajangpan, S., Chisholm, B.J., Khan, E., and Bermudez, J.J.E. (2009a). Entrapement of iron nanoparticles in calcium alginate beads for groundwater remediation applications. *Journal of Hazardous Materials,* 166:1339-1343

Bezbaruah, A.N., Shanbhogue, S.S., Simsek, S., and Khan, E. (2011). Encapsulation of iron nanoparticles in alginate biopolymer for trichloroethylene remediation. *J Nanopart Res,* 13: 6673-6681

Bezbaruah, N. A., Thompson, J.M., and Chisholm, B.J. (2009b). Remediation of alachlor and atrazine contaminated water with zero-valent iron nanoparticles. *Journal of Engineering Science and Health, Part B,* 44(6): 518-524

Bian, Z., Inyang, H.I., Daniels, J.L., Otto, F., and Struthers, S. (2010). Environmental issues from coal mining and their solutions. *Mining Science and Technology*, 20: 0215-0223

Biswas, A., Brooks, S.C., Miller, C.L, Mosher, J.J., Yin, X.L., and Drake, M.M. (2011). Bacterial growth phase influences methylmercury production by the sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132. *Science of the Total Environment,* 409: 3943-3948

Borglin, S., Joyner, D., Jacobsen, J., Mukhopadhyay, A., and Hazen, T.C. (2009). Overcoming the anaerobic hurdle in phenotypic microarrays: Generation and visualization of growth curve data for *Desulfovibrio vulgaris* Hildenborough. *Journal of Microbiological Methods*, 76: 159-168

Bray, E.L. (2011). Bauxite and Alumina. *US Geological Survey*, 10.1-1012

Brioukhanov, A., Pieulle, L., and Dolla, A. (2010). Antioxidative defense systems of anaerobic sulfate-reducing microorganisms. *Applied Microbiology and Microbial Biotechnology,* 148-159

Bussmann, I. and Reichardt, W. (1991). Sulfate-reducing bacteria in temporary oxic sediments with bivalves. *Marine Ecology Progress Series*, 78: 97-102

Cao, J., Zhang, G., Mao, Z.-S., Li, Y., Fang, Z., and Yang, C. (2012). Influence of electron donors on the growth and activity of sulfate reducing bacteria. *International Journal of Mineral Processing*, 106-109: 58-64

Castillo, J., Perez-Lopez, R., Sarmiento, A.M., and Nieto, J.M. (2012). Evaluation of organic substrates to enhance the sulfate reducing bacteria activity in phosphogypsum. *Science of the Total Environment*, 439:106-113

Central Intelligence Agency, CIA (2013). THE WORLD FACTBOOK. Available at https://www.cia.gov/library/publications/the-world-factbook/geos/gv.html. Accessed July 2013.

Chakraborty, R., Hazen, T.C., Joyner, D.C., Kusel, K., Singer, M.E., Sitte, J., and Torok, T. (2011). Use of immunomagnetic separation for the detection of Desulfovobrio vulgaris from environmental samples. *Journal of Microbiological Methods*, 86: 204-209

Chee, S.-Y., Wong, P.-K., and Wong, C.-L. (2011). Extraction and Characterization of alginate from brown seaweeds (Fucales, Phaeophyceae) collected from Port Dickson, Peninsular Malaysia. *J Appl Phycol*, 23: 191-196

Chirico, P.G., Malpeli, K.C., Bockstael, M.V., Diaby, M., Cisse, K., Diallo, T.A., and Sano, M. (2012). Alluvial Diamond Resource Potential and Production Capacity Assessment of Guinea. *US Geological Survey*, Scientific Report 2012-5256

Choudhary, R.P. and Sheoran, A.S. (2012). Performance of single substrate in sulphate reducing bioreactor for the treatment of acid mine drainage. *Mineral Engineering*, 39: 29-35

Choudhary, R.P. and Sheoran, A.S. (2011). Comparative study of cellulose waste versus organic waste as substrate in a sulfate reducing bioreactor. *Bioresource Technology*, 102: 4319-4324

Christia-Lotter, A., Bartoli, C., Percecchi-Marti, M.-D., Demory, D., Pelissier-Alicot, A.-L., Sanvoisin, A., and Leonetti, G. (2007). Fatal occupational inhalation of hydrogen sulfide. *Forensic Science International*, 169: 206-209

Claudet, I., Marcoux, M.-O., Karsenty, C., Rittie, J.-L., Honorat, R., and Lelong-Tissier, M.-C. (2012). Severe hydrogen sulfide intoxication: A pedriatric case of survival. *Annales Francaises d'Anesthesie et de Reanimation*, 31: 255-258

Climatemp (2013). Climate and Temperature, Guinea. Available at http://www.guinea.climatemps.com. Accessed July 2013

Colin, Y., Goni-Urriza, M., Caumette, P., and Guyoneaud, R. (2012). Combination of high throughput cultivation and dsrA sequencing for assessment of sulfate-reducing bacteria diversity in sediments. *FEMS Microbial Ecol*, 83: 26-37

Costa, M.C., Martins, M., Jesus, C., and Duarte, J.C. (2008). Treatment of Acid Mine Drainage by Sulphate-reducing Bacteria Using Low Cost Matrices. *Water Air Soil Pollut.*, 189: 149-162

Coulibaly, S. (2002). Future of Guinea bauxite mining and alumina industry development. *The Minerals, Metals & Materials Society*, 1-3

Cui, M., Jang, M., Cho, S.-H., Khim, J., and Cannon, F.S. (2012). A continuous pilot-scale system using coal-mine drainage sludge to treat acid mine drainage contaminated with high concentrations of Pb, Zn, and other heavy metals. *Journal of Hazardous Materials*, 215-216: 122-128

Davies, H., Weber, P., Lindsay, P., Craw, D., and Pope, J. (2011). Characterisation of acid mine drainage in a high rainfall mountain environment, New Zealand. *Science of the Total Environment*, 409: 2971–2980

Dar, S.A., Kleerebezem, R., Stams, A.J.M., Kuenen, J.G., and Muyzer, G. (2008). Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. *Appl Microbiol Biotechnol*, 78:1045–1055

Dar, S.A., Yao, L., van Dongen, U., Kuenen, J.G., and Muyzer, G. (2006). Analysis of Diversity and Activity of Sulfate-Reducing Bacteria Communities in Sulfidogenic Bioreactors Using 16S rRNA and dsrB Genes as Molecular Markers. *Appl. Environ. Microbiol*., 73(2): 594-604

Del Rio, V., Larrechi, M.S., and Callao, M.P. (2010). Determination of sulphate in water and biodiesel samples by a sequential injection analysis - Multivariate curve resolution method. *Analytica Chimica Acta*, 676: 28-33

Deschamps, T., Benzaazoua, M., Bussière, B., and Aubertin, M. (2011). Laboratory study of surface paste disposal for sulfidic tailings: Physical model testing. *Minerals Engineering* 24: 794–806

Dhargalkar, V.K. and Verlecar, X.N. (2009). Southern Ocean seaweeds: A resource for exploration in food and drugs. *Aquaculture*, 287: 229-242

Dierberg, F.E., Debosk, T.A., Larson, N.R., Kharbanda, M.D., Chan, N., and Gabriel, M.C. (2011). Effect of sulfate amendments on mineralization and phosphorus release from South Florida (USA) wetland soils under anaerobic conditions. *Soil Biology & Biochemistry*, 43: 31-45

Edenborn, H.M. and Brickett, L.A. (2001). Bacteria in gel probes: comparison of the activity of immobilized sulfate-reducing bacteria with in situ sulfate reduction in a wetland sediment. *Journal of Microbiological Methods,* 46: 51-62

El Bayoumy, M.A., Bewtra, J.K., Ali, H.I., and Biswas, N. (1999). Sulfide production by sulfate reducing bacteria with lactate as feed in an upflow anaerobic fixed film reactor. *Water, Air, and Soil Pollution,* 112: 67-84

Faulwetter, J.E., Burr, M.D., Parker, A.E., Stein, O.R., and Camper, A.K. (2013). Influence of Season and Plant Species on the Abundance and Diversity of Sulfate Reducing Bacteria and Ammonia Oxidizing Bacteria in Constructed Wetlands Microcosms. *Environmental Microbiology*, 65: 111-127

Faure, G. (1998). Principles and Applications of Geochemistry, 2nd Edition. *Prentice-Hall, Inc.*, 443-447 pp

Fenoradosoa, T., Ali, G., Delattre, C., Laroche, C., Petit, E., Wadouachi, A., and Michaud P. (2010). Extraction and characterization of an alginate from the brown seaweed Sargassum turbinatioides Grunow. *J Appl Phycol*, 22: 131-137

Finnie, K.S., Bartlett, J.R., and Woolfrey, J.L. (2000). Encapsulation of sulfate-reducing bacteria in a silica host. *J. Mater. Chem*., 10: 1099-1101

Fischer, J., Quentmeier, A., Gansel, S., Sabados, V., and Friedrich, C.G. (2002). Inducible aluminum resistance of *Acidiphilium cryptum* and aluminum tolerance of other acidophilic bacteria. *Arch Microbiol*, 178: 554-558

Fortin, D., Roy, M., Rioux, J.-P., and Thibault, P.-J. (2000). Occurence of sulfate-reducing bacteria under a wide range of physico-chemical conditions in Au and Cu-Zn mine tailings. *FEMS Microbiology Ecology*, 33: 197-208

Foucher, S., Battaglia-Brunet, F., Ignatiadis, I., and Morin, D. (2001). Treatment by sulfate-reducing bacteriaof Chessy acid-mine drainage and metals recovery. *Chemical Engineering Science,* 56: 1639-1645

Franco, J.L., Posser, T., Dunkley, P.R., Dickson, P.W., Mattos, J.J., Martins, R., Bainy, A.C.D., Marques, M.R., Dafre, A.L., and Farina, M. (2009). Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. *Free Radical Biology & Medecine,* 47: 449-457

Garcia, C., Moreno, D.A., Ballester, A., Blasquez, M.L., and Gonzalez, F. (2001). Bioremediation of an industrial acid mine water by metal-tolerant sulphate-reducing bacteria. *Minerals Engineering*, 14(9): 997-1008

Garcia-Lorenzo, M.L., Perez-Sirvent, C., Martinez-Sanchez, M.J., and Molina-Ruiz, J. (2012). Trace elements contamination in an abandoned mining site in semiarid zone. *Journal of Geochemical Exploration*, 113: 23-35

Geurts, J.J.M., Sarneel, J.M., Willers, B.J.C., Reolofs, J.G.M., Verhoeven, J.T.A., and Lamers, L.P.M. (2009). Interacting effects of sulfphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: A mesocosm experiment. *Environmental Pollution*, 157: 2072-2081

Gibert, O., Rotting, T., Cortina, J.L., de Pablo, J., Ayora, C., Carrera, J., and Bolzicco, J. (2011). In-situ remediation of acid mine drainage using a permeable reactive barrier in Aznalcollar (Sw Spain). *Journal of Hazardous Materials*, 191: 287-295

Gilmour, C.C., Elias, D.A., Kuchen, A.M., Brown, S.D., Palumbo, A.V., Schadt, C.W., and Wall, J.D. (2011). Sulfate-Reducing Bacterium Desulfovibrio desulfuricans ND132 as a Model for Understanding Bacteria Mercury Methylation. *Appl. Environ. Microbiol.*, 77(12): 3938-3951

Ghosh, S., Moitra, M., Woolverton, C.J., and Leff, L.G. (2012). Effects of remediation on the bacterial community of an acid mine drainage impacted stream. *Can. J. Microbiol.*, 58: 1316-1326

Gomez, C.G., Lambrecht, M.V.P., Lozano, J.E., Rinaudo, M., and Villar, M.A. (2009). Influence of extraction-purification conditions on final prperties of alginates obtained from brown algae (*Macrocystis pyrifera*). *International Journal of Biological Macromolecules*, 44:365-371

Guan, J., Xia, L.-P., Wang, L.-Y., Liu, J.-F., Gu, J.-D., and Mu, B.-Z. (2013). Diversity and distribution of sulfate-reducing bacteria in four petrouleum reservoirs detected by using 16S rRNA and dsrAB genes. *International Biodeterioration & Biodegradation*, 76: 58-66

Hammack, R.W., de Vegt, A.L., and Schoeneman, A.L. (2006) The removal of sulfate and metals from mine waters using bacterial sulfat ereduction: Pilot plant results, *Mine Water and the Environment*, 17:8-27

Hard, B.C., Walther, C., and Babel, W. (2010). Sorption of Aluminum by Sulfate-Reducing Bacteria Isolated from Uranium Mine Tailings. *Geomicrobiology Journal*, 16(4): 267-275

Hernandez-Carmona, G., McHugh, D.J., Arvizu-Higuera, D.L., and Rodriguez-Montesinos, Y.E. (1999). Pilot plant scale extraction of alginate from Macrocystis pyrifera. 1. Effect of pre-extraction treatments on yield and quality of alginate. *Journal of Applied Phycology*, 10: 507-513

Hill, C.B., and Khan, E. (2008). A Comparative Study of Immobilized Nitrifying and Co-Immobilized Nitrifying and Denitrifying Bacteria for Ammonia Removal from Sludge Digester Supernatant. *Water Air Soil Pollut*, 195: 23-33

Hogsden, K.L. and Harding J.S. (2012). Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. *Freshwater Science*, 31(1): 108-120

Huang, S.S.-Y., Strathe, A.B., Fadel, J.G., Johnson, M.L., Lin, P., Liu, T.-Y., and Hung, S.S.O. *Aquatic Toxicology*, 126: 274-282

Ima, C.S. and Mann, D.D. (2007). Physical Properties of Woodchip: Compost Mixtures used as Biofilter Media. *Agricultural Engineering International*, Manuscript BC 07 005. Vol. IX.

Jalali, K. and Baldwin, S.A. (2000). The role of sulphate reducing bacteria in copper removal from aqueous sulphate solutions. *Wat., Res.*34(3): 797-806

Janssen, P.H. and Schink, B. (1995). Catabolic and Anabolic Enzyme Activities and Energetics of Acetone Metabolism of Sulfate-Reducing Bacterium *Desulfococcus biacutus*. *Journal of Bacteriology*, 177(2): 277-282

Johnson, D.B. (2012). Reductive dissolution of minerals and selective recovery of metals using acidophilic iron- and sulfate-reducing acidophiles. *Hydrometallurgy*, 127-128: 172-177

Johnson, D.B. and Hallberg, K.B. (2005). Acid mine drainage remediation options: a review. *Science of the Total Environment*, 338: 3-14

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

Karabika, E., Kallimanis, A., Dados, A., Pilidis, G., Drainas, C., and Koukkou, A.I. (2009). Taxonomic Identification and Use of Free and Entrapped Cells of a New Mycobacterium sp., Strain Spyr1 for Degradation of Polycyclic Aromatic Hydrocarbons (PAHs). Appl *Biochem Biotechnol*, 159: 155-167

Khin, M.M., Nair, A.S., Babu, V.J., Murugan, R., and Ramakrishna, S. (2012). A review on nanomaterials for environmental remediation. *Energy Environ. Sci.*, 5: 8075-8109

Kieu, H.T.Q., Muller, E., and Horn, H. (2011). Heavy metal removal in anaerobic semi-continuous stirred tank reactors by a consortium of sulfate-reducing bacteria. *Water Research*, 45: 3863-3870

King, J.K., Kostka, J.E., Frischer, M.E., and Saunders, F.M. (2000). Sulfate-Reducing Bacteria Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments. *Appl. Environ. Microbiol.*, 66(6): 2430-2437

Kongchum, M., Devai, I., DeLaune, R.D., and Jugsujinda, A. (2006). Total mercury and methylmercury in freshwater and salt marsh soils of the Mississippi river deltaic plain. *Chemosphere*, 63: 1300-1303

Koschorreck, M. and Wendt-Potthoff, K. (2012). A sediment exchange experiment to assess the limiting factors of microbial sulfate reduction in acidic mine pit lakes. *J Soils Sediments* 12: 1615-1622

Krajangpan, S., Kalita, H., Chisholm, B.J., and Bezbaruah, A.N. (2012). Iron Nanoparticles Coated with Amphiphilic Polysiloxane Graft Copolymers: Dispersibility and Contaminant Treatability. *Environ. Sci. Technol.,* 46: 10130-10136

Kuo, W.-C. and Shu, T.-Y. (2004). Biological pre-treatment of wastewater containing sulfate using anaerobic immobilized cells. *Journal of Hazardous Materials,* B113: 147-155

Lamers, L.P.M., Falla, S.-J., Samborska, E.M., van Dulken, I.A.R., van Hengstum, G., and Roelofs, J.G.M. (2002). Factors Controlling the Extend of Entrophication and Toxicity in Sulfate-Polluted Freshwater Wetlands. *Limnology and Oceanography,* 47(2): 585-593

Lamers, L.P.M., Tomassen, H.B.M., and Roelofs, J.G.M. (1998). Sulfate-Induced Eutrophication and Phytotoxicity in Freshwater Wetlands. *Environ. Sci. Technol.,* 32: 199-205

Leal-Egana, A., Fritsch, A., Heidebrecht, F., Diaz-Cuenca, A., Nowicki, M., Bader, A., and Kas, J. (2012). Tuning liver stiffness against tumours: An in vitro study using entrapped cells in tumour-like microcapsules. *Journal of the Mechanical Behavior of Biomedical Materials,* 9: 113-121

Lee, D.-J., Lee, C.-Y., and Chang, J.-S. (2012). Treatment and electricity harvesting from sulfate/ sulfide-containing wastewaters using fuel cell with enriched sulfate-reducing mixed culture. *Journal of Hazardous Materials,* 243: 67-72

Li, X., McInerney, M.J., Stahl, D.A., and Krumholz, L.R. (2011). Metabolism of H2 by Desulfovibrio alaskensis G20 during syntrophic growth on lactate. *Microbiology,* 157: 2912-2921

Liljeqvist, M., Sundkvist, J.-E., Saleh, A., and Dopson, M. (2011). Low Temperature Removal of Inorganic Sulfur Compounds From Mining Process Waters. *Biotechnology & Bioengineering,* 108(6): 1251-1259

Lobo, S.A.L., Melo, A.M.P., Carita, J.N., Teixeira, M., and Saraiva, L.M. (2007). The anaerobe *Desulfovibrio desulfuricans* ATCC 27774 grows at nearly atmospheric oxygen levels. *FEBS Letters,* 581: 433-436

Lu, J., Chen, T., Wu, J., Wilson, P.C., Hao, X., and Qian, J. (2011). Acid tolerance of an acid mine drainage bioremediation system based on biological sulfate reduction. *Bioresource Technology,* 102: 10401-10406

Matin, A.H., Goddard, E., Vandermoere, F., Blanchemanche, S., Bieberstein, A., Marette, S., and Roosen, J. (2012). Do environmental attitudes and food technology neophobia affect

perceptions of the benefits of nanotechnology? *International Journal of Consumer Studies*, 36: 149-157

Martins, M., Taborda, R., Silva, G., Assuncao, A., Matos, A.P., and Costa, M.C. (2012). Aluminum and sulphate removal by a highly Al-resistant dissimilatory sulphate-reducing bacteria community. *Biodegradation,* 23: 693-703

Martin, M., Faleiro, M.L., Silva, G., Chaves, S., Tenreiro, R., and Costa M.C. (2011). Dynamics of bacterial community in up-flow anaerobic packed bed system for acid mine drainage treatment using wine wastes ascarbon source. *International Biodeterioration & Biodegradation,* 65: 78-84

Martins, M., Faleiro, M.L., Barros, R.J., Verissimo, A.R., Barreiros, M.A., and Costa, M.C. (2009). Characterization and activity studies of highly heavy metal resistant sulphate-reducing bacteria to be used in acid mine drainage decontamination. *Journal of Hazardous Materials,* 166: 706-713

Matlock, M.M., Howerton, B.S., and Atwood, D.A. (2002). Chemical precipitation of heavy metals from acid mine drainage. *Water Research,* 36: 4757-4764

Matsui, T., Kojima, H., and Fukui, M. (2013). Effectsof temperature on anaerobic decomposition of high-molecular weight organic matter under sulfate-reducing conditions. *Estuarine, Coastal and Shelf Science*, 119: 139-144

Meulepas, R.J.W., Jagersma, C.G., Khadem, A.F., Buisman, C.J.N., Stams, A.J.M., and Lens, P.N.L. (2009). Effect of Environmental Conditions on Sulfate Reduction with Methane as Electron Donor by an Eckernforde Bay Enrichment. *Environ. Sci. Technol.*, 43: 6553-6559

Mitsch, W.J. and Gosselink, J.G. (2007). Wetlands, 4th Edition. *John Wiley & Sons, Inc*., 184-187 pp

Mobbs, P. (1997). The Mineral Industry of Guinea. R1-R4

Mogensen, G.L., Kjeldsen, K.U., and Ingvorsen, K. (2005). *Desulfovibrio aerotolerans* sp. nov., an oxygen tolerant sulphate-reducing bacterium isolated from activated sludge. *Anaerobe,* 11: 339-349

Mudd, G.M. (2010). Global trends and environmental issues in nickel mining: Sulfides versus laterites. *Ore Geology Reviews*, 38: 9–26

Mulopo, J., Greben, H., Sigama, J., Radebe, V., Mashego, M., and Burke, L. (2011). The Relationships Between Sulphate Reduction and COD/VFA Utilisation Using Grass Cellulose as Carbon and Energy Sources. *Appl Biochem Biotechnol*, 163: 393-403

Murillo-Alvarez, J.I. and Hernandez-Carmona, G. (2007). Monomer composition and sequence of sodium alginate extracted at pilot plant scale from three commercially important seaweeds from Mexico. *J Appl Phycol*, 19: 545-548

Nagpal, S., Chuichulcherm, S., Livingston, A. and Peeva, L. (2000). Ethanol Utilization by Sulfate-Reducing Bacteria: An Experimental and Modeling Study. *Biotechnol Bioeng*, 70: 533-543

Neculita, C.M., Zagury, G.J., and Bussiere, B. (2007). Passive Treatment of Acid Mine Drainage in Bioreactors using Sulfate-Reducing Bacteria: Critical Review and Research Needs. *J. Environ. Qual.*, 36: 1-16

Nelsen, J.L., Scoble, M., and Ostry, A. (2010). Sustainable socio-economic development in mining communities north-central British Columbia perspectives. *International Journal of Mining, Reclamation and Environment*, 24(2): 163-179

Nordstrom, D.K. (2000). Negative pH and Extremely Acidic Mine Waters from Iron Mountain, California. *Environ. Sci. Technol.,* 34: 254-258

Noubactep, C., Care, S., and Crane, R. (2012). Nanoscale Metallic Iron for Environmental Remediation: Prospects and Limitations. *Water Air Soil Pollut*, 223: 1363-1382

Office Nationale du Tourisme de Guinée, ONTG. (2013). Données physiques de la Guinée. Available at http://www.ontguinee.org/web/index.php?option=com_content&view=article&id=147&Itemid=108

Olsvik, P.A., Amlund, H., and Torstensen, E. (2011). Dietary lipids modulate methylmercury toxicity in Atlantic salmon. *Food and Chemical Toxicity*, 49: 3258-3271

Pedersen, K. (2012). Influence of H_2 and O_2 on sulphate-reducing activity of a subterranean community and the coupled response in redox potential. *FEMS Microbiol Ecol*, 82: 653-665

Pramanik, S., McEvoy, J., Siripattanakul, S., and Khan, E. (2011). Effects of cell entrapment on nucleic acid content and microbial diversity of mixed cultures in biological wastewater treatment. *Bioresource Technology*, 102: 3176-3183

Pramanik, S., and Khan, E. (2009). Effects of cell entrapment on growth rate and metabolic activity of pure cultures commonly found in biological wastewater treatment. *Biochemical Engineering Journal*, 46: 286-293

Quan, L.M., Khanh, D.P., Hira, D., Fujii, T., and Furukawa, K. (2011). Reject water treatment by improvement of whole cell anamox entrapment using polyvinyl alcohol/alginate gel. *Biodegradation*, 22: 1155-1167

Ramamoorthy, S., Piotrowski, J.S., Langner, H.W., Holben, W.E., Morra, M.J., and Rosenzweig, R.F. (2009). Ecology of Sulfate-Reducing Bacteria in an Iron-Dominated, Mining-Impacted Freshwater Sediment. *J. Environ. Qual.*, 38: 675-684

Regmi, G., Indraratna, B., ASCE, F., Nghiem, L.D., Golab, A., and Prasad, B.G. (2011). Treatment of Acidic Groundwater in Acid Sulfate Soil Terrain Using Recycled Concrete: Column Experiments. *Journal of environmental Engineering*, 137(6): 433-443

Rio Tinto (2013). Simandou project overview. Available at http://www.riotintosimandou.com/ENG/index_projectoverview.asp. Accessed July 2013

Rivera-Becerril, F., Juarez-Vasquez, L.V., Harnandez-Cervantes, S.C., Acevedo-Sandoval, O.A., Vela-Correa, G., Cruz-Chavez, E., Moreno-Espindola, I.P., Esquivel-Herrera, A, and de Leon-Gonzalez, F. (2013). Impacts of Manganese Mining Activity on the Environment: Interactions Among soil, Plants, and Arbuscular Mycorrhiza. *Arch Environ Contam Toxicol*, 64: 219-227

Roy, A., Das, B.K., and Bhattacharya, J. (2011). Development and Validation of a Spectrophotometric Method to Mesure Sulfate Concentrations in Mine Water without Interference. *Mine Water Environ*, 30: 169-174

Rozek, A., Kowalczyk, P., and Wolicka, D. (2013). Revealing Sulfate-Reducing Microorganisms in Oilfield Waters (Flysch Carpathians, South -eastern Poland). *Geomicrobiology Journal*, 30(3): 268-277

Ryu, A., Jeong, S.-W., Jang, A., and Choi, H. (2011). Reduction of highly concentrated nitrate using nanoscale zero-valent iron: Effects of aggregation and catalyst on reactivity. *Applied Catalysis B: Environmental*, 105: 128-135

Sahinkaya, E., Dursun, N., Kilic, A., Demirel, S., Uyanik, S., and Cinar, O. (2011). Simultaneous heterotrophic and sulfur-oxidizing autotrophic dinitrification process for drinking waater treatment: Control of sulfate production. *Water Research*, 45: 6661-6667

Schmidtova, J. and Baldwin S.A. (2011). Correlation of bacterial communities supported by different organic materials with sulfate reduction in metal-rich landfill leachate. *Water Research*, 45: 1115-1128

Seth, A.D., and Edyvean, R.G.J. (2006). The function of sulfate-reducing bacteria in corrosion of portable water mains. *International Biodeterioration & Biodegradation*, 58: 108-111

Singh, R., Kumar, A., Kirrolia, A., Kumar, R., Yadav, N., Bishnoi, N.R., and Lohchab, R.K. (2011). Removal of sulfate, COD, and Cr(VI) in simulated and real wastewater by sulphate reducing bacteria enrichment in small bioreactor and FTIR study. *Bioresource Technology*, 102: 677-682

Sim, M.S., Ono, S., and Bosak, T. (2012). Effects of Iron and Nitrogen Limitation on Sulfur Isotope Fractionation during Microbial Sulfate Reduction. *Appl. Environ. Microbiol*. 78(23): 8368-8376

Siripattanakul, S., Pochnant, C.J., and Khan, E. (2010). Nitrate Removal from Agricultural Infiltrate by Biaugmented Free and Alginate Entrapped Cells. *Environ. Res.*, 82: 617-621

Soto-Viruet, Y. (2011). The Mineral Industry of Guinea. *U.S. Geological Survey Minerals Yearbook*, 20.1-20.6

Stubner, S., Wind, T., and Conrad, R. (1998). Sulfur Oxidation in Rice Field Soil: Activity, Enumeration, Isolation and Characterization of Thiosulfate-oxidazing Bacteria. *System. Appl. Microbiol*. 21: 569-578

Szczesniak, P.A. (2001). The Mineral Industries of Cote D'Ivoire, Guinea, Liberia, and Sierra Leone. *US Geological Survey Yearly Minerals Book,* 11.1-11.7

Thompson, J.M., Chisholm, B.J., and Bezbaruah, A.N. (2010). Reductive Dechlorination of Chloroacetanilide Herbicide (Alachlor) Using Zero-Valent Iron Nanoparticles. *Environmental Engineering Science*, 27(3): 227-232

U.S. Department of State (2012). Summary of the Diamond Resource Potential and Production Capacity Assessment of Guinea. *US Geological Survey*, Fact Sheet 2012–3129

USEPA, Methods for the Chemical Analysis of Water and Wastes (MCAWW) (EPA/600/4-79/020). Issued 1971; Editorial Revision 1978

Venzlaff, H., Enning, D., Srinivasan, J., Mayrhofer, K.J.J., Hassel, A.W., Widdel, F., and Stratmann. (2013). Accelerated cathodic reaction in microbial corrosion of iron due to direct electron uptake by sulfate-reducing bacteria. *Corrosion Science*, 66: 88-96

Vester, F. and Ingvorsen, K. (1998). Improved Most-Probable-Number Method To Detect Sulfate-reducing Bacteria with Natural Media and a Radiotracer. *Applied and Environmental Microbiology*, 64(5): 1700-1707

Viggi, C.C., Pagnanelli, F., Cibati, A., Uccelletti, D., Palleschi, C., and Toro, L. (2010). Biotreatment and bioassessment of heavy metal removal by sulphate reducing bacteria in fixed bed reactors. *Water Research*, 44: 151-158

Wadhawan, T., Maruska, Z.B., Siripattanakul, S., Hill, C.B., Gupta, A., Prub, B.M., McEvoy, J.M., and Khan, E. (2011). A new method to determine initial viability of entapped cells using fluorescent nucleic acid staining. *Bioresource Technology*, 102: 1622-1627

Wadhawan, T., McEvoy, J.M., Prub, B.M., and Khan, E. (2010). Assessing tetrazolium and ATP assays for rapid in situ viability quantification of bacterial cells entrapped in hydrogel beads. *Enzyme and Microbial Technology*, 47: 166-173

Wahsha, M., Bini, C., Fontana, S., Wahsha, A., and Zilioli, D. (2012). Toxicity assessment of conataminated soils from a mining area in Northeast Italy by using lipid peroxidation assay. *Journal of Geochemical Exploration,* 113: 112-117

Wan, Y., Zhang, D., Liu, H. Li, Y., and Hou, B. (2010). Influence of sulfate-reducing bacteria on environmental parameters and marine corrosion behavior of Q235 steel in aerobic conditions. *Electrochimica Acta,* 55: 1528-1534

Wang, J., Li, S.-J., Chen, T.-H., Zhang, H., Zhang, N., and Yue, Z.-B. (2012). Effects of heavy metals on the performance of anaerobic sulfidogenic reactor using rape straw as carbon source. *Enviro Earth Sci*, 67: 2161-2167

Wen, J., Zhao, K., Gu, T., and Raad, I. (2010). Chelators enhanced biocide inhibition of planktonic sulfate-reducing bacteria growth. *World J Microbiol Biotechnol*, 26: 1053-1057

White, C., Sayer, J.A., and Gadd, G.M. (1997). Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiology Reviews*, 20: 503-516

White, R.A., Freeman, C., and Kang, H. (2011). Plant-derived phenolic compounds impair the remediation of acid mine drainage using treatment wetlands. *Ecological Engineering*, 37: 172-175

Wilkin, R.T. and McNeil, M.S. (2003). Laboratory evaluation of zero-valent iron to treat water impacted by acid mine drainage. *Chemosphere*, 53: 715-725

World Press (2012). Guinea Lacks Running Water and Electricity, Yet Bauxite, Gold, Diamond, and Iron Ore Mines are Booming, Available at http://guineaoye.wordpress.com/2012/04/03/guinea-lacks-running-water-and-electricity-yet-bauxite-gold-diamond-and-iron-ore-mines-are-booming-articles/. Accessed July 2013.

Xin, Y., Yong, K., Dujong, L., and Ying, F. (2008). Bioaugmented sulfate reduction using enriched anaerobic microflora in the presence of zero valent iron. *Chemosphere,* 73: 1436-1441

Yager, T.R., Bermudez-Lugo, O., Mobbs, P.M., Newman, H.R., Taib, M., Wallace, G.J., and Wilburn, D.R. (2012). The Mineral Industries of Africa. *US Geological Survey Mineral Yearbook,* 1.1-1.24

Yager, T.R. (2003). The Mineral Industries of Cote D'Ivoire, Guinea, Liberia, and Sierra Leone. U.S. Geological Survey Minerals Yearbook, 11.1-11.7

Yangguo, Z., Aijie, W., Nanqi, R., Qiushi, Z., and Maryam, Z. (2007). Impacts of Alkalinity Drops on Shifting of Functional Sulfate-Reducers in a Sulfate-Reducing Bioreactor Characterized by FISH. *Chin J. Chem. Eng*., 15(2): 276-280

Yenilmez, F., Kuter, N., Emil, M.K., and Aksoy, A. (2011). Evaluation of pollution levels at an abandoned mine site in Turkey with the aid of GIS. *International Journal of Coal Geology*, 86: 12-19

Yin, K., Lo, I.M.C., Dong, H., Rao, P., and Mak, M.S.H. (2012). Lab-scale simulation of the fate and transport of nano zero-valent iron in subsurface environments: Aggregation, sedimentation, and contaminant desorption. *Journal of Hazardous Materials*, 227-228: 118-125

Yan, W., Vasic, R., Frenkel, A.I., and Koel, B.E. (2012). Intraparticle Reduction of Arsenite [As(III)] by Nanoscale Zerovalent Iron (nZVI) Investigated with In Situ X-ray Absorption Spectroscopy. *Environ. Sci. Technol.*, 46: 7018-7026

APPENDIX A. TABLES OF RAW AND NORMALIZED DATA FROM SULFATE REDUCTION EXPERIMENTS

Table A.1. Raw data from sulfate reduction experiments using entrapped SRB (see normalized data in Table A.2). Initial sulfate concentration 250 mg/L.

6/22/2012 Blank (Sulfate only)										
Time, days	**pH**	**Dilution rate**	**λ2**	**1- SO_4^{2-}**	**λ3**	**2- SO_4^{2-}**	**λ4**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.074	252.903226	0.073	249.677419	0.073	249.677419	250.752688	1.86242022
Day-1		10/100	0.07	240	0.071	243.225806	0.069	236.774194	240	3.22580645
Day-2		10/100	0.071	243.225806	0.067	230.322581	0.069	236.774194	236.774194	6.4516129
Day-3		10/100	0.067	230.322581	0.068	233.548387	0.07	240	234.623656	4.92750075
Day-5		10/100	0.065	223.870968	0.069	236.774194	0.066	227.096774	229.247312	6.71505161
Day-7		10/100	0.069	236.774194	0.067	230.322581	0.073	249.677419	238.924731	9.85500149
6/22/2012 Control (Ca-alginate beads)										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.074	252.903226	0.073	249.677419	0.073	249.677419	250.752688	1.86242022
Day-1		10/100	0.045	159.354839	0.072	246.451613	0.067	230.322581	212.043011	46.3364762
Day-2		10/100	0.069	236.774194	0.068	233.548387	0.07	240	236.774194	3.22580645
Day-3		10/100	0.07	240	0.058	201.290323	0.054	188.387097	209.892473	26.8602064
Day-5		10/100	0.072	246.451613	0.043	152.903226	0.047	165.806452	188.387097	50.6975279
Day-7		10/100	0.059	204.516129	0.052	181.935484	0.057	198.064516	194.83871	11.6308106
6/22/2012 No Carbon (with Entrapped SRB beads)										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.074	252.903226	0.073	249.677419	0.073	249.677419	250.752688	1.86242022
Day-1		10/100	0.061	210.967742	0.069	236.774194	0.063	217.419355	221.72043	13.4301032
Day-2		10/100	0.031	114.193548	0.028	104.516129	0.026	98.0645161	105.591398	8.11810154
Day-3		10/100	0.022	85.1612903	0.03	110.967742	0.022	85.1612903	93.7634409	14.8993618
Day-5		10/100	0.025	94.8387097	0.025	94.8387097	0.026	98.0645161	95.9139785	1.86242022
Day-7		10/100	0.027	101.290323	0.031	114.193548	0.03	110.967742	108.817204	6.71505161

Table A.2. Normalized raw data from sulfate reduction experiments using entrapped SRB (see raw data in Table A.1). Initial sulfate concentration 250 mg/L.

6/22/2012 Blank (sulfate only)							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-1		10/100	0.94897959	0.97416021	0.94832041	0.957153404	0.01473201
Day-2		10/100	0.96173469	0.92248062	0.94832041	0.944178576	0.01995211
Day-3		10/100	0.91071429	0.93540052	0.96124031	0.935785038	0.02526521
Day-5		10/100	0.88520408	0.94832041	0.90956072	0.91436174	0.03183088
Day-7		10/100	0.93622449	0.92248062	1	0.952901703	0.04136316
6/22/2012 Control (With Ca-alginate beads)							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-1		10/100	0.63010204	0.9870801	0.92248062	0.846554255	0.19021552
Day-2		10/100	0.93622449	0.93540052	0.96124031	0.944288439	0.01468653
Day-3		10/100	0.94897959	0.80620155	0.75452196	0.836567702	0.10072249
Day-5		10/100	0.9744898	0.6124031	0.66408269	0.750325195	0.19584438
Day-7		10/100	0.80867347	0.72868217	0.79328165	0.776879098	0.04244331
6/22/2012 No Carbon (with Entrapped SRB beads)							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-1		10/100	0.83418367	0.94832041	0.87080103	0.88443504	0.05827704
Day-2		10/100	0.45153061	0.41860465	0.39276486	0.420966707	0.029454
Day-3		10/100	0.33673469	0.44444444	0.34108527	0.374088137	0.06096917
Day-5		10/100	0.375	0.37984496	0.39276486	0.382536606	0.00918321
Day-7		10/100	0.4005102	0.45736434	0.44444444	0.43410633	0.02980362

Table A.3. Raw data from sulfate reduction experiments using entrapped SRB (see normalized data in Table A.4). Sulfate degradation by entrapped SRB beads in presence of ethanol and lactose as organic substrates.

1/25/2013 Blank (Sulfate only)										
Sample ID	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.154	500	0.156	505.128205	0.156	505.128205	503.4188034	2.96077061
Day-3		10/100	0.152	494.871795	0.151	492.307692	0.152	494.871795	494.017094	1.48038531
Day-6		10/100	0.153	497.435897	0.121	415.384615	0.149	487.179487	466.6666667	44.7066558
Day-9		10/100	0.153	497.435897	0.13	438.461538	0.151	492.307692	476.0683761	32.6692562
Day-12		10/100	0.15	489.74359	0.143	471.794872	0.15	489.74359	483.7606838	10.3626971
Day-15		10/100	0.147	482.051282	0.14	464.102564	0.149	487.179487	477.7777778	12.117476
1/25/2013 Control (With Ca-alginate beads)										
Sample ID	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.154	500	0.156	505.128205	0.156	505.128205	503.4188034	2.96077061
Day-3		10/100	0.101	364.102564	0.125	425.641026	0.102	366.666667	385.4700855	34.8126698
Day-6		10/100	0.128	433.333333	0.086	325.641026	0.099	358.974359	372.6495726	55.1331741
Day-9		10/100	0.077	302.564103	0.09	335.897436	0.106	376.923077	338.4615385	37.2457411
Day-12		10/100	0.08	310.25641	0.085	323.076923	0.091	338.461538	323.9316239	14.1219758
Day-15		10/100	0.072	289.74359	0.097	353.846154	0.099	358.974359	334.1880342	38.5753302
1/25/2013 No Carbon (With Entrapped SRB beads)										
Sample ID	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.154	500	0.156	505.128205	0.156	505.128205	503.4188034	2.96077061
Day-3		10/100	0.104	371.794872	0.151	492.307692	0.15	489.74359	451.2820513	68.8498543
Day-6		10/100	0.088	330.769231	0.085	323.076923	0.098	356.410256	336.7521368	17.4534853
Day-9		10/100	0.014	141.025641	0.073	292.307692	0.09	335.897436	256.4102564	102.275234
Day-12		10/100	0.013	138.461538	0.072	289.74359	0.088	330.769231	252.991453	101.284756
Day-15		10/100	0.056	248.717949	0.015	143.589744	0.094	346.153846	246.1538462	101.306391

Table A.4. Normalized raw data from sulfate reduction experiments using entrapped SRB (see raw data in Table A.3) 500 mg/L of sulfate were initially prepared.

1/25/2013 Blank (Sulfate only)							
Sample ID	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.98974359	0.974619289	0.979695431	0.98135277	0.007697155
Day-6		10/100	0.994871795	0.822335025	0.964467005	0.927224608	0.092100387
Day-9		10/100	0.994871795	0.868020305	0.974619289	0.94583713	0.068147888
Day-12		10/100	0.979487179	0.934010152	0.969543147	0.961013493	0.023908288
Day-15		10/100	0.964102564	0.918781726	0.964467005	0.949117098	0.026271835
1/25/2013 Control (With Ca-alginate beads)							
Sample ID	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.728205128	0.842639594	0.725888325	0.765577682	0.066747626
Day-6		10/100	0.866666667	0.644670051	0.710659898	0.740665539	0.113999466
Day-9		10/100	0.605128205	0.664974619	0.746192893	0.672098573	0.070801657
Day-12		10/100	0.620512821	0.639593909	0.670050761	0.64338583	0.024985714
Day-15		10/100	0.579487179	0.700507614	0.710659898	0.663551564	0.072978646
1/25/2013 No Carbon (With Entrapped SRB beads)							
Sample ID	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.743589744	0.974619289	0.969543147	0.895917393	0.131944028
Day-6		10/100	0.661538462	0.639593909	0.705583756	0.668905376	0.033606079
Day-9		10/100	0.282051282	0.578680203	0.664974619	0.508568701	0.200858875
Day-12		10/100	0.276923077	0.573604061	0.654822335	0.501783158	0.198923714
Day-15		10/100	0.497435897	0.284263959	0.685279188	0.488993015	0.200640886

Table A.5. Raw data from sulfate reduction experiments using entrapped SRB in presence of two different organic substrates (see normalized data in Table A.6). Initial sulfate concentration of 500 mg/L.

01/12/2013 Lactose with Entrapped SRBs										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.153	507.741935	0.152	504.516129	0.151	501.290323	504.516129	3.22580645
Day-3		10/100	0.021	81.9354839	0.022	85.1612903	0.024	91.6129032	86.23655914	4.92750075
Day-6		10/100	0.02	78.7096774	0.031	114.193548	0.03	110.967742	101.2903226	19.6218146
Day-9		10/100	0.021	81.9354839	0.023	88.3870968	0.023	88.3870968	86.23655914	3.72484045
Day-12		10/100	0.017	69.0322581	0.021	81.9354839	0.022	85.1612903	78.70967742	8.53468165
Day-15		10/100	0.017	69.0322581	0.007	36.7741935	0.018	72.2580645	59.35483871	19.6218146
01/12/2013 Ethanol with Entrapped SRBs										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.153	507.741935	0.152	504.516129	0.151	501.290323	504.516129	3.22580645
Day-3		10/100	0.101	340	0.099	333.548387	0.098	330.322581	334.6236559	4.92750075
Day-6		10/100	0.048	169.032258	0.038	136.774194	0.078	265.806452	190.5376344	67.1505161
Day-9		10/100	0.027	101.290323	0.022	85.1612903	0.021	81.9354839	89.46236559	10.3695169
Day-12		10/100	0.005	30.3225806	0.006	33.5483871	0.005	30.3225806	31.39784946	1.86242022
Day-15		10/100	0.005	30.3225806	0.003	23.8709677	0.004	27.0967742	27.09677419	3.22580645

Table A.6. Normalized raw data from sulfate reduction experiments using entrapped SRB in the presence of two different organic substrates (see raw data in Table A.5). Initial sulfate concentration of 500 mg/L.

01/12/2012 Lactose with Entrapped SRB beads							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.1613723	0.168797954	0.182754183	0.170974812	0.01085589
Day-6		10/100	0.15501906	0.226342711	0.221364221	0.200908664	0.03981944
Day-9		10/100	0.1613723	0.175191816	0.176319176	0.170961097	0.00832325
Day-12		10/100	0.135959339	0.162404092	0.16988417	0.156082534	0.017824
Day-15		10/100	0.135959339	0.072890026	0.144144144	0.117664503	0.03899119
01/12/2012 Treatment2 Ethanol with Entrapped SRB beads							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.669631512	0.66112532	0.658944659	0.66323383	0.00564682
Day-6		10/100	0.332909784	0.271099744	0.53024453	0.378084686	0.13534986
Day-9		10/100	0.199491741	0.168797954	0.163449163	0.177246286	0.01944987
Day-12		10/100	0.059720457	0.066496164	0.06048906	0.062235227	0.00371004
Day-15		10/100	0.059720457	0.047314578	0.054054054	0.053696363	0.00621067

Table A.7. Raw data from sulfate reduction experiments using entrapped SRB in presence of two different organic substrates (see normalized data in Table A.8). Initial sulfate concentration was 1,000 mg/L.

01/12/2012 Lactose with Entrapped SRBs										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.306	1001.29032	0.305	998.064516	0.306	1001.29032	1000.215054	1.86242022
Day-3		10/100	0.122	407.741935	0.096	323.870968	0.132	440	390.5376344	59.9456338
Day-6		10/100	0.078	265.806452	0.081	275.483871	0.075	256.129032	265.8064516	9.67741935
Day-9		10/100	0.061	210.967742	0.076	259.354839	0.074	252.903226	241.0752688	26.2726704
Day-12		10/100	0.057	198.064516	0.056	194.83871	0.046	162.580645	185.1612903	19.6218146
Day-15		10/100	0.046	162.580645	0.044	156.129032	0.036	130.322581	149.6774194	17.0693633
01/12/2012 Ethanol with Entrapped SRBs										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.306	1001.29032	0.305	998.064516	0.306	1001.29032	1000.215054	1.86242022
Day-3		10/100	0.129	430.322581	0.136	452.903226	0.12	401.290323	428.172043	25.8735686
Day-6		10/100	0.062	214.193548	0.072	246.451613	0.061	210.967742	223.8709677	19.6218146
Day-9		10/100	0.014	59.3548387	0.018	72.2580645	0.015	62.5806452	64.7311828	6.71505161
Day-12		10/100	0.004	27.0967742	0.004	27.0967742	0.002	20.6451613	24.94623656	3.72484045
Day-15		10/100	0.006	33.5483871	0.003	23.8709677	0.002	20.6451613	26.02150538	6.71505161

Table A.8. Normalized raw data from sulfate reduction experiments using entrapped SRB in presence of two different organic substrates (see raw data in Table A.7). Initial sulfate concentration was 1,000 mg/L.

01/12/2012 Lactose with Entrapped SRB beads							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.407216495	0.32449903	0.43943299	0.390382838	0.05928729
Day-6		10/100	0.265463918	0.2760181	0.255798969	0.265760329	0.01011282
Day-9		10/100	0.210695876	0.259857789	0.25257732	0.241043662	0.02653285
Day-12		10/100	0.197809278	0.195216548	0.162371134	0.18513232	0.01975435
Day-15		10/100	0.162371134	0.156431803	0.130154639	0.149652526	0.01714481
01/12/2012 Ethanol with Entrapped SRB beads							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.429768041	0.453781513	0.400773196	0.428107583	0.02654314
Day-6		10/100	0.213917526	0.246929541	0.210695876	0.223847648	0.0200543
Day-9		10/100	0.059278351	0.07239819	0.0625	0.064725514	0.00683719
Day-12		10/100	0.027061856	0.027149321	0.020618557	0.024943245	0.00374554
Day-15		10/100	0.033505155	0.023917259	0.020618557	0.026013657	0.0066942

Table A.9. Raw data from sulfate reduction experiments using entrapped SRB under different pH conditions, ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see normalized data in Table A.10).

1/17/2012 Entrapped SRB + Ethanol at pH 2.0										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.2	500	0.2	500	0.201	502.12766	500.7092199	1.22840483
Day-3		10/100	0.135	361.702128	0.154	402.12766	0.154	402.12766	388.6524823	23.3396917
Day-6		10/100	0.15	393.617021	0.133	357.446809	0.15	393.617021	381.5602837	20.8828821
Day-9		10/100	0.157	408.510638	0.152	397.87234	0.157	408.510638	404.964539	6.14202414
Day-12		10/100	0.157	408.510638	0.167	429.787234	0.155	404.255319	414.1843972	13.6789372
Day-15		10/100	0.152	397.87234	0.168	431.914894	0.181	459.574468	429.787234	30.9060405
1/17/2012 Entrapped SRB + Ethanol at pH 4.0										
Time, days	**pH**	**Dilution rate**	**λ2**	**1- SO_4^{2-}**	**λ3**	**2- SO_4^{2-}**	**λ4**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.2	500	0.2	500	0.201	502.12766	500.7092199	1.22840483
Day-3		10/100	0.112	312.765957	0.146	385.106383	0.125	340.425532	346.0992908	36.5024375
Day-6		10/100	0.096	278.723404	0.105	297.87234	0.1	287.234043	287.9432624	9.59414841
Day-9		10/100	0.103	293.617021	0.104	295.744681	0.104	295.744681	295.035461	1.22840483
Day-12		10/100	0.116	321.276596	0.113	314.893617	0.119	327.659574	321.2765957	6.38297872
Day-15		10/100	0.064	210.638298	0.145	382.978723	0.1	287.234043	293.6170213	86.3473357
1/17/2013 Entrapped SRB + Ethanol at pH 7.5										
Time, days	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day 0		10/100	0.228	501	0.2276	500.2	0.228	501	500.7333333	0.46188022
Day 3		10/100	0.114	273	0.132	309	0.142	329	303.6666667	28.3783955
Day 6		10/100	0.005	55	0.055	155	0.001	47	85.66666667	60.1775152
Day 9		10/100	0.115	275	0.004	53	0.001	47	125	129.938447
Day 12		10/100	0.038	121	0.004	53	0.002	49	74.33333333	40.4639757
Day 15		10/100	0	45	0.002	49	0	45	46.33333333	2.30940108

Table A.10. Normalized raw data from sulfate reduction experiments using entrapped SRB under different pH conditions, ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see raw data in Table A.9).

1/17/2013 Entrapped SRB + Ethanol at pH 2.0							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.72340426	0.80425532	0.80084746	0.776169011	0.04572738
Day-6		10/100	0.78723404	0.71489362	0.78389831	0.762008655	0.04083689
Day-9		10/100	0.81702128	0.79574468	0.81355932	0.808775093	0.01141666
Day-12		10/100	0.81702128	0.85957447	0.80508475	0.82722683	0.02864258
Day-15		10/100	0.79574468	0.86382979	0.91525424	0.858276235	0.05994802
1/17/2013 Entrapped SRB + Ethanol at pH 4.0							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.62553191	0.77021277	0.6779661	0.691236928	0.07324768
Day-6		10/100	0.55744681	0.59574468	0.5720339	0.575075129	0.01932922
Day-9		10/100	0.58723404	0.59148936	0.58898305	0.589235485	0.00213886
Day-12		10/100	0.64255319	0.62978723	0.65254237	0.641627599	0.01140577
Day-15		10/100	0.4212766	0.76595745	0.5720339	0.586422647	0.17279033
1/17/2013 Entrapped SRB + Ethanol at pH 7.5							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.54491018	0.6177529	0.65668663	0.606449902	0.05673898
Day-6		10/100	0.10978044	0.30987605	0.09381238	0.171156288	0.12039985
Day-9		10/100	0.5489022	0.10595762	0.09381238	0.249557396	0.25931132
Day-12		10/100	0.24151697	0.10595762	0.09780439	0.148426325	0.08072186
Day-15		10/100	0.08982036	0.09796082	0.08982036	0.092533845	0.00469989

Table A.11. Raw data from sulfate reduction experiments using entrapped SRB under different temperature, ethanol was the organic substrates, and initial sulfate concentration was 500 mg/L (see normalized data in Table A.12).

1/17/2013 Entrapped SRB + Ethanol at 30°C										
Sample ID	**pH**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.161	517.948718	0.158	510.25641	0.152	494.871795	507.6923077	11.7501941
Day-3		10/100	0.032	187.179487	0.008	125.641026	0.064	269.230769	194.017094	72.0386581
Day-6		10/100	0.023	164.102564	0.019	153.846154	0.01	130.769231	149.5726496	17.0726362
Day-9		10/100	0.003	112.820513	0.006	120.512821	0.005	117.948718	117.0940171	3.91673136
Day-12		10/100	0.01	130.769231	0.009	128.205128	0.008	125.641026	128.2051282	2.56410256
Day-15		10/100	0.004	115.384615	0.006	120.512821	0	105.128205	113.6752137	7.83346273
2/05/2013 Entrapped SRB + Ethanol at 15°C										
Sample ID	**pH**	**Dilution rate**	**λ2**	**1- SO_4^{2-}**	**λ3**	**2- SO_4^{2-}**	**λ4**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-18		10/100	0.228	501	0.227	499	0.228	501	500.3333333	1.15470054
Day-21		10/100	0.159	363	0.155	355	0.175	395	371	21.1660105
Day-24		10/100	0.143	331	0.105	255	0.143	331	305.6666667	43.8786205
Day-27		10/100	0.073	191	0.089	223	0.061	167	193.6666667	28.0950767
Day-30		10/100	0.074	193	0.082	209	0.015	75	159	73.1846979
Day-33		10/100	0.002	49	0.003	51	0.003	51	50.33333333	1.15470054
3/07/2013 Entrapped SRB + Ethanol at 5°C										
Sample ID	**pH**	**Dilution rate**	**λ3**	**1- SO_4^{2-}**	**λ4**	**2- SO_4^{2-}**	**λ5**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	0.125	501.666667	0.125	501.666667	0.125	501.666667	501.6666667	6.9619E-14
Day-3		10/100	0.07	318.333333	0.095	401.666667	0.073	328.333333	349.4444444	45.5013227
Day-6		10/100	0.105	435	0.095	401.666667	0.083	361.666667	399.4444444	36.717137
Day-9		10/100	0.077	341.666667	0.091	388.333333	0.078	345	358.3333333	26.0341656
Day-12		10/100	0.113	461.666667	0.116	471.666667	0.099	415	449.4444444	30.2459058
Day-15		10/100	0.075	335	0.071	321.666667	0.077	341.666667	332.7777778	10.1835015

Table A.12. Normalized raw data from sulfate reduction experiments using entrapped SRB under different temperature, ethanol was the organic substrates, and initial sulfate concentration was 500 mg/L (see raw data in Table A.11).

1/17/2013 Entrapped SRB + Ethanol at 30°C							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.36138614	0.24623116	0.54404145	0.383886248	0.15017468
Day-6		10/100	0.31683168	0.30150754	0.2642487	0.294195975	0.02704324
Day-9		10/100	0.21782178	0.2361809	0.23834197	0.230781552	0.01127538
Day-12		10/100	0.25247525	0.25125628	0.25388601	0.25253918	0.00131603
Day-15		10/100	0.22277228	0.2361809	0.21243523	0.223796138	0.0119059
2/05/2013 Entrapped SRB + Ethanol at 15°C							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.7245509	0.71142285	0.78842315	0.741465633	0.04119275
Day-6		10/100	0.66067864	0.51102204	0.66067864	0.61079311	0.08640428
Day-9		10/100	0.38123752	0.44689379	0.33333333	0.387154882	0.05701101
Day-12		10/100	0.38522954	0.41883768	0.1497006	0.317922605	0.14665047
Day-15		10/100	0.09780439	0.10220441	0.10179641	0.100601736	0.00243115
3/07/2013 Entrapped SRB + Ethanol at 5°C							
Time, days	**pH**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0		10/100	1	1	1	1	0
Day-3		10/100	0.6345515	0.80066445	0.65448505	0.696566999	0.09070031
Day-6		10/100	0.86710963	0.80066445	0.72093023	0.796234773	0.07319031
Day-9		10/100	0.68106312	0.77408638	0.68770764	0.714285714	0.05189535
Day-12		10/100	0.92026578	0.94019934	0.82724252	0.895902547	0.06029084
Day-15		10/100	0.66777409	0.64119601	0.68106312	0.663344408	0.02029934

Table A.13. Raw data from sulfate reduction experiments using entrapped SRB with interference from Aluminum. Ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see normalized data in Table A.14).

1/17/2013 Entrapped SRB + Ethanol with 0.5 mg/L of Al										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/7/2013	10/100	0.186	503.26087	0.188	507.608696	0.184	498.913043	503.2608696	4.34782609
Day-3	2/10/2013	10/100	0.004	107.608696	0.005	109.782609	0.038	181.521739	132.9710145	42.0602083
Day-6	2/13/2013	10/100	0.006	111.956522	0.005	109.782609	0.005	109.782609	110.5072464	1.25510928
Day-9	2/16/2013	10/100	0.006	111.956522	0.003	105.434783	0.002	103.26087	106.884058	4.52536087
Day-12	2/19/2013	10/100	0.003	105.434783	0.001	101.086957	0.003	105.434783	103.9855072	2.51021856
Day-15	2/22/2013	10/100	0.007	114.130435	0.007	114.130435	0.006	111.956522	113.4057971	1.25510928
1/17/2013 Entrapped SRB + Ethanol 25 mg/L of Al										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/16/2013	10/100	0.185	501.086957	0.185	501.086957	0.185	501.086957	501.0869565	0
Day-3	2/19/2013	10/100	0.114	346.73913	0.102	320.652174	0.119	357.608696	341.6666667	18.9932499
Day-6	2/22/2013	10/100	0.071	253.26087	0.1	316.304348	0.178	485.869565	351.8115942	120.300768
Day-9	2/25/2013	10/100	0.089	292.391304	0.023	148.913043	0.091	296.73913	246.0144928	84.1204166
Day-12	2/28/2013	10/100	0.099	314.130435	0.059	227.173913	0.162	451.086957	330.7971014	112.883108
Day-15	3/3/2013	10/100	0.119	357.608696	0.087	288.043478	0.101	318.478261	321.3768116	34.8730708
1/17/2013 Entrapped SRB + Ethanol 50 mg/L of Al										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/16/2013	10/100	0.185	501.086957	0.185	501.086957	0.185	501.086957	501.0869565	0
Day-3	2/19/2013	10/100	0.171	470.652174	0.17	468.478261	0.109	335.869565	425	77.1968735
Day-6	2/22/2013	10/100	0.135	392.391304	0.149	422.826087	0.113	344.565217	386.5942029	39.4511814
Day-9	2/25/2013	10/100	0.157	440.217391	0.174	477.173913	0.099	314.130435	410.5072464	85.4857502
Day-12	2/28/2013	10/100	0.173	475	0.153	431.521739	0.183	496.73913	467.7536232	33.2070703
Day-15	3/3/2013	10/100	0.168	464.130435	0.116	351.086957	0.16	446.73913	420.6521739	60.8695652

Table A.14. Normalized raw data from sulfate reduction experiments using entrapped SRB with interference from Aluminum. Ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see raw data in Table A.13).

		1/17/2013	Entrapped SRB + Ethanol 0.5 mg/L of Al				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/7/2013	10/100	1	1	1	1	0
Day-3	2/10/2013	10/100	0.213822894	0.21627409	0.363834423	0.264643802	0.08591034
Day-6	2/13/2013	10/100	0.222462203	0.21627409	0.220043573	0.219593289	0.003118534
Day-9	2/16/2013	10/100	0.222462203	0.207708779	0.206971678	0.212380887	0.008738451
Day-12	2/19/2013	10/100	0.20950324	0.199143469	0.211328976	0.206658562	0.00657197
Day-15	2/22/2013	10/100	0.226781857	0.2248394	0.224400871	0.22534071	0.001267185
		1/17/2013	Entrapped SRB + Ethanol 25 mg/L of Al				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/16/2013	10/100	1	1	1	1	0
Day-3	2/19/2013	10/100	0.69197397	0.639913232	0.713665944	0.681851048	0.0379041
Day-6	2/22/2013	10/100	0.505422993	0.631236443	0.969631236	0.702096891	0.240079623
Day-9	2/25/2013	10/100	0.5835141	0.297180043	0.592190889	0.490961678	0.167875886
Day-12	2/28/2013	10/100	0.626898048	0.453362256	0.90021692	0.660159074	0.225276485
Day-15	3/3/2013	10/100	0.713665944	0.57483731	0.635574837	0.641359364	0.069594848
		1/17/2013	Entrapped SRB + Ethanol with 50 mg/L of Al				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/16/2013	10/100	1	1	1	1	0
Day-3	2/19/2013	10/100	0.939262473	0.934924078	0.670281996	0.848156182	0.154058836
Day-6	2/22/2013	10/100	0.78308026	0.843817787	0.687635575	0.771511208	0.078731208
Day-9	2/25/2013	10/100	0.878524946	0.952277657	0.626898048	0.81923355	0.170600629
Day-12	2/28/2013	10/100	0.947939262	0.861171367	0.99132321	0.933477946	0.066270075
Day-15	3/3/2013	10/100	0.926247289	0.700650759	0.89154013	0.839479393	0.121475054

Table A.15. Raw data from sulfate reduction experiments using entrapped SRB with interference from Copper. Ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see normalized data in Table A.16).

1/17/2013	Entrapped SRB + Ethanol with 0.5 mg/L of Cu									
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/2/2013	10/100	0.228	501	0.227	499	0.227	499	499.6666667	1.15470054
Day-3	5/5/2013	10/100	0.114	273	0.132	309	0.142	329	303.6666667	28.3783955
Day-6	5/8/2013	10/100	0.065	175	0.137	319	0.06	165	219.6666667	86.1703739
Day-9	5/11/2013	10/100	0	45	0.004	53	0.001	47	48.33333333	4.163332
Day-12	5/14/2013	10/100	0	45	0.002	49	0	45	46.33333333	2.30940108
Day-15	5/17/2013	10/100	0.002	49	0.003	51	0.003	51	50.33333333	1.15470054
1/17/2013	**Entrapped SRB + Ethanol with 25 mg/L of Cu**									
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/9/2013	10/100	0.228	501	0.227	499	0.228	501	500.3333333	1.15470054
Day-3	5/12/2013	10/100	0.14	325	0.05	145	0.125	295	255	96.4365076
Day-6	5/15/2013	10/100	0	45	0.003	51	0	45	47	3.46410162
Day-9	5/18/2013	10/100	0.006	57	0.007	59	0.007	59	58.33333333	1.15470054
Day-12	5/21/2013	10/100	0.004	53	0.007	59	0.008	61	57.66666667	4.163332
Day-15	5/24/2013	10/100	0.007	59	0.015	75	0.008	61	65	8.71779789
1/17/2013	**Entrapped SRB + Ethanol with 50 mg/L of Cu**									
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/14/2013	10/100	0.228	501	0.227	499	0.228	501	500.3333333	1.15470054
Day-3	5/17/2013	10/100	0.132	309	0.161	367	0.166	377	351	36.7151195
Day-6	5/20/2013	10/100	0.057	159	0.105	255	0.121	287	233.6666667	66.613312
Day-9	5/23/2013	10/100	0.033	111	0.091	227	0.007	59	132.3333333	86.0077516
Day-12	5/26/2013	10/100	0.007	59	0.157	359	0.049	143	187	154.764337
Day-15	5/29/2013	10/100	0.007	59	0.019	83	0.007	59	67	13.8564065

Table A.16. Normalized raw data from sulfate reduction experiments using entrapped SRB with interference from Copper. Ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see raw data in Table A.15).

		1/17/2013	Entrapped SRB + Ethanol with 0.5 mg/L of Cu				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/7/2013	10/100	1	1	1	1	0
Day-3	2/10/2013	10/100	0.54491018	0.61923848	0.65931864	0.607822431	0.05805229
Day-6	2/13/2013	10/100	0.3493014	0.63927856	0.33066132	0.439747092	0.17305048
Day-9	2/16/2013	10/100	0.08982036	0.10621242	0.09418838	0.096740387	0.00848879
Day-12	2/19/2013	10/100	0.08982036	0.09819639	0.09018036	0.092732371	0.0047354
Day-15	2/22/2013	10/100	0.09780439	0.10220441	0.10220441	0.100737736	0.00254035
		1/17/2013	Entrapped SRB + Ethanol with 25 mg/L of Cu				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/16/2013	10/100	1	1	1	1	0
Day-3	2/19/2013	10/100	0.64870259	0.29058116	0.58882236	0.509368704	0.19182649
Day-6	2/22/2013	10/100	0.08982036	0.10220441	0.08982036	0.093948376	0.00714993
Day-9	2/25/2013	10/100	0.11377246	0.11823647	0.11776447	0.116591133	0.00245243
Day-12	2/28/2013	10/100	0.10578842	0.11823647	0.12175649	0.115260461	0.00838971
Day-15	3/3/2013	10/100	0.11776447	0.1503006	0.12175649	0.12994052	0.01774496
		1/17/2013	Entrapped SRB + Ethanol with 50 mg/L of Cu				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	2/16/2013	10/100	1	1	1	1	0
Day-3	2/19/2013	10/100	0.61676647	0.73547094	0.75249501	0.701577473	0.07394008
Day-6	2/22/2013	10/100	0.31736527	0.51102204	0.57285429	0.467080535	0.13329217
Day-9	2/25/2013	10/100	0.22155689	0.45490982	0.11776447	0.264743726	0.17267187
Day-12	2/28/2013	10/100	0.11776447	0.71943888	0.28542914	0.37421083	0.31050711
Day-15	3/3/2013	10/100	0.11776447	0.16633267	0.11776447	0.133953869	0.02804086

Table A.17. Raw data from sulfate reduction experiments using entrapped SRB with interference from Zinc. Ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see normalized data in Table A.18).

5/2/2013 Entrapped SRB beads + Ethanol + 0.5 mg/L of Zn										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/2/2013	10/100	0.227	499	0.228	501	0.228	501	500.3333333	1.15470054
Day-3	5/5/2013	10/100	0.14	325	0.148	341	0.147	339	335	8.71779789
Day-6	5/8/2013	10/100	0.045	135	0.049	143	0.022	89	122.3333333	29.143324
Day-9	5/11/2013	10/100	0.005	55	0.002	49	0	45	49.66666667	5.03322296
Day-12	5/14/2013	10/100	0.004	53	0.001	47	0	45	48.33333333	4.163332
Day-15	5/17/2013	10/100	0	45	0.003	51	0.003	51	49	3.46410162
4/15/2013 Entrapped SRB beads + Ethanol + 25 mg/L of Zn										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	4/15/2013	10/100	0.227	499	0.228	501	0.228	501	500.3333333	1.15470054
Day-3	4/18/2013	10/100	0.193	431	0.198	441	0.192	429	433.6666667	6.42910051
Day-6	4/21/2013	10/100	0.201	447	0.182	409	0.206	457	437.6666667	25.3245599
Day-9	4/24/2013	10/100	0.197	439	0.196	437	0.196	437	437.6666667	1.15470054
Day-12	4/27/2013	10/100	0.197	439	0.194	433	0.196	437	436.3333333	3.05505046
Day-15	4/30/2013	10/100	0.195	435	0.187	419	0.197	439	431	10.5830052
4/15/2013 Entrapped SRB beads + Ethanol + 50 mg/L of Zn										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	4/15/2013	10/100	0.227	499	0.228	501	0.228	501	500.3333333	1.15470054
Day-3	4/18/2013	10/100	0.212	469	0.18	405	0.211	467	447	36.3868108
Day-6	4/21/2013	10/100	0.188	421	0.198	441	0.2	445	435.6666667	12.858201
Day-9	4/24/2013	10/100	0.192	429	0.197	439	0.2	445	437.6666667	8.08290377
Day-12	4/27/2013	10/100	0.193	431	0.191	427	0.198	441	433	7.21110255
Day-15	4/30/2013	10/100	0.195	435	0.198	441	0.198	441	439	3.46410162

Table A.18. Normalized raw data from sulfate reduction experiments using entrapped SRB with interference from Zinc. Ethanol was the organic substrate, and initial sulfate concentration was 500 mg/L (see raw data in Table A.17).

4/15/2013 Entrapped SRB beads + Ethanol + 0.5 mg/L of Zn							
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	4/15/2013	10/100	1	1	1	1	0
Day-3	4/18/2013	10/100	0.65130261	0.68063872	0.67664671	0.669529345	0.01591052
Day-6	4/21/2013	10/100	0.27054108	0.28542914	0.17764471	0.244538311	0.05840787
Day-9	4/24/2013	10/100	0.11022044	0.09780439	0.08982036	0.09928173	0.01027997
Day-12	4/27/2013	10/100	0.10621242	0.09381238	0.08982036	0.096615053	0.00854788
Day-15	4/30/2013	10/100	0.09018036	0.10179641	0.10179641	0.097924392	0.00670653
4/15/2013 Entrapped SRB beads + Ethanol + 25 mg/L of Zn							
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	4/15/2013	10/100	1	1	1	1	0
Day-3	4/18/2013	10/100	0.86372745	0.88023952	0.85628743	0.866751467	0.01225905
Day-6	4/21/2013	10/100	0.89579158	0.81636727	0.91217565	0.874778166	0.05124436
Day-9	4/24/2013	10/100	0.87975952	0.87225549	0.87225549	0.874756832	0.00433245
Day-12	4/27/2013	10/100	0.87975952	0.86427146	0.87225549	0.872095488	0.00774527
Day-15	4/30/2013	10/100	0.87174349	0.83632735	0.8762475	0.861439446	0.02186401
4/15/2013 Entrapped SRB beads + Ethanol + 50 mg/L of Zn							
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	4/15/2013	10/100	1	1	1	1	0
Day-3	4/18/2013	10/100	0.93987976	0.80838323	0.93213573	0.893466241	0.07378571
Day-6	4/21/2013	10/100	0.84368737	0.88023952	0.88822355	0.870716816	0.02374614
Day-9	4/24/2013	10/100	0.85971944	0.8762475	0.88822355	0.874730166	0.01431251
Day-12	4/27/2013	10/100	0.86372745	0.85229541	0.88023952	0.865420795	0.0140488
Day-15	4/30/2013	10/100	0.87174349	0.88023952	0.88023952	0.87740751	0.00490519

Table A.19. Raw data from sulfate reduction experiments using entrapped SRB in presence of NZVI (see normalized data in Table A.20). Initial sulfate concentration was 500 mg/L.

5/9/2013 Entrapped SRB + Ethanol + 0.05 g of NZVI										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/9/2013	10/100	0.177	501.25	0.177	501.25	0.177	501.25	501.25	0
Day-3	5/12/2013	10/100	0.106	323.75	0.116	348.75	0.12	358.75	343.75	18.0277564
Day-6	5/15/2013	10/100	0.105	321.25	0.101	311.25	0.1	308.75	313.75	6.61437828
Day-9	5/18/2013	10/100	0.097	301.25	0.094	293.75	0.097	301.25	298.75	4.33012702
Day-12	5/21/2013	10/100	0.052	188.75	0.045	171.25	0.09	283.75	214.583333	60.5358021
Day-15	5/24/2013	10/100	0.009	81.25	0.01	83.75	0.01	83.75	82.9166667	1.44337567
5/13/2013 Entrapped SRB + Ethanol + 0.1 g of NVZI										
Time, days	**Test date**	**Dilution rate**	**λ1**	**1- SO_4^{2-}**	**λ2**	**2- SO_4^{2-}**	**λ3**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/13/2013	10/100	0.177	501.25	0.177	501.25	0.177	501.25	501.25	0
Day-3	5/16/2013	10/100	0.158	453.75	0.135	396.25	0.124	368.75	406.25	43.3733789
Day-6	5/19/2013	10/100	0.113	341.25	0.153	441.25	0.136	398.75	393.75	50.1871497
Day-9	5/22/2013	10/100	0.115	346.25	0.106	323.75	0.108	328.75	332.916667	11.8145391
Day-12	5/25/2013	10/100	0.052	188.75	0.071	236.25	0.113	341.25	255.416667	78.0357824
Day-15	5/28/2013	10/100	0.008	78.75	0.015	96.25	0.002	63.75	79.5833333	16.2660177

Table A.20. Normalized raw data from sulfate reduction experiments using entrapped SRB in presence of NZVI. Initial sulfate concentration was 500 mg/L (see raw data in Table A.19).

		5/9/2013	Entrapped SRB + Ethanol + 0.05 g of NZVI				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/9/2013	10/100	1	1	1	1	0
Day-3	5/12/2013	10/100	0.64588529	0.6957606	0.71571072	0.68578554	0.0359656
Day-6	5/15/2013	10/100	0.64089776	0.62094763	0.6159601	0.62593516	0.01319577
Day-9	5/18/2013	10/100	0.60099751	0.58603491	0.60099751	0.59600998	0.00863866
Day-12	5/21/2013	10/100	0.3765586	0.34164589	0.56608479	0.42809643	0.12076968
Day-15	5/24/2013	10/100	0.16209476	0.16708229	0.16708229	0.16541978	0.00287955
		5/13/2013	**Entrapped SRB + Ethanol + 0.1 g of NVZI**				
Time, days	**Test date**	**Dilution rate**	**1- SO_4^{2-}**	**2- SO_4^{2-}**	**3- SO_4^{2-}**	**Average- SO_4^{2-}**	**St. Dev.**
Day-0	5/13/2013	10/100	1	1	1	1	0
Day-3	5/16/2013	10/100	0.90523691	0.79052369	0.73566085	0.81047382	0.08653043
Day-6	5/19/2013	10/100	0.680798	0.88029925	0.79551122	0.78553616	0.10012399
Day-9	5/22/2013	10/100	0.69077307	0.64588529	0.65586035	0.6641729	0.02357015
Day-12	5/25/2013	10/100	0.3765586	0.4713217	0.680798	0.50955943	0.15568236
Day-15	5/28/2013	10/100	0.15710723	0.19201995	0.12718204	0.15876974	0.03245091

APPENDIX B. STATISTICAL ANALYSES DATA

B.1. Test 1

Testing for difference between treatments with and without carbon sources in sulfate removal by entrapped SRB in batch experiments. Initial sulfate concentration: 500 mg/L.

Two-way ANOVA: Sulfate versus Source, Day

Source	DF	SS	MS	F	P
Source	4	1317370	329342	189.07	0.000
Day	5	832269	166454	95.56	0.000
Interaction	20	416871	20844	11.97	0.000
Error	60	104517	1742		
Total	89	2671027			

S = 41.74 R-Sq = 96.09% R-Sq(adj) = 94.20%

Tukey's pairwaise comparison test

Individual 95% CIs For Mean Based on Pooled StDev

Pooled StDev = 126.2

Grouping Information Using Tukey Method (Means that do not share a letter are significantly different).

Source	N	Mean	Grouping
Blank	18	483.6	A
Control	18	376.4	A B
No carbon	18	341.2	B
Ethanol	18	196.3	C
Lactose	18	152.7	C

B.2. Test 2

Testing for difference between treatments with pH interference on sulfate removal by SRB entrapped in Ca-alginate beads in batch experiments. Initial sulfate concentration: 500 mg/L.

Two-way ANOVA: Sulfate versus Source, Day

Source	**DF**	**SS**	**MS**	**F**	**P**
Source	3	690359	230120	142.69	0.000
Day	5	825636	165127	102.39	0.000
Interaction	15	321644	21443	13.30	0.000
Error	48	77410	1613		
Total	71	1915050			

S = 40.16 R-Sq = 95.96% R-Sq(adj) = 94.02%

Tukey's pairwaise comparison test

Grouping Information Using Tukey Method (Means that do not share a letter are significantly different).

Source	**N**	**Mean**	**Grouping**
pH 2.0	18	420.0	A
pH 4.0	18	340.8	A
pH 5.1	18	196.3	B
pH 7.5	18	189.3	B

B.3. Test 3

Testing for difference between treatments with temperature interference on sulfate removal by SRB entrapped in Ca-alginate beads in batch experiments. Initial sulfate concentration: 500 mg/L.

Two-way ANOVA: Sulfate versus Source, Day

Source	**DF**	**SS**	**MS**	**F**	**P**
Source	3	478065	159355	159.33	0.000
Day	5	1057508	211502	211.47	0.000
Interaction	15	293560	19571	19.57	0.000
Error	48	48008	1000		
Total	71	1877140			

S = 31.63 R-Sq = 97.44% R-Sq(adj) = 96.22%

Tukey's pairwaise comparison test

Grouping Information Using Tukey Method (Means that do not share a letter are significantly different).

Source	**N**	**Mean**	**Grouping**
5^{o}C	18	398.5	A
15^{o}C	18	263.3	B
30^{o}C	18	201.7	B
22^{o}C	18	196.3	B

B.4. Test 4

Testing for difference between treatments with aluminum interference on sulfate removal by SRB entrapped in Ca-alginate beads in batch experiments. Initial sulfate concentration: 500 mg/L.

Two-way ANOVA: Sulfate versus Source, Day

Source	**DF**	**SS**	**MS**	**F**	**P**
Source	3	823948	274649	106.35	0.000
Day	5	723660	144732	56.04	0.000
Interaction	15	332537	22169	8.58	0.000
Error	48	123965	2583		
Total	71	2004110			

S = 50.82 R-Sq = 93.81% R-Sq(adj) = 90.85%

Tukey's pairwaise comparison test

Grouping Information Using Tukey Method (Means that do not share a letter are significantly different).

Source	**N**	**Mean**	**Grouping**
50 mg/L	18	435.3	A
25 mg/L	18	348.8	A
No metal	18	196.3	B
0.5 mg/L	18	178.5	B

B.5. Test 5

Testing for difference between treatments with copper interference on sulfate removal by SRB entrapped in Ca-alginate beads in batch experiments. Initial sulfate concentration: 500 mg/L.

Two-way ANOVA: Sulfate versus Source, Day

Source	**DF**	**SS**	**MS**	**F**	**P**
Source	3	61045	20348	8.19	0.000
Day	5	1845142	369028	148.46	0.000
Interaction	15	83390	5559	2.24	0.018
Error	48	119315	2486		
Total	71	2108892			

S = 49.86 R-Sq = 94.34% R-Sq(adj) = 91.63%

Tukey's pairwaise comparison test

Grouping Information Using Tukey Method (Means that do not share a letter are significantly different).

Source	**N**	**Mean**	**Grouping**
50 mg/L	18	245.2	A
No metal	18	196.3	A
0.5 mg/L	18	194.7	A
25 mg/L	18	163.9	A

B.6. Test 6

Testing for difference between treatments with zinc interference on sulfate removal by SRB entrapped in Ca-alginate beads in batch experiments. Initial sulfate concentration: 500 mg/L.

Two-way ANOVA: Sulfate versus Source, Day

Source	**DF**	**SS**	**MS**	**F**	**P**
Source	3	1192616	397539	1181.79	0.000
Day	5	674710	134942	401.15	0.000
Interaction	15	434118	28941	86.04	0.000
Error	48	16147	336		
Total	71	2317591			

S = 18.34 R-Sq = 99.30% R-Sq(adj) = 98.97%

Tukey's pairwaise comparison test

Grouping Information Using Tukey Method (Means that do not share a letter are significantly different).

Source	**N**	**Mean**	**Grouping**
50 mg/L	18	448.8	A
25 mg/L	18	446.1	A
No metal	18	196.3	B
0.5 mg/L	18	184.1	B