Biologically-mediated, Simultaneous Removal of Nitrate and Arsenic from Drinking Water Sources

by

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This dissertation is dedicated to my parents,

Acharya Khem Raj Keshavasharan Dahal and Sharada Devi Upadhyaya

without whom nothing would have been possible.

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Abstract

Nitrate and arsenic frequently co-exist in natural water sources. While conventional drinking water treatment technologies fail to provide simultaneous removal of these contaminants, advanced technologies, such as reverse osmosis and ion exchange often are cost prohibitive. Furthermore, prevailing arsenic removal technologies are not sustainable as the arsenic-laden sludge releases arsenic under landfill conditions. It is therefore imperative to develop a treatment system that simultaneously removes these contaminants with minimum waste production.

Utilizing microorganisms originating from natural groundwater, a train of two fixed-bed biologically active carbon (BAC) reactors removed 50 mg/L NO_3^- and 200 to 300 µg/L As to below the detection limit of 0.2 mg/L NO_3^- and less than 10 µg/L As, respectively, at a total empty bed contact time (EBCT) of 30 min. Dissolved oxygen, nitrate, arsenate, and sulfate were utilized sequentially along the flow direction. Arsenic was removed by co-precipitation and adsorption on biologically generated iron sulfides (mackinawite) or precipitation of arsenic sulfides. While sulfate reducing bacteria (SRB) closely related to complete oxidizers from the *Desulfobacteraceae* family dominated the system, three distinct clusters of

dissimilatory arsenate reducing bacteria (DARB) were detected with a predominance of *Geobacter uraniireducens-like* DARB. Both SRB and DARB were distributed throughout the reactors. After complete denitrification in the upper part of reactor A, sulfate and arsenate reducing activity co-existed and increased along the flow direction. After attaining a maximum level in the middle of the second reactor, both sulfate- and arsenate- reducing activity declined. The microbial community responded to changes in operational parameters and lowering the EBCT of reactor A resulted in a shift of sulfate reducing zone towards the second reactor. The co-location of sulfate- and arsenate reduction, iron(II) availability, and the generation of fresh iron sulfides were the key parameters for sustained arsenic removal. Lowering the phosphorus level in the influent from 0.5 to 0.2 and to 0.1 mg/L P resulted in improved arsenic removal. Reactor performance was unaffected when air replaced nitrogen gas during backwashing of the first reactor. Overall, this research demonstrated the effectiveness of anaerobic bioreactors for the simultaneous removal of nitrate and arsenic and emphasized the need for the integration of molecular studies in understanding reactor performance.

Chapter 1

Introduction

1.1 Introduction

With the increasing population and urbanization throughout the world, water has become one of the most critical resources. The profligate use and unabated pollution of water resources aggravates the pressure on fresh water resource management. To cope with the ever increasing demand of water supply for domestic, agricultural and industrial needs, sustainable development calls for more efficient and equitable allocations of groundwater and surface water sources. In this context, it is paramount to regenerate contaminated water sources while continuing to explore new alternative sources utilizing environmentally sustainable technologies.

Regeneration of existing water sources contaminated with various oxy-anionic pollutants including arsenic (arsenate and arsenite), nitrate, perchlorate, bromate, chromate, selenate, and uranium (uranate) has been a top priority in the context of providing safe drinking water. Originating from anthropogenic

and/or geogenic sources, occurrence of these contaminants is a global problem. For example, nitrate levels more than the regulated concentration (maximum contaminant level (MCL) 10 mg/L NO_3^- as N) have been reported in developed (Hudak, 2003; van Maanen et al., 2001) as well as developing countries (Guha et al., 2005; Khatiwada et al., 2002). Likewise, the presence of arsenic in groundwater ranging from 0.5 to 5,000 µg/L (Smedley and Kinniburgh, 2002) has been reported around the world (Dou et al., 2006; Yokota et al., 2001; Zahid et al., 2008). The co-existence of two or more of these contaminants (Hudak, 2003; USGS, 2004) aggravates the problem and water utilities are facing increased challenges in providing safe drinking water. Lack of knowledge, inadequate technologies, and improper management practices have compounded the challenges in developing countries as millions of people are exposed to these contaminants through their drinking water (Argos et al., 2010). For example, in several countries in South East Asia, including India, Bangladesh, and Nepal, high concentrations of arsenic exist in groundwater (Bittner et al., 2002; Zahid et al., 2008). In addition, extensive fertilization and unmanaged irrigation (Behera et al., 2003) in these countries result in the presence of nitrate in groundwater. Depth-specific profile studies have shown the co-existence of arsenic and nitrate in groundwater in Kathmandu Valley in Nepal (Khatiwada et al., 2002) and West Bengal in India (Guha et al., 2005). Poor sanitary practices and sewage management add to the problem of nitrate leaching into the groundwater in these areas (Dongol et al., 2005). The presence of one or a combination of these contaminants in drinking water sources often results in closure of wells

(Jahagirdar, 2003; Rosen et al., 2004) or the need for expensive, multi-step treatment.

Regulatory pressures or anticipated regulations have resulted in the development of technologies that are suitable for treating nitrate (Gros et al., 1986; Kappelhof et al., 1992) or arsenic (Lehimas et al., 2001; Takanashi et al., 2004) in isolation. However, the co-existence of multiple contaminants necessitates the development of a single-unit treatment system with a small footprint that is affordable and can remove multiple contaminants while producing limited and safely disposable wastes. As such, the crux of this research is an extensive effort to assess the possibility of utilizing a fixed-bed biologically active carbon (BAC) reactor system for simultaneous removal of nitrate and arsenic from drinking water sources.

Conventional treatment technologies, such as coagulation and filtration fail to provide simultaneous removal of nitrate and arsenic. Advanced treatment technologies, such as reverse osmosis and ion exchange may be successful in this regard (Min et al., 2005), but these processes are limited due to the requirement of regeneration of exhausted materials and treatment of concentrated waste streams (Nerenberg and Rittmann, 2004). In contrast, biological processes often achieve consistent contaminant removal while avoiding the need for regeneration of solid phase sorbents or treatment of the generated wastes. In addition, many organic and inorganic contaminants can be converted to innocuous compounds (Brown, 2007).

Besides the inadequacy of the conventional technologies for simultaneous removal of nitrate and arsenic, prevailing arsenic removal technologies are not sustainable. Existing arsenic removal technologies generally utilize oxy-hydroxides of iron (Driehaus et al., 1998; Tyrovola et al., 2007) or aluminum (Singh and Pant, 2004; Takanashi et al., 2004), which are very effective in sequestering arsenic. However, under landfill conditions, arsenic sorbed to iron or aluminum oxy-hydroxides is released due to microbially mediated iron(III) (Ghosh et al., 2006; Irail et al., 2008) or arsenate (As(V)) (Sierra-Alvarez et al., 2005; Zobrist et al., 2000) reduction. Therefore, it is imperative to develop a treatment system that simultaneously removes nitrate and arsenic while preventing the release of arsenic from the generated sludge under landfill conditions.

Biological denitrification is a long established treatment technology that utilizes microorganisms to convert nitrate to dinitrogen gas using organic or inorganic electron donor substrates (Li et al., 2010; Mateju et al., 1992; Soares, 2000). Arsenic, however, can only be removed from drinking water through phase transfer, i.e., by converting soluble arsenic into solid phase arsenic. Arsenate reducing bacteria reduce arsenate (As(V)) to arsenite (As(III)) species, which may react with sulfides resulting in the precipitation of an arsenic sulfide phase such as orpiment (As_2S_3) (Newman et al., 1997) or realgar (AsS) (Ledbetter et al., 2007). In addition, in an environment containing both iron and sulfide, arsenic can be removed from water through adsorption/co-precipitation with iron sulfides (Bostick and Fendorf, 2003; Wilkin and Ford, 2006).

1.2 Hypotheses and Objectives

Capitalizing on the biologically mediated transformations of nitrate, sulfate, and arsenic followed by the precipitation of arsenic or iron sulfides, the overarching objective of this study was to develop a train of two biologically active carbon (BAC) bioreactors for the simultaneous removal of nitrate and arsenic from groundwater. It was hypothesized that biological nitrate, sulfate and arsenate reduction can be promoted in the system by using microbial inocula originating from natural groundwater and that the generation of a stable redox gradient across the filter beds would result in the sequential use of dissolved oxygen, nitrate, arsenate, and sulfate. It was further hypothesized that iron(II) would react with biologically generated sulfides resulting in the precipitation of iron sulfides, which concomitantly would remove arsenic through co-precipitation or adsorption mechanisms. Precipitation of arsenic sulfides would further enhance arsenic removal.

Two fixed-bed biofilm reactors were set up and operated in series to remove nitrate and arsenic simultaneously from a synthetic groundwater. Combining different methodologies developed by a variety of disciplines, including water quality process engineering, environmental chemistry, material science, microbial ecology, and molecular biology, this research evaluated bioreactor process parameters, including the addition of electron donor (acetate), iron(II), and phosphorous, selection of empty bed contact time (EBCT), and backwash strategy to study the potential of the system to remove the contaminants.

Microbial communities were characterized and reactor performance was linked to microbial information to optimize the reactor system.

1.3 Dissertation organization

This dissertation consists of eight chapters. Chapters 3-6 were written as independent chapters and were prepared for publication as peer-reviewed journal publications. In addition to the background information and literature review provided in Chapter 2, each of these chapters provides an introduction with literature review relevant to the topics covered in the respective chapters.

This introductory chapter provides a brief description of the problem and the motivation for the research and describes the objectives and hypotheses. Chapter 2 provides detailed background on arsenic and nitrate contamination of groundwater and the related health effects of long-term exposure to these contaminants through drinking water. The available treatment technologies and the associated problems are also discussed providing the rationale behind the current research. Chapter 3, recently published in the journal *Water Research* (Upadhyaya et al., 2010), provides the proof of concept of the bioreactor system for the simultaneous removal of nitrate and arsenate from contaminated drinking water sources. Characterization of the microbial community present in the system and the spatial distribution and activity of sulfate and arsenate reducing bacteria are presented in Chapter 4. This chapter was prepared for consideration for publication in the journal *Applied and Environmental Microbiology*. Chapter 5 was prepared for publication in the journal *Water*

Research and explores the optimization of the EBCT for arsenic and nitrate removal. Relating microbial information to reactor performance, this study identified the minimum EBCT at which the reactor could be operated without compromising reactor performance. Additional operational parameters considered include influent concentrations of electron donor, iron, nitrate, and arsenic. Chapter 6 covers a comparative study utilizing either nitrogen gas or compressed air for backwashing the reactors. The overall goal of this analysis was to evaluate the feasibility of using air rather than nitrogen gas during backwashing, which would be preferable for full-scale operation due to the associated advantages, such as ease of operation, safety, and low operation cost. Chapter 7 explores the impact of phosphorus levels on reactor performance. Integrating computer simulations (MINEQL+), this chapter evaluates the effects of phosphate levels in the influent on the production of arsenic and iron sulfide solids that are considered to be the primary solids needed for effective arsenic removal. This chapter was prepared for consideration for publication in the journal *Environmental Science and Technology.* Finally, Chapter 8 summarizes the conclusions, discusses the practical implications of the research, and provides future research needs motivated by the result of this study.

1.4 References

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Chapter 2

Background

The purpose of this chapter is to provide background on nitrate and arsenic contamination of groundwater, health effects associated with these contaminants, microbially mediated reactions and existing treatment technologies and associated problems. With this background, this chapter establishes the research context. Biological denitrification is a well-studied and proven technology and is not covered in detail in this chapter. Rather the emphasis here is given to the potential of biologically mediated arsenic removal under reducing conditions in comparison to existing technologies for arsenic removal.

2.1 Problem Statement

Contamination of natural water sources with various oxy-anionic pollutants, including arsenic (arsenate and arsenite), nitrate, perchlorate, bromate, chromate, selenate, and uranium (urinate, (U(VI)), has been of major concern throughout the world in the context of providing safe drinking water. Regulatory pressures and anticipated regulations have resulted in the development of many treatment technologies (Mohan and Pittman Jr, 2007; Pintar and Batista,

2006; Pintar et al., 2001; Takanashi et al., 2004) for the removal of these contaminants. However, not only has the isolated existence of these contaminants been reported, but two or more of these contaminants commonly co-exist in natural water bodies (Fytianos and Christophoridis, 2004; Ghurye et al., 1999; Hudak, 2003; Hudak and Sanmanee, 2003; Seidel et al., 2008; Tellez et al., 2005). The co-existence of multiple contaminants in source waters for drinking water production makes it imperative to develop treatment systems that provide simultaneous removal of multiple contaminants.

2.2 Prevalence of Nitrate and Arsenic Contamination

Contamination of groundwater with nitrate is a global problem. Nitrate concentrations greater than the regulated level (maximum contaminant level (MCL) 10 mg/L as NO_3^--N) have been reported not only in the United States (Hudak, 1999; Hudak and Sanmanee, 2003), but also in other parts of the world, including in the Netherlands (van Maanen et al., 2001), Nigeria (Egereonu and Ibe, 2004), South Africa (Tredoux and du Plessis, 1992), Palestine (Almasri and Ghabayen, 2008), Chile (Arumi et al., 2005), Nepal (Shrestha and Ladah, 2002), and India (Guha et al., 2005). Nitrate contamination of water sources may result from human activities as well as non-anthropogenic causes, such as evaporative deposition, biological N-fixation, or geological sources (Stadler et al., 2008). Anthropogenic activities may include non-point sources, such as runoff from agricultural fields after application of fertilizers, and point sources, such as concentrated animal feeding operations and municipal wastewater treatment plants (Behera et al., 2003; Dongol et al., 2005; Khatiwada et al., 2002).

The problem of arsenic contamination of water bodies is equally widespread (Mandal and Suzuki, 2002; Nordstrom, 2002). In Bangladesh alone about 40 million people are at risk of arsenic poisoning (Argos et al., 2010; Zahid et al., 2008). Many other countries, including India (Gault et al., 2005), the United States (Utsunomiya et al., 2003), Argentina (Paoloni et al., 2005), China (Dou et al., 2006), Botswana (Huntsman-Mapila et al., 2006), Canada (Wang and Mulligan, 2006), Greece (Kouras et al., 2007), Taiwan (Liu et al., 2006), Nepal (Shrestha et al., 2003), Belgium (Cappuyns et al., 2002), Croatia (Habuda-Stanic et al., 2007), Mexico (Planer-Friedrich et al., 2001), and Germany (Zahn and Seiler, 1992), are also severely affected by arsenic contamination of water bodies.

Localized point sources, including industrial waste disposal, coal combustion, runoff from mine tailings, pigment production for paints and dyes, and processing of pressure-treated wood are a few of the anthropogenic sources of arsenic contamination (Oremland and Stolz, 2003). In contrast, wide spread arsenic contamination is often related to geogenic sources, such as weathering of arsenic bearing rocks, geothermal waters, and volcanic eruptions (Oremland and Stolz, 2003). Arsenic present in natural environments may be mobilized due to biological activities (Bose and Sharma, 2002; Ghosh et al., 2006), reductive dissolution of oxides (Guha et al., 2005; Keimowitz et al., 2005; Smedley and Kinniburgh, 2002), and oxidative dissolution of sulfides (Guha et al., 2005).

Adding complexity to the problem of groundwater contamination with nitrate or arsenic in isolation is their co-existence in many locations. For example, the

groundwater of Atacama Desert in Northern Chile (Cities of Taltal, Chanaral, and Antofagasta) (Tellez et al., 2005) and the Ogallala aquifer of Texas contain both nitrate and arsenic along with perchlorate (Huston et al., 2002). Groundwaters in Northern Greece (Fytianos and Christophoridis, 2004), Ripon (California) (Seidel et al., 2008), Oakland County (Michigan) (USGS, 2004), Gulf Coast Aquifer (South Central Texas) (Hudak, 2003), and McFarland (California) (Ghurye et al., 1999) also contain both arsenic and nitrate. In several South Asian countries (e.g., Bangladesh, India, and Nepal), where arsenic contamination of groundwater exposes tens of millions of people to this contaminant through drinking water (Argos et al., 2010) as discussed above, nitrate leaching to groundwater is also likely widespread due to mismanaged fertilization and irrigation practices (Behera et al., 2003). For example, in Kathmandu Valley (Nepal) and West Bengal (India), depth-specific profile studies have shown arsenic and nitrate contamination (Guha et al., 2005; Khatiwada et al., 2002). In addition to this, poor sanitary practices and sewage management add to the problem of nitrate leaching into the groundwater in these areas (Dongol et al., 2005). The common co-existence of nitrate and arsenic in source waters for drinking water production makes it desirable to develop treatment systems that provide simultaneous removal of these contaminants.

2.3 Arsenic in the Environment

Arsenic is a ubiquitous metalloid (Mohan and Pittman Jr, 2007) and exists in -III, 0, +III, and +V oxidation states (Oremland and Stolz, 2003). In natural environments, inorganic arsenic exists primarily in the As(V) and As(III) forms

(Cullen and Reimer, 1989). The pentavalent forms of arsenic (i.e., H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-}) are the most abundant species in oxidizing environments, while the trivalent forms of arsenic (i.e., H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$ and AsO_3^{3-}) are the dominant species under reducing conditions (Oremland and Stolz, 2003). Iron(III)- and aluminum hydroxides are most commonly involved in adsorption of arsenic in natural environments (Cheng et al., 2009). However, under sulfate reducing conditions, amorphous sulfides and sulfide minerals, such as greigite (Fe_3S_4), mackinawite (tetragonal iron sulfide, FeS_{1-x}), and pyrite (FeS_2) can be important sinks for arsenic (Welch et al., 2000). In the presence of sulfides, generated biologically or chemically, arsenic may also exist as thioarsenate ($HAsO_3S^{2-}$, $HAsO_2S_2^{2-}$, $AsOS_3^{3-}$) (Stauder et al., 2005) and/or thioarsenite ($As(OH)_2(HS)$, $As(OH)_2S^-$, AsS_3^{3-}, AsS_3H^{2-}, and $As(HS)_4^-$) complexes. In addition, biomethylation of arsenic can result in the formation of monomethylarsonic acid ($CH_3AsO(OH)_2$; MMA(V)), dimethylarsinic acid ($(CH_3)_2AsO(OH)$; DMA(V)), trimethylarsine oxide ($(CH_3)_3AsO$; TMAO(V)), monomethylarsinous acid ($CH_3As(OH)_2$; MMA(III)), dimethylarsinous acid ($(CH_3)_2As(OH)$; DMA(III)), monomethylarsine (AsH_2CH_3; MMA), dimethylarsine ($AsH(CH_3)2$; DMA), and trimethylarsine ($As(CH_3)_3$; TMA) (Bright et al., 1994; Challenger, 1945).

2.4 Health Effects of Nitrate and Arsenic

The presence of high levels of nitrate in drinking water can lead to blue-baby syndrome (Knobeloch et al., 2000), diuresis, and hemorrhaging of the spleen (http://www.epa.gov/safewater/pdfs/factsheets/ioc/tech/nitrates.pdf).

Reduction of nitrate into nitrite in saliva may contribute to the formation of nitrosamines, which are known carcinogens (Mateju et al., 1992; Soares, 2000). The World Health Organization's (WHO) guideline value for nitrate in drinking water is 50 mg/L as NO_3^- (Chettri and Smith, 1995). Based on this guideline, the U.S. EPA has set a maximum contaminant level (MCL) for nitrate in drinking water at 10 mg/L NO_3^- as N. The European Union (EU) standard for nitrate in drinking water is 50 mg/L as NO_3^- (Chettri and Smith, 1995).

The toxicity of arsenic varies dramatically with the chemical form in which arsenic exists. While inorganic arsenite and arsenate are highly toxic, MMA(V) and DMA(V) are slightly less toxic (Nriagu, 1994). However, the methylated trivalent arsenicals, MMA(III)) and DMA(III), are more toxic than the inorganic arsenicals as they are more efficient in causing DNA damage (Wang and Mulligan, 2006). Compared to the inorganic As(V) and As(III) species, MMA(III) and DMA(III) impart more enzyme inhibition and cytotoxicity (Styblo et al., 2002). The greater toxicity of MMA(III) compared to As(III) may be due to its higher affinity for thiol ligands in biological binding sites (Sharma and Sohn, 2009). Wang and Mulligan (Wang and Mulligan, 2006) listed the order of DNA damaging capacity of the arsenic compounds as DMA(III) > MMA(III) > As(III) or As(V) > MMA(V) > DMA(V) > TMAO(V). Trivalent arsenic compounds, such as arsenic trioxide (As_2O_3), orpiment (As_2S_3), and sodium arsenite ($NaAsO_2$) are generally more toxic than pentavalent arsenic compounds, such as arsenic pentoxide (As_2O_5), sodium arsenate (Na_2HAsO_4), and calcium arsenate $Ca_3(AsO_4)_2$. The trivalent form of arsenic is about 60 times more poisonous than arsenate (Kundu

et al., 2004). Arsine gas (AsH_3) is the most toxic among all the arsenic compounds of the trivalent form (Planer-Friedrich, 2004).

In reference with epidemiological data, inorganic arsenicals have been classified as Group I carcinogens (DeSesso et al., 1998; Pontius et al., 1994). A wide variety of adverse health effects, including several cancers, cardiovascular diseases, and neurological effects have been attributed to chronic exposure to high levels of arsenic, primarily through drinking water (Mohan and Pittman Jr, 2007). Cancer end-point diseases, typically skin, bladder, and lung cancers, and non-cancerous diseases, such as hypertension, cardiovascular diseases and diabetes are some of the clinical manifestations of chronic arsenic exposure. Long-term exposure to inorganic arsenic has also been linked to peripheral neuropathy (Ng et al., 2003). Black foot disease has been the most severe manifestation associated with chronic exposure to high levels of arsenic in drinking water (Ng et al., 2003; Sun, 2004).

Arsenate (As(V)) is a molecular analog of phosphate and inhibits oxidative phosphorylation. Arsenate enters the body through phosphate transporters (Salmassi et al., 2002). Since arsenite (As(III)) binds to sulfhydryl groups, many proteins are inactivated by As(III) (Oremland and Stolz, 2003). Thioarsenic species, which already have –SH groups, are thought to be less toxic than other As(III) solution complexes (Stauder et al., 2005; Wilkin and Ford, 2006).

The WHO guideline value, the U.S. EPA established MCL, and the European Union limit for arsenic in drinking water is 10 µg/L (Mohan and Pittman

Jr, 2007). While India has adopted an MCL of 10 µg/L for arsenic in drinking water (Mohan and Pittman Jr, 2007), the permissible level of arsenic in drinking water in Bangladesh and Nepal is 50 µg/L (Mohan and Pittman Jr, 2007; Shrestha et al., 2003).

2.5 Microbiologically Mediated Processes and Contaminant Removal

To utilize microbiological reduction processes for contaminant removal from water sources, it is necessary to stimulate and maintain desired active microbial populations in bioreactors. In general, this is accomplished by supplying an appropriate energy source (an electron donor), such as acetate. The available electron acceptors are utilized sequentially, depending on the metabolic capabilities of the microorganisms established in a reactor system.

Redox or electron transfer reactions involve the transfer of an electron from a reductant (electron donor) to an oxidant (electron acceptor). In natural or engineered environments, the presence of various electron donors, electron acceptors, and microorganisms can be exploited to facilitate contaminant removal. Microbially mediated redox reactions can be effectively controlled by providing electron donors and acceptors (Lovley and Chapelle, 1995). Microorganisms have developed various strategies for energy generation based on the availability of a suitable electron acceptor. Such strategies include aerobic respiration (oxygen reduction), denitrification (nitrate reduction), iron(III) reduction, manganese(IV) reduction, sulfate reduction, arsenate reduction, and CO_2 reduction. While these redox conversions involve a series of complex

electron transfers within the microorganisms, they ultimately result in the transfer of electrons from the substrate (electron donor) to the available electron acceptor. Such microbiologically driven electron transfer processes are called terminal electron accepting processes (TEAPs) (Lovley and Chapelle, 1995).

In groundwater, the thermodynamically dictated sequential uptake of the commonly available electron acceptors (dissolved oxygen (DO), nitrate, iron(III), manganese, and sulfate) results in segregation of different TEAP zones spanning from aerobic to anaerobic conditions (Lovley and Chapelle, 1995). However, physiological constraints and competition for the available substrates may modify the theoretically determined TEAPs sequence. For example, facultative bacteria can utilize oxygen under aerobic conditions, while growth can still be sustained utilizing nitrate in the absence of oxygen. However, strict anaerobic bacteria are inhibited in an aerobic environment. Additionally, concentrations of the available electron acceptors may also modify the TEAPs sequence. Canfield et al. (1993) reported iron and sulfate utilization prior to Mn(IV), the thermodynamically preferable electron acceptor, when manganese levels were lower in the sediments. In contrast, only manganese reduction occurred when manganese levels were relatively high.

In general, when DO, nitrate, iron(III), sulfate, and arsenate are present and an electron donor (e.g., acetate) is available, a series of sequential and energetically favorable TEAPs will be established starting with aerobic respiration.

2.5.1 Aerobic Respiration

$CH_3COO^- + 2O_2 \rightarrow 2HCO_3^- + H^+$ ($\Delta G°' = -844$ kJ/mole Ac^-) (Lovley and Phillips, 1988)

Aerobic respiration, coupling the oxidation of an electron donor with oxygen as the electron acceptor, is thermodynamically the most favorable of the TEAPs. Microorganisms gain substantial energy for cell growth through the mediation of this redox reaction (Lovley and Chapelle, 1995). Aerobic as well as facultative bacteria have the capability to mediate this reaction and are ubiquitous in natural environments. Such bacteria can completely oxidize a plethora of organic substrates ranging from natural to manmade compounds (Lovley and Chapelle, 1995). Additionally, some of these microorganisms can utilize inorganic electron donors, such as Fe(II), ammonium, elemental sulfur, and Mn(II) (Lovley and Chapelle, 1995).

2.5.2 Iron(III) Respiration

$CH_3COO^- + 8Fe^{3+} + 3H_2O \rightarrow HCO_3^- + 8Fe^{2+} + 8H^+ + CO_2$ ($\Delta G°' = -814$ kJ/mole Ac-) (Lovley and Phillips, 1988)

Iron is universally present in most of the aquatic ecosystems and dissimilatory iron(III) reduction is recognized as one of the key microbiological processes that define the biogeochemistry of such ecosystems. Microorganisms with the capacity of Fe(III) reduction are phylogenetically dispersed throughout the domains of Bacteria and Archaea (Lovley et al., 2004). Many fermentative

bacteria, such as *Clostridium pasteurianum,* and *Lactobacillus lactis* (Lovley et al., 2004), are capable of Fe(III) reduction (Lovley et al., 2004). In contrast, dissimilatory iron(III) reducing bacteria (DIRB) conserve substantial energy from the mediation of electron transfer from an organic substrate to Fe(III).

DIRB are generally grouped in accordance with their substrate requirement and their capability to completely oxidize an organic compound to CO_2 (Coates et al., 1996; Nielsen et al., 2002). Members of *Geobacter*, *Geovibrio*, *Desulfuromonas*, and *Desulfuromusa* are examples of DIRB that completely oxidize an organic substrate to CO_2, while *Pelobacter* and *Shewanella* species are incomplete oxidizers (Coates et al., 1996). Most of the known DIRB are members of the *Deltaproteobacteria* (Geobacter, *Desulfuromonas*, and *Pelobacter*) and *Gammaproteobacteria* (*Shewanella* and *Pseudomonas*), and the *Geovibrio* genus (Lonergan et al., 1996; Nielsen et al., 2002). A few DIRB exhibit diverse metabolic capabilities and can utilize DO, nitrate (Coates et al., 1998; Lovley et al., 2004), manganese (Mn(IV)) (Coates et al., 1998; Lovley et al., 2004; Roden and Lovley, 1993), and sulfate (Ramamoorthy et al., 2006) as electron acceptors.

2.5.3 Biological Denitrification

$CH_3COO^- + 8/5NO_3^- + 3/5H^+ \rightarrow 2HCO_3^- + 4/5H_2O + 4/5N_2$ ($\Delta G^{\circ\prime}$ = -792 kJ/mole Ac^-) (Rikken et al., 1996)

Denitrifying bacteria, a ubiquitous and phylogenetically diverse group of facultative anaerobic bacteria, mediate the transfer of electrons from an electron

donor to nitrate and acquire energy for growth (Mateju et al., 1992; Soares, 2000). Both autotrophic (Gros et al., 1986; Ho et al., 2001) and heterotrophic (Gibert et al., 2008; Kappelhof et al., 1992; Satoh et al., 2006) denitrifying bacteria have been described. *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Azospirillum*, *Beggiatoa*, *Clostridium*, *Desulfovibrio*, *Propionibacterium*, *Pseudomonas*, *Azospira*, *Dechloromonas*, and *Thiobacillus* are a few of the genera that include nitrate reducing bacteria (Mateju et al., 1992).

Denitrifying bacteria exhibit diverse metabolic capability with respect to electron acceptors, including capabilities to utilize DO, nitrate, iron (III), bromate (Hijnen et al., 1999), selenate (Lortie et al., 1992), selenite (Lortie et al., 1992), and perchlorate (Li et al., 2010a; Nerenberg and Rittmann, 2002). Though denitrifying bacteria can utilize a wide variety of organic electron donors, including methanol, ethanol, acetate, glucose, aspartate, formic acid, molasses, and whey, most of the denitrification processes related to drinking water treatment systems utilize methanol, ethanol and acetate (Brown et al., 2005; Khardenavis et al., 2007; Li et al., 2010a). Gibert et al. (2008) evaluated the possibility of utilizing natural organic substrates (softwood, hardwood, coniferous twigs and leaves, mulch, willow wood chips, compost and leaves) in permeable reactive barrier for the bioremediation of groundwater contaminated with nitrate. Operating batch and continuous flow reactors, they demonstrated >95% nitrate removal with all the substrates evaluated. Softwood was the substrate of choice as complete denitrification was observed without the generation of nitrite or ammonia. Autotrophic denitrifying bacteria can utilize H_2 (Chung et al., 2006;

Hoeft et al., 2007), arsenite (Hoeft et al., 2007; Sun et al., 2009), iron(II) (Sun et al., 2009), and sulfide (Hoeft et al., 2007) as an electron donor.

Conversion of nitrate to N_2 gas proceeds through intermediates: NO_3^-, NO_2^- NO, and N_2O in sequence (Aslan and Cakici, 2007) and each step is catalyzed by a different enzyme (Mateju et al., 1992). The first step is catalyzed by membrane-bound nitrate reductase (NaR), while nitrite reductase (NiR) (membrane bound or cytoplasmic) mediates the conversion of nitrite (NO_2^-) to nitric oxide (NO). Nitrous oxide (N_2O) is produced by nitric oxide reductase (NOR). Finally, nitrous oxide reductase (N_2OR) mediates the final step converting N_2O to dinitrogen gas (N_2).

Though biological denitrification has been practiced for years in wastewater treatment (Dhamole et al., 2008; Mateju et al., 1992) and water treatment (Aslan and Cakici, 2007; Gros et al., 1986), more recently the production of NO and N_2O gases has drawn attention. N_2O has a greenhouse gas effect equivalent to 300 times that of CO_2 (IPCC, 2000). Both N_2O (Ravishankara et al., 2009) and NO (Huijie and Chandran, 2010) contribute to the depletion of the ozone layer.

N_2O emission has been linked to agricultural soils (Whalen, 2000), landfills (Borjesson and Svensson, 1997; Rinne et al., 2005), rivers (McMahon and Dennehy, 1998), and biological denitrification in wastewater treatment plants (Ahn et al., 2010; Kimochi et al., 1998). N_2O emission is observed both during nitrification and denitrification (Tallec et al., 2006) and both autotrophic and heterotrophic bacteria mediate the release of N_2O gas during denitrification

(Tallec et al., 2006; Yu et al., 2010). While minimal N_2O emission is generally observed during optimum operational conditions, change in operational parameters such as pH (Daum and Schenk, 1998; Focht, 1974), electron donor limitation, increase in concentrations of nitrite, and DO may enhance N_2O emission. However, Huijie and Chandran (2010) recently observed that the limitation of electron donor as well as increased nitrite levels did not increase N_2O emission in two sequencing batch reactors fed with methanol and ethanol, respectively. Instead, increased levels of DO resulted in substantial emission of N_2O from the reactor fed with ethanol, while no effect was observed in the methanol-fed reactor. Adouani et al. (2010) also observed that N_2O emission varied with the electron donor used; acetate caused more N_2O release compared to ethanol, casein extract, and meat extract. Additionally, they reported that NO levels may also impact N_2O emission. Interestingly, Ahn et al. (2010) reported higher N_2O emission in the aerobic zone of a biological nutrient removal (BNR) system compared to the anoxic zone. The recovery from low DO conditions might trigger N_2O emission, while a sudden increase in DO levels in the presence of high levels of ammonia resulted in the generation of NO_2^-, which consequently enhanced N_2O production (Ahn et al., 2010).

2.5.4 Microbiologically Mediated Arsenic Transformations

Biological processes can significantly affect distribution of arsenic species in natural environments through the processes of accumulation (Joshi et al., 2008; Say et al., 2003) and transformation (Oremland et al., 2005; Rhine et al., 2008). Many reviews can be found on arsenic biogeochemical cycling starting in the

1970s (Ferguson and Gavis, 1972; Peterson and Carpenter, 1983). Lièvremont et al. (2009) recently presented an extensive review on arsenic cycling in natural environments. In addition to conversion processes for detoxification, some microorganisms also facilitate arsenic species transformation reactions, such as arsenate reduction and arsenite oxidation, to generate energy for their growth.

2.5.4.1 Arsenate Reduction

Arsenate reduction can be related to the derivation of energy for metabolism (Macy et al., 1996; Newman et al., 1997b) or for detoxification (Chang et al., 2007; Li and Krumholz, 2007). These two processes are described in the following two paragraphs.

2.5.4.1.1 Arsenate Reduction: a Detoxification Process

Arsenic is toxic to microorganisms and the detoxification mechanism utilized by a wide variety of microorganisms involves the reduction of As(V) to As(III) within the cytoplasm and the subsequent expulsion of the reduced product utilizing a transmembrane efflux pump (Lièvremont et al., 2009; Rosen, 2002). Though microbial As(V) reduction generates the more toxic As(III), the ability of microorganisms to transport arsenite across the cell membrane apparently is an effective method of detoxification. The *ars* operon, implicated in detoxification, is the most extensively studied arsenic resistance mechanism and consists of at least three protein-coding genes: the transcriptional repressor *arsR*, the transmembrane efflux pump *arsB*, and the arsenate reductase *arsC (Oremland and Stolz, 2003; Páez-Espino et al., 2009)*. The *ars* operon in Gram negative

bacteria, such as *Escherichia coli*, encodes for arsenate reductase (ArsC) and a two-component ATPase complex consisting of an ATPase subunit, ArsA, associated with an integral membrane subunit ArsB (Cervantes et al., 1994; Rosen, 2002). Both plasmid and chromosomal loci have been found in the *ars* operon in *E. coli* (Stolz et al., 2006). While the plasmid locus contains five genes, *arsA*, *arsB*, *arsC*, *arsD*, and *arsR*, the chromosomal locus consists of only *arsB*, *arsC*, and *arsR* (Stolz et al., 2006). Gram positive bacteria lack the ArsA ATPase subunit (Cervantes et al., 1994). The ArsC enzyme produced by Gram positive bacteria, such as *Staphylococcus aureus* (with operon located on plasmid pI258), has only 20% amino acids sequence identity with the ArsC enzyme of Gram negative bacteria (Ji et al., 1994). The two enzymes differ in their energy coupling mechanism: the ArsC from *E. coli* receives reducing equivalents from glutathione and glutaredoxin (Shi et al., 1999), whereas the ArsC from *S. aureus* couples with thioredoxin to receive reducing equivalents (Cervantes et al., 1994; Ji et al., 1994). Once arsenate is transported into the cell through phosphate transporters, the protein product of *arsC* gene reduces As(V) to As(III) in the cytoplasm, and then the transmembrane protein ArsB or the ArsAB complex transports the arsenite across the membrane. Differing from the Gram positive and Gram negative arsenate reductase, the arsenate reductase Acr2p in fungi, such as *Saccharomyces cerevisiae,* acquires reducing equivalents from glutathione and glutaredoxin with the reduction product (As(III)) extruded from the cell by Acr3p (Mukhopadhyay et al., 2000).

2.5.4.1.2 Arsenate Respiration: an Energy Generating Process

$CH_3COO^- + 2HAsO_4^{2-} + 2H_2AsO_4^- + 5H^+ \rightarrow 2HCO_3^- + 4H_3AsO_3$ ($\Delta G^{\circ\prime}$ = -252.6 kJ/mole Ac^-) (Macy et al., 1996)

Besides the detoxification mechanism discussed above, microorganisms can reduce arsenate to generate energy. Thermodynamic calculations for arsenate reduction coupled to acetate or lactate oxidation indicate that arsenate reduction is energetically favorable and should precede sulfate reduction (Stolz and Oremland, 1999). Many members of *Archaea*, *Alpha*-, *Beta*-, and *Gamma*-*Proteobacteria*, *Firmicutes*, and *Chrysiogenes,* which show varying physiological characteristics, can respire arsenate (Páez-Espino et al., 2009).

All the arsenate reducing bacteria described to date are not obligate arsenate respirer except strain MLMS-1(Hoeft et al., 2004) and can use other electron acceptors such as oxygen, nitrate, selenate, Fe(III), fumarate, sulfate, thiosulfate, and sulfur (Stolz et al., 2006). Few sulfate reducing bacteria have been shown to mediate dissimilatory arsenate reduction (Newman et al., 1997b). In addition to heterotrophic arsenate reduction, chemolithoautotrophic arsenate reduction has also been reported (Stolz et al., 2006). Arsenate respirer MLMS-1 couples oxidation of hydrogen sulfide to arsenate reduction, generating arsenite and sulfate (Hoeft et al., 2004). Chung et al. (Chung et al., 2006) observed arsenate reduction in a hydrogen-based hollow-fiber membrane bioreactor when H_2 was used as the sole electron donor.

The dissimilatory arsenate reductase is a membrane bound protein closely related to the dimethyl sulfoxide (DMSO) reductase family. The arsenate reductase *arr* operon is invariably encoded by two genes: a*rrA* and *arrB*. Respiratory arsenate reductase enzymes (Arr) have been purified and characterized from *Chrysiogenes arsenatis* (Krafft and Macy, 1998)*, Bacillus selenitireducens* (Afkar et al., 2003), and *Shewanella* sp. strain ANA-3 (Malasarn et al., 2008). Richey et al. (2009) recently reported that the Arr enzymes from *Shewanella* sp. ANA-3 and *Alkaliphilus oremlandii* are bidirectional and can function as an oxidase or a reductase depending on the electron potential of the molybdenum center and [Fe-S] cluster, the other subunits, or the constitution of the electron transfer chain.

The arsenate reductase of *C. arsenatis* consists of two heterodimers ArrA and ArrB subunits of 87 and 29 kDa, respectively (Krafft and Macy, 1998). Similarly, the ArrA and ArrB subunits of the heterodimer arsenate reductase from *B. selenitireducens* are 110 kDa and 34 kDa, respectively (Afkar et al., 2003). The arsenate reductase enzyme from *S. sp.* ANA-3 contains a 95 kDA ArrA subunit and a 27 kDa ArrB subunit (Malasarn et al., 2008). Regardless of the difference in size, ArrA is the molybdopterin catalytic subunit and contains an iron-sulfur [4Fe-4S] center, while the small subunit ArrB contains three to four iron-sulfur [4Fe-4S] clusters (Krafft and Macy, 1998; Richey et al., 2009).

The catalytic subunit ArrA is highly conserved among arsenate reducing prokaryotes and has been utilized as a molecular marker (Malasarn et al., 2004) for the detection of dissimilatory arsenate reducing prokaryotes (DARP) from

different environments (Hoeft et al., 2002; Lear et al., 2007; Song et al., 2009). However, Islam et al. (2005) reported that the primers (Malasarn et al., 2004) designed for the amplification of partial *arrA* gene from arsenate reducing bacteria amplified a 170 bp product from the genomic DNA of *Geobacter sulfurreducens* even though *G*. *sulfurreducens* did not grow on arsenate. This indicates that one must utilize these primers cautiously while amplifying the *arrA* genes from environmental samples.

2.5.5 Arsenite Oxidation

Arsenite (As(III)) oxidizing prokaryotes are phylogenetically diverse. Both heterotrophic and chemolithotrophic prokaryotes that can oxidize arsenite have been reported (Oremland and Stolz, 2003; Silver and Phung, 2005). Arsenite-oxidizing prokaryotes spanning the *Alpha*-, *Beta*-, *Gamma- Proteobacteria*, and the genus *Thermus* have been described (Oremland and Stolz, 2003). The facultative chemoautotrophic strain MLHE-1 isolated from Mono Lake (California) oxidized As(III) to arsenate As(V) when incubated with nitrate or nitrite (Oremland et al., 2002). Nitrate dependent autotrophic growth with H_2 or sulfide (oxidized to sulfate) as well as heterotrophic growth with acetate was observed with this strain. MLHE-1 was identified as a member of the haloalkaliphilic *Ectothiorhodospira* family (genus *Alkalilimnicola)* of *Gammaproteobacteria* (Hoeft et al., 2007).

Arsenite oxidase (Aox), which is a member of the DMSO reductase family, is the mediator of arsenite oxidation, whether the microorganisms oxidize

arsenite to gain energy or to detoxify (Richey et al., 2009). Aox is also a heterodimer comprised of a catalytic subunit AoxB (~90 kDa) and an associated subunit AoxA (~14 kDa) (Ellis et al., 2001). However, the subunit structure may vary among the arsenite oxidases. For example, the native molecular mass of the arsenite oxidase in *Hydrogenophaga* sp. Strain NT-14 is 316 kDa, whereas the molecular mass of the two subunits are 86 kDa and 16 kDa, respectively, suggesting a possible $\alpha_3\beta_3$ configuration (vanden Hoven and Santini, 2004). Similarly, the native molecular mass of arsenite oxidase from the chemolithoautotroph NT-26 is 219 kDa, while the individual masses of the subunits are 98 kDa and 14 kDa, respectively (Santini and vanden Hoven, 2004). Compared to the associated subunit AoxA, which has a single Rieske-type [2Fe-2S] cluster, the subunit AoxB contains a [3Fe-3S] cluster and molybdenum bound to the pyranopterin cofactor (Ellis et al., 2001; Richey et al., 2009).

Besides the Aox mediated arsenite oxidation, recent findings have indicated the presence of an alternative arsenite oxidizing mechanism in chemoautotrophic microorganism *Alkalilimnicola ehrlichii* (Hoeft et al., 2007). In fact, two operons that encode two putative dissimilatory arsenate reductase genes are detected in *A. ehrlichii* and one of these two homologs exhibits both arsenate reductase and arsenite oxidase activities (Richey et al., 2009).

2.5.6 Biomethylation of Arsenic

Methylation of metals and metalloids by microorganisms is a well-known process (Bright et al., 1994; Ridley et al., 1977). A broad group of

microorganisms, including iron and sulfate reducing bacteria are capable of producing methylarsenicals (Bright et al., 1994). Though primarily attributed to the detoxification mechanism, biomethylation of arsenic has recently been described as a process that generates genotoxic arsenic compounds, such as MMA(III) and DMA(III) (Qin et al., 2006). Since the end product of microbial methylation of arsenic is a volatile species that is more bio-available and toxic, biomethylation is of an environmental concern. The arsenic methylation mechanism suggested by Challenger (Challenger, 1945) involves As(V) reduction to As(III) and subsequent oxidative incorporation of methyl groups to generate MMA(V), MMA(III), DMA(V), and DMA(III), TMAO(V), and TMA in sequence (Dombrowski et al., 2005). S-adenosylmethionine (SAM) is the methyl group donor in the reaction (Dombrowski et al., 2005).

2.6 Sulfate Reduction

$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$ ($\Delta G^{\circ\prime}$ = -47.6 kJ/mole Ac^-) (Celis-Garcia et al., 2007)

Biological sulfate reduction is mediated by sulfate reducing prokaryotes (SRP) that use sulfate as the electron acceptor for the oxidation of an organic or inorganic electron donor. Dissimilatory sulfate reducing microbes are ubiquitous and phylogenetically diverse, including both *Bacteria* and *Archaea* (Loy et al., 2002). The dissimilatory sulfate reducing bacteria (SRB) described to date (based on 16S rRNA gene sequences) fall into five bacterial lineages (*Deltaproteobacteria*, *Nitrospirae*, *Thermodesulfobacteria,*

Thermodesulfobiaceae, and *Clostridia (Muyzer and Stams, 2008)*, but most of the species described so far belong to the class *Deltaproteobacteria* (23 genera) and the phylum *Firmicutes* (family *Peptococcaceae*) (Muyzer and Stams, 2008). SRB within *Archaea* domain belong to *Euryarchaeota* (genus *Archaeoglobus*) and *Crenarachaeota* (genus *Thermocladium* and *Caldirvirga*) (Muyzer and Stams, 2008). Sulfate reducers can utilize various electron acceptors, including sulfate, sulfite, thiosulfate, elemental sulfur (Kaksonen et al., 2007), nitrate (Moura et al., 1997), arsenate (Macy et al., 2000; Newman et al., 1997a), and iron(III) (Coleman et al., 1993). They can oxidize organic compounds, such as C2-C18 fatty acids, alcohols, formate, aromatic hydrocarbons, and chlorinated compounds as well as H_2 (Celis-Garcia et al., 2007; Christensen, 1984). Several SRB can couple the oxidation of acetate to the reduction of sulfate (Muthumbi et al., 2001; Oude Elferink et al., 1999).

The enzyme dissimilatory (bi)sulfite reductase (DSR) catalyzes the final steps in sulfate reduction and is ubiquitous in all known SRB (Karr et al., 2005). Its ubiquity and high sequence conservation has made this enzyme ideal for assessing the diversity of sulfate reducing communities and genes encoding DSRA (α-subunit) and DSRB (β subunit) of DSR are generally amplified using PCR for this purpose (Karr et al., 2005; Klein et al., 2001).

The products of microbial sulfate reduction are H_2S, HS^-, and S^{2-}, which can be toxic to microorganisms. However, sulfide toxicity depends on total concentration of sulfides produced and pH of the system. Celis Garcia et al. (2007) reported that total sulfide concentrations as high as 1200 mg/L did not

affect the chemical oxygen demand (COD) and sulfate removal efficiency of a down-flow fluidized bed bioreactor treating sulfate-rich wastewater in a pH range of 6.5-8.4. However, in another experiment with a hybrid bioreactor using granular sludge and polyethylene rings, SRB were seriously impacted with a total sulfide concentration of 1000 mg/L; the sulfate removal rate dropped from 87.5% to 50% (Celis-Garcia et al., 2007). While growth of a bacterium isolated from an anaerobic digester and related to the *Desulfovibrio* was optimum at pH 6.6, 547 mg/L H_2S inhibited growth completely (Reis et al., 1992).

2.7 Biotic and Abiotic Oxidation of Iron(II)

Besides the microbiologically mediated iron reduction presented in section 2.5.2, abiotic as well as biotic processes may oxidize iron(II) to iron(III). Under aerobic conditions, microorganisms indigenous to groundwaters, such as *Gallionella ferruginea* and *Leptothrix ochracea* (Katsoyiannis and Zouboulis, 2004) are capable of Fe(II) oxidation. Bacteria that can couple the oxidation of Fe(II) with the reduction of nitrate under anoxic environments have also been described (Lack et al., 2002; Straub et al., 1996). In activated sludge system, biologically mediated oxidation of Fe(II) utilizing nitrate or nitrite as the electron acceptors was observed (Nielsen and Nielsen, 1998). A bacterial strain isolated from the Field Research Center, Oak Ridge, TN and identified to be closely related to *Klebsiella oxytoca* oxidized FeS and soluble Fe(II) resulting in the precipitation of amorphous iron(III) hydroxides and geothite, respectively, when grown in a medium containing nitrate (Senko et al., 2005). Weber et al. (Weber et al., 2006) isolated an anaerobic lithoautotrophic bacterium closely related to

Chromobacterium violaceum that oxidized iron(II) to iron(III) utilizing nitrate as the electron acceptor. While the end product of nitrate reduction was nitrite in a no-growth control experiment (washed cells suspended in a medium lacking acetate), N_2 and N_2O gases were released when acetate was present (growth experiment). When washed cells of *Dechlorosoma suillum* strain PS were added to a bicarbonate buffer medium, nitrate-dependent Fe(II) oxidation was observed even though growth was not observed (Lack et al., 2002) resulting in the precipitation of amorphous Fe(III) hydroxides. However, when the same strain was used in a growth medium that contained acetate as the co-substrate, nitrate-dependent Fe(II) oxidation resulted in the precipitation of magnetite (Fe_3O_4) (Chaudhuri et al., 2001). Fe(II) oxidation started only after acetate was completely consumed. This different Fe(III) end product formation was explained by the difference in reaction kinetics: the precipitation was faster in the no-growth conditions compared to the growth conditions.

Nitrite-dependent abiotic Fe(II) oxidation has also been reported. In oxygen-free batch reactors, when ionic Fe(II) was added to lepidocrocite (γ-FeOOH), H^+ was released with the formation of magnetite-containing reactive complex, which resulted in the reduction of NO_2^- to N_2O (Sørensen and Thorling, 1991). Nitrite reduction was not observed in the absence of lepidocrocite. Tai and Dempsey (2009) reported similar observation when Fe(II) oxidation with nitrite reduction was evaluated in the presence or absence of hydrous ferric oxide (HFO). Fe(II) oxidation was negligible in the absence of HFO.

Nitrite mediated Fe(II) oxidation, both biotic and abiotic, is of environmental concern as this reaction may result in the generation of NO and N_2O gases (Moraghan and Buresh, 1977; Tai and Dempsey, 2009; Weber et al., 2006). Additionally, since the geochemistry of metals and metalloids are affected by Fe(III) oxy-hydroxides, nitrate/nitrite mediated Fe(II) oxidation is important in the context of evaluating arsenic mobility.

2.8 Iron Sulfide Precipitation

The reaction between Fe(II) and S(-II) in aqueous solutions at ambient temperatures results in the precipitation of black-colored nanoparticles of iron sulfides (Mullet et al., 2002; Rickard et al., 2006; Wolthers et al., 2003a). This solid has been described as kansite (Fe_9S_8), hydrotroilite ($FeS.nH_2O$), precipitated iron sulfide, amorphous iron sulfide, and mackinawite (FeS_{1-x}) in the literature (Rickard et al., 2006). Mackinawite is typically the first iron sulfide to precipitate in aqueous solutions and may transform into more stable solids of iron sulfide, such as greigite (Fe_3S_4), and pyrite (FeS_2) (Wolthers et al., 2003b). Mackinawite has a tetragonal structure with the Fe atoms linked in tetrahedral coordination with four equidistant sulfur atoms (Wolthers et al., 2003b) forming sheets of Fe weakly held by Van der Waals bonding between the sulfur atoms at a distance of 0.5 nm (Mullet et al., 2002; Wolthers et al., 2003a).

Mackinawite has been reported as slightly sulfur-rich mineral (FeS_{1+x}), slightly iron-rich mineral (FeS_{1-x}), and nearly stoichiometric (FeS) (Gallegos, 2007). Mackinawite can be synthesized at low temperature by the reaction of

aqueous sulfide with metallic iron or aqueous Fe(II), and by the reaction of aqueous ferrous iron with biologically generated sulfides (Wolthers et al., 2003b). Besides mackinawite, other iron sulfides, such as greigite (Wilkin and Ford, 2006), and pyrite (Farquhar et al., 2002) can also form by the reaction of S(-II) with Fe(II).

As discussed in Section 2.6, SRP mediate dissimilatory sulfate reduction in anaerobic environments resulting in the production of sulfides, which control the geochemistry of metals and metalloids, including arsenic (Kaksonen et al., 2003; Kirk et al., 2004; O'Day et al., 2004). In recently formed sediments in natural environments, the formation of mackinawite takes place by the action of SRP that results in hydrogen sulfide, which reacts with iron species from detritus or other sources to form an amorphous precipitate. This amorphous precipitate crystallizes to more stable mackinawite within days (Mullet et al., 2002). Gallegos et al. (2007) chemically prepared fresh amorphous nano-particles of mackinawite with very high specific surface area, which imparted high reactivity to mackinawite for sequestering metals and metalloids.

Biogenic iron sulfides other than metastable mackinawite have also been reported in the literature. Herbert et al. (1998) reported precipitation of greigite and mackinawite when Fe(II) was added to a medium containing SRB. However, in an experiment with *Desulfovibrio desulfuricans*, Neal et al. (2001) found precipitation of pyrrhotite on the surface of heamatite (α-Fe_2O_3). Matsuo et al. (2000) observed pyrrhotite ($Fe_{1-x}S$) within 5 days when *Desulfovibrio* sp. were

incubated in a system containing lactate, sulfate and iron(II), which successively transformed into mackinawite and pyrite with prolonged incubation.

As discussed in sections 2.5.2 and 2.6, it is possible to generate iron(II) and sulfides biologically in an controlled engineered system. Biologically generated iron(II) and sulfides then subsequently react resulting in the precipitation of iron sulfides.

2.9 Interaction of Arsenic with Sulfides (Including Iron Sulfides)

The presence of redox active iron, sulfur, and arsenic species under sulfate reducing conditions results in the existence of complex arsenite speciation and solid phase partitioning (Gallegos, 2007). In natural settings, higher concentrations of arsenic are observed where sulfate levels are low and vice versa suggesting the existence of an inverse relationship between sulfate and dissolved arsenic concentrations (Kirk et al., 2004). Biological sulfate reduction has been demonstrated to sequester arsenic through the generation of arsenic sulfides, such as realgar (AsS) (Ledbetter et al., 2007) and orpiment (As_2S_3) (Newman et al., 1997a). In the presence of pyrite, arsenic may also be precipitated as arsenopyrite (FeAsS) and orpiment (Bostick and Fendorf, 2003). However, in a system containing iron(II), sulfides, and arsenic, the difference in the solubility of iron and arsenic sulfides results in the precipitation of iron sulfides, which dictate the arsenic removal through adsorption and co-precipitation mechanisms (Kirk et al., 2010; O'Day et al., 2004). Rittle et al. (1995) observed a decrease in As(III) and Fe(II) concentrations in a laboratory

microcosm with biogenic sulfides. Various iron sulfides, including mackinawite, greigite, pyrite, have been suggested to be effective scavengers of arsenic (Gallegos, 2007; Rittle et al., 1995; Wilkin and Ford, 2006). The reactivity of mackinawite comes from the amorphous nature of freshly prepared mackinawite, which consists of nano-scale particles with high specific surface area leading to a relatively high solubility at lower pH (Wolthers et al., 2003b).

Arsenic uptake by troilite (FeS) and pyrite (Bostick and Fendorf, 2003), and mackinawite (Gallegos et al., 2007a) is pH dependent. While arsenic uptake by mackinawite increased with acidic conditions ($2x10^{-3}$, $2x10^{-4}$ and $5x10^{-5}$ moles As/g FeS at pH 5, 7 and 9, respectively) (Gallegos, 2007), sorption increased significantly beyond pH 5 and 6 with troilite and pyrite, respectively (Bostick and Fendorf, 2003). Adsorption on iron sulfides is the principal arsenic removal mechanism under highly reducing conditions with low arsenic levels (below the solubility limit of realgar) (O'Day et al., 2004). When As(III) was reacted with mackinawite, arsenic removal was observed through reduction and subsequent precipitation of realgar when the concentration of arsenic was $5.0X10^{-4}$ M (Gallegos et al., 2007a). However, with an order of magnitude lower arsenic level, realgar precipitation and arsenic adsorption were the arsenic removal mechanisms; adsorption dominated at pH 9 (Gallegos et al., 2007a). Wolthers et al. (2007) reported inhibition of transformation of FeS precipitated in a system containing Fe(II) and sulfide (Wolthers et al., 2007) to mackinawite and pyrite by arsenic. At a S:As(V) ratio of 1:1 and 2:1, As(V) inhibited the transformation of

FeS to mackinawite and pyrite. Iron sulfides were oxidized by As(V) and As(III) resulting in green rust, elemental sulfur, and Fe(III).

Besides the iron and arsenic sulfides, other researchers have suggested the formation of thioarsenate and thioarsenite species depending on pH and the relative concentration of dissolved sulfides and arsenic (Beak et al., 2008; Bostick et al., 2005; Stauder et al., 2005). Stauder et al. (2005) reported arsenite, arsenate and thioarsenate species only in groundwater highly contaminated with arsenic. A 1:1 ratio of As(III):S resulted in mono- and dithioarsenates, while increased sulfide levels (a ratio of 1:1.5 of As(III):S) resulted tri- and tetrathioarsenates. Reaction of As(III) with sulfides also resulted in thioarsenates, which was explained by the high affinity of As(III) for sulfur that results in addition of a sulfur atom to As(III), while As(III) partly gets reduced to elemental As(0) in accordance with the following reaction.

$$5H_3AsO_3 + 3H_2S = 2As + 3H_2AsO_3S^- + 6H_2O + 3H^+$$

Bostick et al., (2005) reported varying fractions of thioarsenite species with different S:As(III) ratio in liquid phase. Thioarsenite species were the predominant arsenic species when S:As(III) ratio was more than 3. However, in the presence of high levels of Fe(II) and reducible solid Fe(III) phase, the sulfide concentration may be maintained at low levels preventing thioarsenate formation and arsenite and arsenate might control the adsorption/co-precipitation reactions (Wilkin and Ford, 2006).

2.10 Overview of Available Treatment Technologies

Regulatory pressures have resulted in the development of technologies suitable for the treatment of arsenic, both for *ex situ* drinking water treatment and for subsurface *in situ* treatment of groundwater. Since arsenic cannot be destroyed either chemically or biologically, it needs to be transformed or combined with other elements to form insoluble (Essig and A., 2008) or volatile compounds (Bright et al., 1994).

Effectiveness of any arsenic removal technology depends on various feed water characteristics, such as pH, arsenic species, total dissolved solids, and competing ions, especially sulfate, phosphate, silicate, and fluoride. At a pH of environmental relevance (i.e., near neutral pH), As(V) exists in mono- or divalent anionic form, while arsenite exists in uncharged form. As a consequence, As(V) is removed more efficiently and effectively from water by several existing technologies (adsorption, ion-exchange, and co-precipitation processes) than As(III), and pre-oxidation of As(III) to As(V) is practiced in many arsenic treatment techniques (http://www.epa.gov/ogwdw/arsenic/pdfs/handbook_arsenic_treatment-tech.pdf). Arsenic usually is removed through sorption processes (Kundu and Gupta, 2007; Mohan and Pittman, 2007; Tyrovola et al., 2007). Recently, biologically mediated arsenic removal has been recognized as a potential treatment technology (Ito et al., 2001) and has been studied by a number of researchers (Halttunen et al., 2007; Kirk et al., 2010; Lehimas et al., 2001). Recent reviews on arsenic removal techniques discussed the available treatment technologies in detail (Mohan and Pittman Jr, 2007; Mondal et al.,

2007; Sharma and Sohn, 2009; Uddina et al., 2007). The review provided below presents brief descriptions of each of the available arsenic removal technologies.

2.10.1 Ion Exchange

Ion exchange processes rely on differential affinity of the functional groups present in synthetic or natural organic and inorganic or polymeric materials used as the ion exchange resin. Ion exchange has been widely used to remove arsenic (Ghurye et al., 1999; Kim and Benjamin, 2004; Kim et al., 2003) from water. Ion exchange processes have two main disadvantages: (i) competition with other non-contaminant ions, and (ii) requirement of regeneration of the ion exchange resins, which results in a concentrated waste stream that must be treated (Gingras and Batista, 2002; Mateju et al., 1992).

2.10.2 Membrane Processes

Membrane separation requires application of high pressure that allows only water molecules to pass through the membrane, while contaminants are retained on the influent side of the membrane. In the case of reverse osmosis (RO), high pressure is applied to reverse the natural osmotic pressure gradient in a system having a semi-permeable membrane that separates the contaminant ions from water. RO is an attractive drinking water treatment technology as it provides higher contaminant removal efficiencies and requires minimal amount of chemicals while ensuring limited accumulation of contaminants on the membrane (Shih, 2005). Waypa et al. (1997) evaluated RO and nanofiltration (NF) membranes for arsenic removal and reported equal rejection of As(III) and As(V)

within the pH range of 4-8. However, while comparing RO, NF, and ultrafiltration (UF) membranes for the removal of chromate, arsenate, and perchlorate, Yoon et al. (2005) reported increasing rejection efficiency with increasing pH. They concluded that increasing negative surface charge due to increased pH and decreasing conductivity improves arsenic rejection. The rejection of targeted ions is directly related to the ionic state of the contaminants; higher efficiency of separation is achieved for multi-charge ionic species (Mateju et al., 1992). High capital and operating costs, requirement of highly skilled operators, and lack of selectivity of RO membranes for mono-ionic contaminants over multi-ionic species are a few of the drawbacks of this technology. Membrane fouling and the generation of concentrated brines are the potentially greatest drawbacks of this technology.

2.10.3 Sorption

The loss of a chemical species of interest from a liquid phase to a solid phase is termed sorption (Sposito, 1987), which encompasses the uptake of a solute from solution by adsorption, absorption, coprecipitation, and surface precipitation mechanisms. Adsorption implies removal of an adsorbate by an adsorbent that is prepared separately (in the absence of the adsorbate) (Crawford et al., 1993) and is, in general, a two-dimensional accumulation of the adsorbate at the interface between the bulk liquid and the solid phase (Sposito, 1987). However, the deposition of solid phases, which have inherent three-dimensional structure, at the interface between a bulk liquid and solid phase still is considered adsorption (Sposito, 1987). Absorption, on the other hand, refers

to the diffusion of an aqueous chemical species into a solid phase (Sposito, 1987). Removal of an adsorbate by an adsorbent during solid solution formation is termed as coprecipitation (Crawford et al., 1993). Surface precipitation refers to a multilayer precipitation of adsorbate (e.g., arsenate or phosphate) and adsorbent (e.g., iron hydroxides), which requires the dissolution of the adsorbent to generate the successive layers (Li and Stanforth, 2000).

Arsenic removal by adsorption onto iron oxyhydroxides (Driehaus et al., 1998; Jain et al., 1999), aluminum hydroxides (Gulledge and O'Connor, 1973), and iron sulfides (Farquhar et al., 2002; Gallegos et al., 2006) has been widely reported. However, only a few of the studies have presented the direct detailed comparison of these processes for arsenic removal (Fuller et al., 1993; Waychunas et al., 1993). In general, contaminants removal through coprecipitation with iron oxy-hydroxides is more efficient and rapid compared to adsorption (Fuller et al., 1993). Interestingly, Arakaki and Morse (1993) observed a dominance of adsorption over coprecipitation for the removal of Mn(II) with mackinawite; this was attributed to the higher specific surface area achieved due to the fine-grained nature of mackinawite.

A detailed review of the sorption mechanisms involved in arsenic removal is beyond the scope of this document and only coagulation/filtration and adsorption as arsenic removal technologies are discussed in the three sections below.

2.10.3.1 Coagulation/Filtration

Co-precipitation or adsorption and subsequent removal of arsenic from water is enhanced by the use of coagulants such as ferric chloride ($FeCl_3$), and alum ($Al_2(SO_4)_3$) (Baskan et al., 2010; Lakshmanan et al., 2008). In water, $FeCl_3$ salt hydrolyzes and precipitates resulting in the formation of pH-dependent positively charged solid phase iron hydroxides. As discussed above, As(V) species are better removed compared to As(III) species due to their respective chemical characteristics near neutral pH (Gregor, 2001; Lakshmanan et al., 2008). Accordingly, chemical oxidation of As(III) with strong oxidizing agents such as chlorine is performed prior to removal through coagulation/filtration. Iron hydroxide solids are positively charged at a pH lower than their point of zero charge (PZC) (near pH of 8). Arsenate, which exists as a negatively charged ion near neutral pH, is thus effectively adsorbed by forming surface complexes with iron hydroxides (Chwirka et al., 2004). Alum works similarly and removes arsenic at pH<6.5 as aluminum hydroxides exist in strong cationic form (Lakshmanan et al., 2008). However, alum is less effective for arsenate removal above pH 6.5 and is ineffective for the removal of arsenite (Lakshmanan et al., 2008).

2.10.3.2 Sorption on Biomass and Biomaterials

Physical-chemical interactions, such as entrapment, ion exchange, or adsorption on living or dead biomass and/or biomass-derived products (White et al., 1995) may be utilized for contaminant removal . For example, sorption on

biological materials such as chitin, chitosan, cellulose, and alginate have been used for arsenic removal (Halttunen et al., 2007; Kartal and Imamura, 2005). Chitin and chitosan have a high number of amine and hydroxyl groups in their structure (White et al., 1995), which promotes the removal of metals through adsorption. Kartal et al. (2005) reported only 63% and 30% removal of arsenic from chromated copper arsenate (wood-preservative) treated wood packed in teabags and dipped in deionized water containing chitin and chitosan. These biopolymers removed copper more efficiently compared to arsenic. Even though Doshi et al. (2009) reported arsenic sorption capacity of 525 and 402 mg As(V)/g of live and dead biomass of blue-green algae *Spirulina* sp., respectively, arsenic removal by native and methylated (to impart a more positive surface charge) biomass of three different *Lactobacillus* species showed very weak interaction between As(V) and the biomass as arsenic was easily released from the sorbates (Halttunen et al., 2007). Similarly, Loukidou et al. (2003) reported that As(V) removal from wastewater by fungal biomass of *Penicillium chrysogenum* was enhanced when the biomass was modified with hexadecyl trimethylammonium bromide, polyelectrolyte Magnafloc-463, and dodecylamine resulting in arsenic removal capacity of 37.85, 56.07 and 33.31 mg/g of modified biomass, respectively. Recently, Ranjan et al. (2009) studied arsenic removal using ‘rice polish’, an agricultural residue, and observed arsenic removal capacity of 138.88 and 147.05 µg As/g absorbent for As(III) (pH 4) and As(V) (pH 7), respectively. In general, the modified biomass shows more effective and efficient removal of arsenic compared to the untreated (native) biomass.

2.10.3.3 Sorption on other materials (Non-biomaterials)

Adsorption on non-biomaterials has been the most studied physico-chemical process for arsenic removal. Various adsorbents, including native and modified granular activated carbon (GAC), iron-based sorbents, and natural materials have been evaluated for arsenic removal. The following paragraphs briefly discuss the effectiveness of these adsorbents for arsenic removal.

GAC in its native form (Huang and Fu, 1984) or chemically modified form (Chen et al., 2007; Gu et al., 2005) has been utilized for arsenic removal. While optimum arsenic removal was obtained at pH 4 for both powdered activated carbon (PAC) and GAC, more arsenic removal was observed with PAC compared to GAC (Huang and Fu, 1984). Compared to the GAC generated by activation of carbon at 1000 ^{o}C either in pure carbon dioxide (CO_2) or under vacuum followed by exposure to oxygen at room temperature, the GAC generated by oxidizing carbon by exposure to oxygen at 200-400 ^{o}C, removed more As(V) (Huang and Fu, 1984). Lorenzen et al. (1994) reported that As(V) was removed more efficiently compared to As(III) with high ash containing activated carbon. Comparing the untreated and Cu(II) treated activated carbon, Rajakovic (1992) reported an arsenic removal capacity of 20.2 and 17.2 mg As(V)/g with the untreated and treated carbon, respectively. Treatment with Cu(II) significantly improved As(III) adsorption; no As(III) removal was observed with untreated carbon, while arsenic removal capacity of 30.71 mg As(III)/g carbon was achieved with the cupper treated activated carbon. .

Iron-based sorption materials have been studied extensively for arsenic removal. Kundo et al. (2004) used iron oxide coated cement (IOCC) and reported very rapid adsorption of As(III) resulting in 0.69 mg As(III)/g of IOCC. Jekel and Seith (2000), while comparing the methods for the coagulation and precipitation with ferric chloride and ferrous sulfate and adsorption on granular ferric hydroxide (GFH) in a full scale water treatment plant, identified adsorption on GFH as the method of choice due to operational reliability and low maintenance requirement. While Driehaus et al. (1998) achieved 1-10 mg As/g of GFH, Badruzzamin et al. (2004) reported 8 mg As/g dry GFH.

Guo et al. (2007a) used natural siderite ($FeCO_3$) in batch and column reactors to remove arsenic and reported arsenic adsorption capacity of 520 and 1040 µg As/g of siderite for As(V) and As(III), respectively. Arsenic co-precipitated with iron oxides formed due to the oxidation of siderite. Arsenic concentration in the final effluent from the column reactor remained below 1 µg/L after 26000 pore volumes of 500 µg/L As. Zero valent iron (ZVI) is also effective in removing arsenic, especially for As(III) in the pH range of 7 to 8 (Xueyuan et al., 2006). Lien et al. (2005) reported 7.5 mg As/g Fe(0) arsenic removal capacity using ZVI. In an experiment with column reactors, Biterna et al. (2010) observed more efficient removal of As(V) from groundwater compared to As(III). They also reported improved arsenite removal after chlorinating the water. Tyrovola et al. (2007) evaluated the effectiveness of arsenic removal with ZVI in the presence of high concentrations of nitrate and phosphate. Arsenic removal occurred due to precipitation/co-precipitation of arsenic onto ZVI and its corrosion

product. The presence of nitrate and phosphate negatively impacted the reactor performance. In a vertical glass column packed with 1.5 g iron filing (ZVI) and 3-4 g quartz sand, Leupin et al. (2005) removed As by re-circulating synthetic groundwater (aerated in between the cycles) containing 500 µg As(III)/L. During the oxidation of the released iron(II), As(III) was oxidized to As(V) and subsequently adsorbed onto the hydrous ferric oxides generated. After four cycles of filtration, total arsenic in the final was less than 50 µg/L.

Activated alumina also removes arsenic significantly. Singh and Pant (2004) reported pH dependent affinity of As(III) towards activated alumina; As(III) removal was highest at pH 7.6. Using aluminum sulfate treated commercially available activated alumina and untreated activated alumina, Takanashi et al. (2004) reported arsenic loading capacity of 10 mg As/g.

Very recently, Maiti et al. (2010) prepared laterite (soils rich in iron and alumina) with a specific surface area of 181±4 m^2/g by treating laterite with acid and alkali in sequence and then tested the material for arsenic removal in batch and column reactors. The arsenic adsorption capacity was found to be 24.8±3.9 and 8±1.4 mg As/g laterite for As(V) and As(III), respectively.

Besides these adsorbents, several other adsorbents have been tested for arsenic removal from water, including coconut husk carbon (2.5-12.5 mg As(III)/g material) (Manju et al., 1998), orange juice residue (70.5 mg As(V)/g and 68.3 mg As(III)/g) (Ghimire et al., 2002), and red mud (0.55-0.6 mg As/g) (Li et al., 2010b).

2.10.4 Small Scale Arsenic Removal Technologies

Small-scale arsenic treatment technologies developed and practiced in rural areas of Bangladesh, India, and Nepal mostly utilize iron-based adsorbents. For example, the arsenic remediation technology (AsRT) developed by Nikolaidis and Lackovic (http://www.engr.uconn.edu/~nikos/asrt-brochure.html) consisted of a simple two column system, where barium sulfate was added to the arsenic contaminated water in the first column and arsenic was removed in the second column that contained iron filings. Ferric hydroxide was generated due to the oxidation of the iron filings, while the reducing equivalents released during iron oxidation resulted in sulfate and arsenate reduction. Arsenic removal occurred due to adsorption and co-precipitation with iron hydroxides, and precipitation as iron-arsenic-sulfides. They reported 97% arsenic removal when the influent concentration ranged between 45 to 8600 µg As/L.

Joshi et al. (1996) developed a two-container arsenic removal system for household use utilizing iron-oxide coated sand and demonstrated efficient arsenic removal resulting in effluent arsenic concentration below 10 µg/L As while producing 625 and 780 L of potable water from 1mg/L As(III) and As(V) contaminated waters, respectively, without regeneration.

A three-pitcher, locally known as three-kolshi, system was tested for arsenic removal in Bangladesh (Khan et al., 2000). While the first pitcher contained iron chips and sand, the second pitcher contained wood charcoal collected from burned firewood and fine sand. The third pitcher was used for the collection of

purified water. The influent arsenic (800 µg As/L) and iron (6 mg Fe/L) were lowered to less than 2 µg As/L and 0.20 mg Fe/L, respectively. The generation of hydrous ferric oxides in the system was responsible for the arsenic removal through precipitation and adsorption. The charcoal in the second pitcher removed organic impurities. The system successfully generated arsenic-free water at a flow rate of 42-148 L/day. However, in another set-up in Nepal, locally known as three-gagri system, Hurd et al. (2001) achieved a purification rate of only 4L/day, which decreased with every successive filtration cycle.

Solar oxidation and removal of arsenic (SORAS) *(http://www.physics.harvard.edu/wilson/arsenic/remediation/sodis/SORAS_Paper.html)* is a technology suitable for the removal of arsenic at the household level. Photolysis of Fe(III)-citrate complex results in the formation of reactive oxidants, such as hydroxyl radical (•OH), superoxide radicals ($•O_2$), and hydrogenperoxide (H_2O_2). Photo-oxidation of As(III) to As(V) and subsequent co-precipitation or adsorption on precipitated iron hydroxides results in arsenic removal. Arsenic removal of 80-90 % was observed in the presence of citrate (50 µM). In rural household settings, lemon juice replaced citrate.

Sarkar et al. (2005) described a well-head arsenic removal filter system managed by local communities in West Bengal (India). Effective arsenic removal was achieved by the precipitation/co-precipitation and adsorption of arsenic with iron hydroxides generated on the surface of spherical activated alumina and hybrid anion exchanger. The arsenic concentration was lowered from the influent levels of 100-500 µg/L As to less than 50 µg/L As.

2.10.5 Biological Treatment Technologies under Oxidizing Conditions

Biologically mediated contaminant removal has gained popularity in recent years and has the potential to be utilized for arsenic removal from water sources. Existing conventional treatment technologies discussed above, such as adsorption/coagulation/filtration, may not completely remove arsenic. Additionally, the requirement of chemical addition to the system makes these technologies costly. The advanced treatment technologies discussed above, such as RO and ion exchange may provide complete arsenic removal. However, the generation of concentrated waste stream, which requires further treatment, and the requirement of regeneration of the exhausted materials are the drawbacks of these technologies. In contrast, multiple contaminants can be removed in a single-step biological treatment system without the requirement of regeneration of the exhausted materials and treatment of the generated wastes (Brown, 2007). In addition, biological processes require limited or no chemical addition.

Biological processes utilize microorganisms to mediate the transfer of electrons from an electron donor to the oxyanionic contaminants of concern. Nutrients (e.g., phosphorus) and trace elements (e.g., molybdenum) might be needed to enhance biological reduction (Chaudhuri et al., 2002). In contrast to other groundwater contaminants such as nitrate, arsenic cannot be destroyed, but it needs to be transformed into solid or gas phase. Biologically mediated arsenic removal has been studied in an oxidizing environment that utilized iron oxidizing bacteria, such as *Gallionella ferruginea* and *Leptotrhix ochracea* to

oxidize Fe(II) to Fe(III), which subsequently traps arsenic (Katsoyiannis et al., 2002; Lehimas et al., 2001). Katsoyannis et al. (2002) used a two-stage up-flow fixed-bed bioreactor containing polystyrene beads as the support medium for bacterial growth. Lehimas et al. (2001) used a sand bed filter to remove arsenic. In both cases, arsenic was removed from water through adsorption on biologically generated iron hydroxides.

Besides these biological arsenic removal processes practiced under oxidizing conditions, bioreactors have been demonstrated to remove arsenic under sulfate reducing conditions. These processes are discussed under section 2.12 in the context of alternative arsenic removal strategy.

2.11 Disposal of Arsenic Contaminated Wastes

In developing countries, wastes generated from both household and community level arsenic-contaminated water treatment units often are disposed inadequately due to lack of guidelines (Afkar et al., 2003). Generally, the arsenic-laden sludge is mixed with cow-dung and dumped into a small pit (1 m^3) lined with bricks and covered with sand (Sullivan et al., 2010). Alternatively, the waste is directly disposed in cow-dung beds (Afkar et al., 2003). Biogeochemical processes initiated by the microorganisms in the cow dung results in significant loss of arsenic from the arsenic-laden sludge (Afkar et al., 2003), possibly through the generation of arsines. The uncovered and unprotected nature of the pits containing arsenic laden sludge increases the potential for arsenic to leach into nearby water sources.

In developed countries, arsenic-containing byproducts of water treatment systems are landfilled. Arsenic-laden iron-hydroxide sludge stored in landfills has the potential to release arsenic due to the reductive dissolution of iron oxy-hydroxides (Guha et al., 2005; Smedley and Kinniburgh, 2002) or due to microbially mediated redox reactions (Bose and Sharma, 2002; Ghosh et al., 2006; Irail et al., 2008). Leaching can also be facilitated by competition with other dissolved species, such as phosphorus and sulfate. Since pH determines the surface speciation and charge of iron hydroxides as well as arsenic solution speciation and species charge, arsenic sorption/desorption is strongly dependent on pH. The competition or enhancement of sorption of arsenic on iron hydroxides depends on the competing, co-adsorbing or precipitating ion to arsenic ratio in solution. For example, readily adsorbing phosphate competes with arsenic for adsorption sites on iron hydroxides (Wilkie and Hering, 1996) and can cause the release of arsenic from arsenic-containing iron hydroxides sludge. In contrast, calcium may help immobilize arsenic through the formation of calcium-arsenic precipitates, such as apatite ($Ca_5(AsO_4)_3^-.OH$) (Bothe and Brown, 1999), calcium arsenate ($Ca_3(ASO_4)_2$) (Vandecasteele et al., 2002), and $NaCaAsO_4.7.5H_2O$ (Akhter et al., 1997).

Recognizing the potential of arsenic re-release from arsenic-laden sludge, Sarkar et al. (2008) described a sludge volume reduction and stabilization scheme, which has been in practice in more than 175 community-based arsenic removal units in West Bengal, India. In the system, arsenic-laden sludge that contains high concentration of iron hydroxides is generated in two stages: (i)

during backwashing (every 24 h) of the spherical activated alumina and (ii) during regeneration at a centrally located regeneration facility. The sludge is disposed in an aerated (passive aeration) coarse sand filter, which minimizes arsenic leaching by preventing reduction of iron(III) hydroxides.

To minimize potential arsenic leaching from spent solids/sludge in landfill environments, other sludge stabilization/solidification technologies have been developed. Minimizing the waste/leachant contact has been the focus of such technologies. Primarily two methods of arsenic stabilization have been used: solidification with pozzolanic material and lime, and encapsulation in polymers. Camacho et al. (2009) reported stabilization of arsenic containing iron hydroxide sludge by treatment with lime ($Ca(OH)_2$) based on the possibility of the formation of calcium-iron compounds with positive surface charge that could prevent the release of arsenic. However, they suggested the need for the use of a protective barrier to prevent the carbonation of the waste and subsequent release of arsenic from the immobilized sludge after long exposure to atmosphere. In general, As(V) is more efficiently stabilized by lime compared to As(III) (Akhter et al., 1997; Buchler et al., 1996; Vandecasteele et al., 2002). Based on toxicity characteristic leaching procedure (TCLP), Akhter et al. (1997) reported that the arsenic leaching from Type I Portland cement-treated arsenic-containing sludge did not vary significantly after curing for 28 days or 3 years.

Jing et al. (2005) performed TCLP, modified TCLP, California wet extraction test (Cal-WET), and modified Cal-WET experiments on arsenic-laden water treatment sludge treated with cement to evaluate leaching of arsenic after

curing for 28 days and 2 years. Compared to the regular TCLP and Cal-WET, the modified tests carried under N_2 environment resulted in more leaching of arsenic. When citrate replaced acetate in the TCLP protocol, arsenic leaching was approximately 20 times more. Additionally, the N_2 purging in the modified Cal-WET resulted in more arsenic leaching compared to the regular Cal-WET. The increased leaching in the modified tests was described as a result of the reduced condition and higher complexing capacity of citric acid that could result in stronger complexation with iron. In disagreement with the study by Akhter et al. (1997), arsenic leaching from the cement treated sludge decreased with increasing curing time, which was explained by the oxidation of As(III) to As(V).

Shaw et al. (2008) demonstrated an alternative stabilization technique through polymer encapsulation of arsenic-laden sludge. Polymer produced through aqueous-based manufacturing process using polystyrene butadiene and epoxy resin was used to encapsulate arsenic containing iron hydroxide sludge. The arsenic concentration in the leachate was well below the hazardous level of 5 mg/L as determined by the TCLP and Cal-WET (Shaw et al., 2008). Similarly, Bankowski et al. (2004) utilized geopolymers having a three dimensional inorganic amorphous structure synthesized by mixing waste materials rich in silica and alumina and activating with alkali metal hydroxide to encapsulate fly ash. They reported lower concentrations of arsenic, calcium, barium, strontium, and selenium in the leachate.

Besides these physico-chemical waste management practices, Banerjee (2010) recently evaluated the possibility of removing arsenic from the arsenic-

laden sludge collected from a water treatment plant through anaerobic digestion. Arsenic loaded sludge (1-10%) was mixed with composite feed slurry containing partially digested garbage/market waste, sludge from primary sedimentation tank of a wastewater treatment plant, and partially digested water hyacinth (1:1:1 ratio) and fed to the digester. A maximum arsenic removal of 99.69% was achieved after digestion for 50 days. The formation of arsine and dimethylarsine was suggested as the possible arsenic removal mechanism; however, this was not supported analytically.

2.12 Alternative Arsenic Removal Strategy

From the sustainable water treatment perspective, the treatment technologies described under section 2.10 may not present technologies of choice. The regeneration of the adsorbent or ion exchange resins and the disposal of the exhausted adsorbents and the sludge generated in these systems are of concern as the waste can contain high levels of arsenic and require further treatment (http://www.epa.gov/ogwdw/arsenic/pdfs/handbook_arsenic_treatment-tech.pdf). The ultimate fate of the arsenic-laden wastes under landfill conditions raises additional questions on the sustainability of the above mentioned technologies.

Based on TCLP, many of the current arsenic removal technologies are characterized as generating non-hazardous (Badruzzaman, 2003; Guo et al., 2007b) wastes. However, the TCLP underestimates arsenic leaching from the arsenic-laden sludge (Ghosh et al., 2004). Additionally, more aggressive

leaching procedures, such as the modified TCLP and Cal-WET tests performed by Jing et al. (2005) resulted in arsenic release even when the arsenic-laden wastes were stabilized. Therefore, arsenic removal technologies practiced under oxidizing environments may not provide a complete solution and alternative arsenic removal technologies need to be explored.

Sequestration of arsenic by sulfides in reducing environments has been reported (Demergasso et al., 2007; Kirk et al., 2004; O'Day et al., 2004) as an important mechanism controlling arsenic mobility in water. This suggests that arsenic removal under reduced conditions has the potential to be exploited as a treatment technology. Recently, researchers have focused on the effectiveness of iron sulfides for the removal of arsenic from water sources under reducing conditions (Gallegos et al., 2007b; Kirk et al., 2010; Teclu et al., 2008).

Belin et al. (1993) demonstrated 88% arsenic removal from the initial concentration of 70 mg As/L in a two stage reactor system (total hydraulic retention time of 24 h) utilizing biogenic sulfides generated by microorganisms indigenous to sulfate-contaminated mine tailings (Dinsdale et al., 1992). Performing batch experiments, Teclu et al. (2008) evaluated arsenic removal through sorption on precipitates generated by a mixed SRB culture and reported 77 and 55% As(III) and As(V) removal, respectively, from the initial concentration of 1 mg As/L. The pH of the system was 6.9 and the contact time was 24 h. Very recently, Kirk et al. (2010) also demonstrated arsenic removal through adsorption on pyrite and greigite generated biologically in a semi-continuous flow bioreactor. When acetate was supplied as the electron donor, microorganisms

originating from fine-grained alluvial sediment converted sulfate to sulfides. The biologically generated sulfides reacted with iron and generated iron sulfides, mackinawite. Interestingly, they reported very low adsorption capacity of mackinawite. After the injection of polysulfide, they reported the formation of greigite and pyrite, which effectively removed arsenic from the aqueous phase.

Arsenic removal utilizing sulfides under reducing environments provides two-fold advantage over treatment by applying iron/aluminum oxy-hydroxides when the ultimate fate is disposal of immobilized arsenic in landfills. First, this approach protects against reductive mobilization of arsenic (Jong and Parry, 2005). Second, should oxidizing conditions occur for short periods of time, the produced ferric oxy-hydroxide solids protect against oxidative mobilization. Under exposure to oxidizing conditions, arsenic-laden iron-sulfide sludge initially releases arsenic due to the oxidation of iron sulfides. However, due to the oxidation of Fe(II) to Fe(III) arsenic again is sequestered from the liquid phase (Jeong et al., 2009).

2.13 References

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Chapter 3

Simultaneous Removal of Nitrate and Arsenic from Drinking Water Sources utilizing a Fixed-bed Bioreactor System

3.1 Abstract

A novel bioreactor system, consisting of two biologically active carbon (BAC) reactors in series, was developed for the simultaneous removal of nitrate and arsenic from a synthetic groundwater supplemented with acetic acid. A mixed biofilm microbial community that developed on the BAC was capable of utilizing dissolved oxygen, nitrate, arsenate, and sulfate as the electron acceptors. Nitrate was removed from a concentration of approximately 50 mg/liter in the influent to below the detection limit of 0.2 mg/liter. Biologically generated sulfides resulted in the precipitation of the iron sulfides mackinawite and greigite, which concomitantly removed arsenic from an influent concentration of approximately 200 μg/liter to below 20 μg/liter through arsenic sulfide precipitation and surface precipitation on iron sulfides. This study showed for the first time that arsenic and nitrate can be simultaneously removed from drinking water sources utilizing a bioreactor system.

3.2 Introduction

Nitrate and arsenic, both regulated drinking water contaminants, have been reported to co-exist in groundwater in various locations around the world (Fytianos and Christophoridis, 2004; Ghurye et al., 1999). In several Asian countries, including Bangladesh (Zahid et al., 2008), India (Guha et al., 2005; Singh, 2006), Nepal (Singh, 2006), and Taiwan (Smedley and Kinniburgh, 2002), arsenic is present in groundwaters at concentrations of several hundreds of μg/liter. As a result, tens of millions of people are exposed to this contaminant through their drinking water (Argos et al., 2010). Excessive application of fertilizers and unmanaged irrigation (Behera et al., 2003), as well as poor sanitation and limited sewage management often result in co-contamination with nitrate in these areas. While the extent of the problem is less severe in the developed world, the presence of these contaminants in drinking water sources often results in closure of wells (Jahagirdar, 2003; Rosen et al., 2004) or the need for expensive, multi-step treatment.

Nitrate is most commonly removed from drinking water using ion-exchange or reverse osmosis (Pintar and Batista, 2006). Biological nitrate removal from drinking water has been widely studied and is commonly applied at the full-scale level in Europe (Aslan and Cakici, 2007; Mateju et al., 1992; Richard, 1989). Denitrifying bacteria convert nitrate to innocuous dinitrogen gas using organic or inorganic electron donor substrates. Arsenic, however, can only be removed from drinking water through phase transfer, i.e., by converting soluble arsenic into solid phase arsenic. The methods commonly applied for arsenic removal are

adsorption of arsenic species on iron or aluminum oxy-hydroxides, ion exchange, and reverse osmosis (Badruzzaman et al., 2004; Greenleaf et al., 2006; Ning, 2002). In a variation of the physico-chemical iron oxy-hydroxide adsorption process, Katsoyiannis et al. (2002) and Lehimas et al. (2001) utilized an aerobic bioreactor and biologically generated iron oxy-hydroxides to remove arsenic from groundwater. Alternatively, anaerobic bioreactors in which dissimilatory sulfate reduction takes place have the potential to remove arsenic from water sources through arsenic sorption by the sulfide solids produced. In addition, such reactors can support dissimilatory arsenate reducing microorganisms, which can enhance arsenic removal through co-precipitation of reduced arsenic species through the sulfide phases generated such as orpiment (As_2S_3) and realgar (As_4S_4).

Sulfate reducing prokaryotes mediate dissimilatory sulfate reduction in anaerobic environments resulting in the production of sulfides, which control the geochemistry of metals and metalloids, including arsenic (Kaksonen et al., 2003; Kirk et al., 2004; O'Day et al., 2004). While this process has mostly been studied in natural environments or subsurface remediation scenarios (Kirk et al., 2004), Belin et al. (1993) investigated the sequestration of arsenic by biogenically produced sulfides under reducing conditions for the treatment of mining and milling wastewater in a two-stage reactor system. They observed arsenic removal from an initial concentration of 70 mg/L to less than 2 mg/L due to the precipitation of orpiment (As_2S_3). Teclu et al. (2008) utilized a sulfate reducing consortium and achieved 55 and 77% arsenic removal from the initial concentration of 1 mg/L As(III) and As(V), respectively, in batch reactors.

Recently, Kirk et al. (2010) observed arsenic removal by sorption to pyrite and greigite in a sulfate reducing semi-continuous bioreactor.

Due to the co-existence of multiple contaminants in drinking water sources, including nitrate and arsenic as indicated above, technologies for their simultaneous removal are desirable. Reverse osmosis and ion exchange allow for simultaneous removal of multiple contaminants (Min et al., 2005), but are costly due to the required further treatment of concentrated waste streams, high energy requirements, and the need for regeneration of ion exchange resins (Nerenberg and Rittmann, 2004). In the current study, we developed a biologically mediated treatment alternative that can remove multiple contaminants in a single system. We demonstrate the potential of this treatment strategy using a laboratory-scale, continuous flow reactor system consisting of two fixed-bed biologically active carbon (BAC) reactors in series. The system can simultaneously remove arsenic and nitrate from a synthetic groundwater amended with acetic acid.

3.3 Materials and Methods

Reactor Set-up and Operation. The biologically active carbon (BAC) reactor system operated in this study consisted of two identical glass columns (reactor A and reactor B) with 4.9 cm inner diameter and 26 cm height (Figure 3.1). Reactor A and reactor B were packed with BAC particles collected from a bench-scale and a pilot-scale nitrate and perchlorate removing bioreactor (Li et al., 2010) to attain a bed volume of 200 cm^3 in each reactor. Granular activated

carbon (GAC) (bituminous F816; Calgon Carbon Corp., PA) with an effective size of 1.4 mm was used to generate the BAC particles in the nitrate and perchlorate removing reactor systems. The microbial communities, which developed in the bench-scale nitrate and perchlorate removing reactor, originated from various sources, including groundwater and a GAC filter operated at a full-scale drinking water treatment plant in Ann Arbor, Michigan (Li et al., 2010).

An arsenic contaminated synthetic groundwater was prepared as the influent solution (Table 3.1). Dissolved oxygen (DO) in the synthetic groundwater was removed to below 1 mg/L by purging with oxygen free N_2 gas for 40 min. To maintain the DO level below 1 mg/L, the influent tank was covered with a floating cover and the synthetic groundwater was purged with oxygen free N_2 gas for 20 min every 24 h. Based on an average net yield of 0.4 g biomass/g COD acetate (Rittmann and McCarty P. L., 2001), 23 mg/L acetate as carbon was estimated to be required to completely remove the electron acceptors (i.e., residual DO, nitrate, arsenate, and sulfate). With a safety factor of 1.5, the influent acetic acid concentration was maintained at 35 mg/L acetic acid as carbon.

The reactors were operated at room temperature (21.5±0.7 ^{o}C), except for the first 50 days of operation when the operating temperature was 18 ^{o}C, with the influent fed to reactor A in a down-flow mode using a peristaltic pump. A syringe pump (Harvard Apparatus, Holliston, MA) was used to feed a concentrated solution of glacial acetic acid and $FeCl_2.4H_2O$ to the influent line to reactor A, so that the acetic acid and Fe(II) concentrations fed to the system were equivalent to those reported in Table 3.1. The concentrated solution of acetic acid was

autoclaved and equilibrated in an anaerobic glove box (Coy, Grass Lake, MI) after which the $FeCl_2.4H_2O$ was added. This solution was then loaded into a syringe by filtering through a 0.22 μm filter. The syringe was placed on the syringe pump and the concentrated solution pumped to the reactor through a 0.22 μm filter. In order to promote complete removal of any sulfide formed by sulfate reduction, a concentrated solution of $FeCl_2.4H_2O$, prepared in an anaerobic chamber using de-ionized (DI) water and acidified to a final concentration of 0.02 N HCl, was directly fed to reactor B through a syringe pump to add an additional 4 mg/L Fe(II). The effluent of reactor A was introduced into reactor B in an up-flow fashion.

Reactor A was backwashed every 48 h with a mixed flow of deoxygenated DI water (50 mL/min) and N_2 gas to completely fluidize the filter bed for 2 min followed by a flow of deoxygenated DI water (500 mL/min) for 2 min to remove the dislodged biomass. Reactor B was backwashed approximately every 3-4 months following the same protocol. During the period for which data are reported in this study, reactor B was backwashed only on day 503.

During the operation of the BAC reactor, changes in the operating conditions were occasionally implemented to maintain or enhance performance. The influent flow rate was maintained at 10 mL/min to achieve an empty bed contact time (EBCT) of 20 min in each reactor (total 40 min EBCT). To optimize the EBCT, the bed volume of reactor A was adjusted to 150 cm^3 (EBCT 15 min), 100 cm^3 (EBCT 10 min), and 70 cm^3 (EBCT 7 min), while keeping the flow rate of

10 mL/min and the bed volume of the second reactor constant. Each EBCT condition was evaluated for a minimum of 30 days. On day 517 of reactor operation, 66% of the BAC in reactor A was replaced with BAC from the same stock used initially to pack the reactors and stored at 4 ^{o}C for approximately 17 months. At the same time, the EBCT of reactor A was increased to 10 min, while maintaining the EBCT of reactor B at 20 min (total 30 min EBCT).

Liquid Sample Collection and Chemical Analyses. Water samples were collected from the influent tank (Inf), the first effluent (EA), and the final effluent (EB) every 24 h. In addition, liquid profile samples were collected from the sampling ports of each reactor on day 538 of operation. The samples were stored at 4^{o} C after filtering through 0.22 µm filters (Fisher, Pittsburgh, PA). Samples for total arsenic and total iron were acidified to a final concentration of 0.02 N HCl before storage. All samples were analyzed for various anionic species and total elemental concentrations within 48 h.

The DO levels in the influent and the effluent from reactor A were measured using WTW multi340 meters with CellOx325 sensors in WTW D201 flow cells (Weilheim, Germany) connected to the inlet and outlet of reactor A. The detection limit for DO was 0.01 mg/L. Acetate, nitrate, nitrite, chloride, and sulfate were measured using an ion chromatography system (Dionex, Sunnyvale, CA) with a Dionex DX 100 conductivity detector. Chromatographic separation was achieved using a Dionex AS-14 column (Dionex, Sunnyvale, CA). Anions were eluted through the column with a mixture of ACS reagent grade 1 mM

bicarbonate and 3.5 mM carbonate at a flow rate of 1 mL/min. The detection limit for each of the anions was determined to be 0.2 mg/L.

Samples for total arsenic and total iron were analyzed using an ion coupled plasma mass spectrometer (ICP-MS) (PerkinElmer ALEN DRC-e, Waltham, MA) with detection limits of 2 µg/L As_T and 0.1 mg/L Fe_T, respectively. Samples for arsenic speciation were acidified to a final concentration of 0.02 N HCl and analyzed within 24 h using a Dionex AS4A-SC column (Dionex, Sunnyvale, CA) combined with ICP-MS (PerkinElmer, Waltham, MA). The eluent contained 1.5 mM oxalic acid and was provided at a flow rate of 2.5 mL/min. Both As(V) and As(III) were detectable at a level of 2.5 µg/L As.

Gas Sample Collection and Aanalyses. Gas samples were collected from the upper part of reactor A using a PressureLok® gas tight syringe (Baton Rouge, LA). The presence of nitrous oxide gas (N_2O), an intermediate of denitrification (Mateju et al., 1992), was assessed using an HP 5890 series II gas chromatograph equipped with a Poraplot-Q column (0.53 mm I.D. X 25 m) and an electron capture detector as described by Lee et al. (Lee et al., 2009). The protocol described by Pantsar-Kallio and Korpela (Pantsar-Kallio and Korpela, 2000) was modified to analyze gas samples collected from the upper part of reactor A for the presence of toxic gases of arsenic, i.e., arsine, monomethylarsine, dimethylarsine, and trimethylarsine. Gaseous samples (250 µL) were injected into an HP 5890 series II GC interfaced to a HP 5972 Mass Spectrometer using a PressureLok® gas tight syringe (Baton Rouge, LA). The system was fitted with a DB-5 capillary column (0.25 mm I.D. X 60 m) with 1

micron film thickness. Helium was used as the carrier gas. The analyses were done isothermally at 36 ^{o}C with the mass spectrometer operated in single ion monitor. The detection limits for arsine, monomethylarsine, dimethylarsine, and trimethylarsine were 1 ng/µL, 3 ng/µL, 2 ng/µL, and 2 ng/µL as As, respectively.

X-ray Absorption Spectroscopy and X-ray Diffraction Analyses. Reactor B was backwashed on day 503 of operation to collect solids deposited in the reactor bed. The backwash waste was collected under a flow of N_2-gas and immediately transferred to an anaerobic chamber (Coy, Grass Lake, Michigan) filled with a mixture of 3% H_2 and 97% N_2. Solids were vacuum-filtered within the anaerobic chamber. A part of the vacuum-filtered solids was kept as a wet paste and was transferred to 20 mL serum bottles, sealed with butyl rubber septa and aluminum crimps, and shipped to the Stanford Synchrotron Radiation Lightsource (SSRL) for arsenic and iron X-ray absorption spectroscopy (XAS) data collection. The remaining vacuum filtered solids were freeze-dried and ground in the anaerobic chamber using a mortar and pestle. X-ray diffraction (XRD) patterns of the freeze-dried powdered samples were obtained using a Rigaku Rotaflex rotating anode X-ray diffractometer (Cu $K\alpha$ radiation, 40 kV, 100 mA).

XAS samples prepared for iron analyses were diluted using boron nitride to obtain a concentration sufficiently high for a good signal but low enough to prevent self-absorption (20:1, boron nitride: sample by mass). Sample preparation and loading were performed in an anaerobic chamber. As K-edge (11867 eV) and Fe K-edge (7112 eV) X-ray absorption spectra were collected at

the beam line 11-2 using a 30-element Ge detector or Lytle detector at the beam energy of 3.0 GeV and maximum beam current of 200 mA. Fluorescence spectra of the wet paste samples were collected using a low temperature cryostat filled with liquid nitrogen. To minimize the contribution from the higher order harmonics, the monochromator was detuned 35 % for As and 50 % for Fe at the highest energy position of the scans. The beam energy was calibrated using the simultaneously measured As or Fe standard foil spectrum. To obtain improved signal to noise ratios, eleven and eight scans were collected for the As and Fe samples, respectively.

Data analyses were performed using FEFF8, IFEFFIT, SIXPAK, and EXAFSPAK codes (Ankudinov et al., 2002; George and Pickering, 2000; Newville, 2001). Acceptable signal channels were selected and the multiple scans were averaged after energy calibration. Backgrounds were removed using linear fits below the absorption edge and spline fits above the edge using the IFEFFIT code. The spectra were then converted from the energy to the frequency space using the photo electron wave vector k in the range of $3<k<11$ for As and $3<k<12$ for Fe. EXAFS fitting was performed using SIXPAK with phase shift and amplitude functions for backscattering paths obtained from FEFF8 calculations with crystallographic input files generated using ATOMS program. To obtain the optimal structural parameters, including coordination numbers (*CN*s) and inter-atomic distances (R), the Debye-Waller factor (σ^2) and energy reference E_0 parameters were also floated during the fitting. The many-body factor S_0^2 was fixed at 0.9 to reduce the number of fitting parameters.

EXAFS fitting was also performed using EXAFSPAK and compared to those obtained by SIXPAK to insure results were consistent and not dependent on the fitting algorithms used.

3.4 Results

Reactor Performance. During the reactor operating period reported herein, the pH of the effluents of reactors A and B was 7.2±0.5 (mean ± standard deviation). DO levels in the influent (Inf) and the first effluent (EA) averaged 0.77±0.50 mg/L and 0.02±0.01 mg/L, respectively. Even though arsenic adsorption on virgin or modified GAC has been reported (Chen et al., 2007; Gu et al., 2005; Mondal et al., 2007), arsenic removal was not observed in the current study during startup as the arsenic concentration in the final effluent remained equivalent to the influent level for the first 50 days of operation. After increasing the operating temperature from 18 ^{o}C to 22 ^{o}C on day 50, sulfate reduction started on day 54 and arsenic removal was observed soon thereafter (data not shown).

From days 503 to 517, reactor A was operated at an EBCT of 7 min. At this low EBCT, nitrate occasionally carried over into reactor B (Figure 3.2). To avoid this, the EBCT in reactor A was increased to 10 min on day 517, which resulted in complete nitrate removal in reactor A (Figure 3.2). Nitrite and nitrous oxide, intermediates of denitrification, were never detected in the effluents of either of the reactors or the gas collected from the upper part of the first reactor, respectively.

Prior to day 517, reactors A and B removed 3.4±1.9 mg/L and 15.8±1.5 mg/L sulfate, respectively. Though aqueous phase arsenic speciation analyses were not performed during the period reported herein, previous speciation analyses indicated that arsenate was reduced to arsenite and removed through precipitation with biogenically produced sulfides or surface precipitation and adsorption on iron sulfides (below). From days 503 to 517, the arsenic concentration in the final effluent averaged 41±22µg/L (Figure 3.2). After increasing the EBCT of reactor A from 7 min to 10 min (total EBCT from 27 min to 30 min) on day 517, sulfate removal in reactors A and B was similar to the previous period (1.5±1.1 and 15.4±1.7 mg/L, respectively). However, the arsenic level in the final effluent decreased to below 20 µg/L on day 532 (Figure 3.2). None of the gaseous arsenic species (arsine, monomethylarsine, dimethylarsine, and trimethylarsine) were detected in the gas collected from the upper part of the first reactor.

Concentration Profiles along the Depth of the Bioreactors. Profile samples collected on day 538 indicated a sequential utilization of DO (data not shown), nitrate, and sulfate (Figure 3.3). Nitrate was completely removed in reactor A as indicated by a nitrate concentration below the detection limit in port A8. Sulfate reduction began after nitrate removal was complete (after port A8 in reactor A). The utilization of the electron acceptors corresponded with acetate consumption. Between the influent and port A8 of reactor A, where DO and nitrate were utilized as the electron acceptors, 18.5±0.1 mg/L of acetate as carbon was consumed. The remainder of acetate consumption between port A8 and the final effluent

(6.3±0.1 mg/L of acetate as carbon) corresponded to the amount of acetate required for the measured amount of sulfate to be reduced. Iron and arsenic depletion from the aqueous phase followed the trend of sulfate reduction (Figure 3.3). Reactor A removed 101±2 µg/L of arsenic, while reactor B further reduced the arsenic level to a final effluent (EB) concentration of 13±0.3 µg/L. The precipitation of iron sulfides removed 0.3±0.1 mg/L iron in reactor A and 4.7±0.1 mg/L of iron in reactor B.

Solids Characterization. XRD analysis indicated the presence of mackinawite (tetragonal iron mono-sulfide, FeS_{1-x}) and greigite (Fe_3S_4) as the solids deposited in the reactor system (Figure 3.4). X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) analyses were also performed on the XAS data collected. Fe XANES and the corresponding first derivative plots of the solids collected from the second reactor and chemically synthesized pure model compounds mackinawite and greigite are presented in Figure 3.5. A comparison of the peak positions and shapes suggests that the major iron phase is mackinawite. EXAFS fitting results and the structural parameters extracted from the fitting are given in Figure 3.6 and Table 3.2. The Fe K-edge EXAFS analysis (Figure 3.6(a) and 6(b)) indicates that Fe atoms are coordinated by 5.5 S atoms at 2.23 Å with σ^2 of 0.0133 and 1.8 Fe atoms at 3.04 Å with σ^2 of 0.0045. These structural parameters match reasonably well with previously reported values for mackinawite. For example, Lennie et al. (1995) have reported a coordination number of 4 S atoms with Fe at 2.25577 Å from XRD structural refinement. The Fe-S distance is also in good

agreement with a previous EXAFS result for synthetic mackinawite of 2.24 Å (Jeong et al., 2008).

The EXAFS analysis of As K-edge X-ray absorption spectrum indicates that As has 2.2 S atoms at 2.29 Å with σ^2 of 0.0048 (Table 3.2 and Figure 3.6(c) and 6(d)). These structural parameters are in good agreement with the arsenic-sulfur bond found in solid phases such as orpiment (As_2S_3) (1 S at 2.27009 Å, 1 S at 2.28935 Å, and 1 S at 2.29186 Å) or realgar (As_4S_4) (1 S at 2.23279 Å and 1 S at 2.24143 Å) reported by XRD structural analysis (Mullen and Nowacki, 1972; Whitfield, 1970) and with the reported As-S bond distance of 2.25 Å from the EXAFS analysis of solid phase products of As reacted with mackinawite at circumneutral pH (Gallegos et al., 2008; Jeong et al., 2010). Taken together, these results indicate the formation of arsenic sulfide, either as a bulk precipitate (i.e., three dimensional structures) or surface precipitate (i.e., two dimensional arrays) on iron sulfide particles, as the primary arsenic removal mechanism in the bioreactor. This, however, does not rule out the possibility of arsenic adsorption on iron sulfides as an additional removal mechanism (Gallegos et al., 2007; Teclu et al., 2008)

3.5 Discussion

To evaluate the possibility of arsenic removal under reduced conditions utilizing biogenically produced sulfides, this research investigated the potential of a fixed-bed bioreactor system to remove arsenic from drinking water sources. Since arsenic is seldom the only contaminant that needs to be removed from

drinking water sources, the simultaneous removal of nitrate, a common co-contaminant of arsenic, was also investigated. Given that this BAC system has also been shown to be effective to simultaneously removing other commonly occurring co-contaminants (e.g., perchlorate, nitrate (Li et al., 2010), and uranium (Ghosh et al., unpublished results), the use of anaerobic BAC reactors has potential for widespread application in drinking water treatment (Brown, 2007).

Another potential advantage of the anaerobic BAC system is the nature of the sulfidic sludge that is produced. Although the use of oxy-hydroxides (i.e., iron (III) hydroxides or aluminum hydroxides) in aerobic treatment systems have been found to effectively remove arsenic from contaminated water (Katsoyiannis et al., 2002; Khan et al., 2002), when arsenic-bearing sludge is landfilled and conditions turn anaerobic, arsenic will leach out. Specifically, dissimilatory reduction of Fe(III) is known to cause the release of sorbed arsenic through the reductive dissolution of the iron (III) oxy-hydroxides phases (Bose and Sharma, 2002; Cummings et al., 1999; Ghosh et al., 2006; Irail et al., 2008). Similarly, dissimilatory reduction of adsorbed arsenate (Sierra-Alvarez et al., 2005; Yamamura et al., 2005; Zobrist et al., 2000) to less strongly sorbing As(III) species will result in the release of arsenic to the aqueous phase. In contrast, arsenic removal by the formation of sulfidic solids avoids this shortcoming in two ways. First, this approach protects against reductive mobilization as demonstrated by Jong and Parry (2005). Performing both short and long term leaching tests, they showed that arsenic leaching from a sulfidic sludge was low enough for the sludge to be characterized as nonhazardous waste. Second, in

the event that such a sludge is subjected to episodes of oxygen exposure in a landfill, the production of ferric oxy-hydroxides will protect against oxidative mobilization. This was demonstrated in a recent study. When samples of arsenic reacted with iron sulfides at cirumneutral pH were exposed to oxygen, the iron hydroxide solid phases formed effectively captured any arsenic temporarily released to solution during the oxidation process (Jeong et al., 2009; Jeong et al., 2010).

The BAC reactor employed in this study relies on coupling the oxidation of an electron donor to the reduction of electron acceptors (DO, nitrate, iron(III), sulfate, and arsenate) to promote the biologically mediated removal of nitrate and arsenic from a synthetic groundwater using an engineered reactor system. This is similar to the terminal electron accepting processes (TEAPs) observed in natural environments (Lovley and Chapelle, 1995). For practical reasons, acetic acid was selected as the sole electron donor in this study as it has been approved for drinking water treatment (National Sanitation Foundation product and service listings, www.nsf.org) and was previously found to be effective for nitrate and perchlorate removal in bioreactors from which inocula were used for this study (Li et al., 2010). In addition, many iron (Coates et al., 1996; Cord-Ruwisch et al., 1998; Roden and Lovley, 1993; Vandieken et al., 2006) and sulfate reducing bacteria (Abildgaard et al., 2004; Devereux et al., 1989; Kuever et al., 2005) can utilize acetic acid as their electron donor (Christensen, 1984; Muthumbi et al., 2001; Oude Elferink et al., 1999; Oude Elferink et al., 1998). Given the desire to biogenically produce iron sulfide solids for arsenic removal,

acetic acid was expected to be a good choice for promoting adequate growth of iron and sulfate reducers.

As the results show, coupled with acetate oxidation, DO, nitrate, arsenate, and sulfate present in the synthetic groundwater were sequentially reduced (Figure 3.3). Iron was present in the influent in the form of Fe(II). Despite the presence of low levels of DO in the influent (< 1 mg/L), no visual presence of Fe(III) hydroxides (e.g., brownish orange particles) were observed at the inlet of the bioreactor. This suggested the rapid utilization of the small residual DO from the influent tank. Though DO was not measured along the depth of the reactors, based on thermodynamic favorability (Lovley and Phillips, 1988; Rikken et al., 1996) DO utilization is expected to be the first TEAP to occur at the inlet of the reactor. As seen in Figure 3.3, effective nitrate removal was also established in the system, with nitrate below detection at sampling port A8 and beyond. Gibb's free energies of reaction calculated at standard conditions and pH of 7 for nitrate, arsenate, and sulfate reduction using acetate as the electron donor are -792, -252.6, and -47.6 kJ/mole of acetate, respectively (Macy et al., 1996; Rikken et al., 1996), indicating arsenate reduction is expected after nitrate reduction under equivalent electron acceptor concentration conditions. Arsenic speciation measurements made during the first part of reactor operation showed a predominance of arsenite (As(III)) in the effluent from reactor A (data not shown), confirming that arsenate reduction took place.

The absence of detectable nitrite and nitrous oxide suggest complete denitrification in reactor A. Prior to day 517, the EBCT in reactor A was 7 min

(total EBCT 27 min) and nitrate was occasionally present in the second reactor. During the episodic periods of nitrate presence in reactor B, the TEAP zones for arsenate and sulfate reduction were likely shifted towards the end of reactor B. Even though total sulfate reduction was not impacted, poor arsenic removal was observed during this time period perhaps due to shifting TEAP zones. It is hypothesized that arsenate reduction, sulfate reduction, and the presence of iron(II) must occur proximally to obtain effective arsenic removal through precipitation/co-precipitation. The poor reactor performance observed during this time period suggests that maintaining stable TEAP zones is important for stable and optimal arsenic removal.

As evidenced by chemical analyses of the liquid samples along the depth of the reactors, sulfate reduction corresponded with arsenic removal. Given that arsenite (As(III)) can react with sulfide (S(-II)) and result in the formation of arsenic sulfides, such as orpiment (Newman et al., 1997) and realgar (O'Day et al., 2004), it is possible that arsenic was removed through the precipitation of these solids. However, in the presence of iron(II), it is equally likely that formation of iron sulfide minerals, including poorly crystalline iron sulfides (Herbert et al., 1998), mackinawite (Farquhar et al., 2002; Gallegos et al., 2007; Jeong et al., 2009; Wolthers et al., 2005), greigite (Wilkin and Ford, 2006), and pyrite (Farquhar et al., 2002) were responsible for lowering the arsenic concentrations. In fact, in a system containing iron(II), sulfides, and arsenic, arsenic removal is expected to take place primarily by adsorption/coprecipitation with iron sulfides rather than by precipitation of arsenic sulfides alone due to the

difference in the solubility of iron and arsenic sulfides (Kirk et al., 2010; O'Day et al., 2004). In our system, iron depletion from the liquid phase followed the pattern of sulfate reduction along the flow direction (Figure 3.3) indicating that iron sulfides were generated, which concomitantly removed arsenic from the liquid phase.

Iron(II) and sulfides in aqueous solutions at ambient temperatures result in the precipitation of black nanoparticulate iron sulfides (Jeong et al., 2009; Rittle et al., 1995; Wolthers et al., 2005), which effectively remove arsenic (Gallegos et al., 2007). Additionally, biogenically produced sulfides can sequester arsenic in aqueous systems due to sorption and precipitation/co-precipitation mechanisms (Kirk et al., 2004; Newman et al., 1997; Rittle et al., 1995). XRD analyses of the solids collected from the second reactor in this study confirmed the presence of mackinawite (FeS_{1-x}; JCPDS 04-003-6935) and greigite (Fe_3S_4; JCPDS 00-016-0713). Mackinawite is typically the first iron sulfide to precipitate in aqueous solutions and may transform into more stable iron sulfides, such as greigite and pyrite (Wolthers et al., 2003). In an acetate-fed semi-continuous bioreactor, Kirk et al. (2010) reported that precipitation of iron sulfides sequestered arsenic from the liquid phase but that arsenic sulfides (i.e., realgar and orpiment) were under-saturated. In the current system, arsenic was likely removed from the liquid phase through surface precipitation on iron sulfide surfaces and direct arsenic sulfide precipitation. Adsorption on iron sulfides may have provided additional arsenic removal. Even though orpiment precipitation requires acidic conditions, arsenic sulfide precipitation could occur in local environments or as a result of

microbial activity (Newman et al., 1997). Previous studies also indicated that realgar can be precipitated in the presence of iron sulfides under sufficiently reducing conditions (Gallegos et al., 2008; Gallegos et al., 2007). EXAFS analyses from this study further supports this interpretation, confirming Fe-S and As-S coordination consistent with the formation of iron sulfide and arsenic sulfide solid phases.

Microbial reductions of arsenate and arsenite have been reported to generate methylated arsenicals (Reimer, 1989). In addition, iron, nitrate, and sulfate reducing bacteria have been shown to be capable of producing methylated arsenic compounds including toxic arsenic gases, such as arsine, monomethylarsine, dimethylarsine, and trimethylarsine (Bentley and Chasteen, 2002; Reimer, 1989). Despite the presence of a diverse microbial community in the present reactor system, including iron, nitrate, arsenate, and sulfate reducing bacteria (Upadhyaya et al.; unpublished results), these toxic arsenic gases were not detected. Interestingly, although sulfate reducing bacteria are known to be the primary producers of methylated mercury species, the presence of iron sulfide has been found to inhibit mercury methylation (Liu et al., 2009). Perhaps iron sulfide is playing a similar role in inhibiting the formation of methyl arsine species in this reactor system.

Biological reduction of arsenate to arsenite and the concomitant interaction of biogenic sulfides with arsenite resulted in the progressive removal of arsenic from the aqueous phase along the depth of the reactors. However, to date, arsenic concentrations in the final effluent are still above the World Health

Organization (WHO)'s provisional guideline value and U.S. EPA maximum contaminant level (MCL) of 10 µg/L. Current efforts are focused on optimizing the system, including adjustment of iron and sulfate additions, to lower arsenic concentrations in the final effluent below 10 µg/L. While achieving substantial arsenic removal, complete nitrate removal was accomplished at all times.

3.6 Conclusions

The fixed-bed bioreactor system described in this study simultaneously removed arsenic and nitrate from synthetic drinking water utilizing an inoculum originating from a mixed community of microbes indigenous to groundwater. The microorganisms utilized DO, nitrate, sulfate, and arsenate as the electron acceptors in a sequential manner in the presence of acetic acid as the electron donor. Biologically produced sulfides effectively removed arsenic from the water, likely through the formation of arsenic sulfides, and/or surface precipitation and adsorption on iron sulfides. This work demonstrates the feasibility of fixed-bed bioreactor treatment systems for achieving simultaneous removal of arsenic and nitrate from contaminated drinking supplies.

3.7 Tables and Figures

Table 3.1: *Composition of the synthetic groundwater fed to reactor A.*

Chemical	Concentration	Unit
$NaNO_3$	50.0	mg/L as NO_3^-
NaCl	13.1	mg/L as Cl^-
$CaCl_2$	13.1	mg/L as Cl^-
$MgCl_2.6H_2O$	13.1	mg/L as Cl^-
K_2CO_3	6.0	mg/l as CO_3^{2-}
$NaHCO_3$	213.5	mg/L as HCO_3^-
Na_2SO_4	22.4	mg/L as SO_4^{2-}
$Na_2HAsO_4.7H_2O$	0.2	mg/L as As
H_3PO_4	0.5	mg/L as P
$FeCl_2.4H_2O$[a,b]	6.0	mg/L as Fe^{2+}
CH_3COOH[a]	35.0	mg/L as C

[a] Added as concentrated solution through a syringe pump. The concentrations in the table represent the concentrations after mixing of the concentrated solution and the influent.

[b] In addition to the supplementation of $FeCl_2.4H_2O$ to reactor A, a concentrated solution of $FeCl_2.4H_2O$ was added to reactor B using a syringe pump to provide an additional 4 mg/L as Fe(II) to the system.

Table 3.2: *Structural parameters extracted from the EXAFS analysis*

Data	Path	*CN*	*R*	σ^2	Fit value (R factor)
Fe K edge	Fe-S	5.5	2.23	0.0133	0.2568
	Fe-Fe	1.8	3.04	0.0045	0.0192
As Kedge	As-S	2.2	2.29	0.0048	0.0845
	As-As	4.4	3.56	0.0184	0.0551

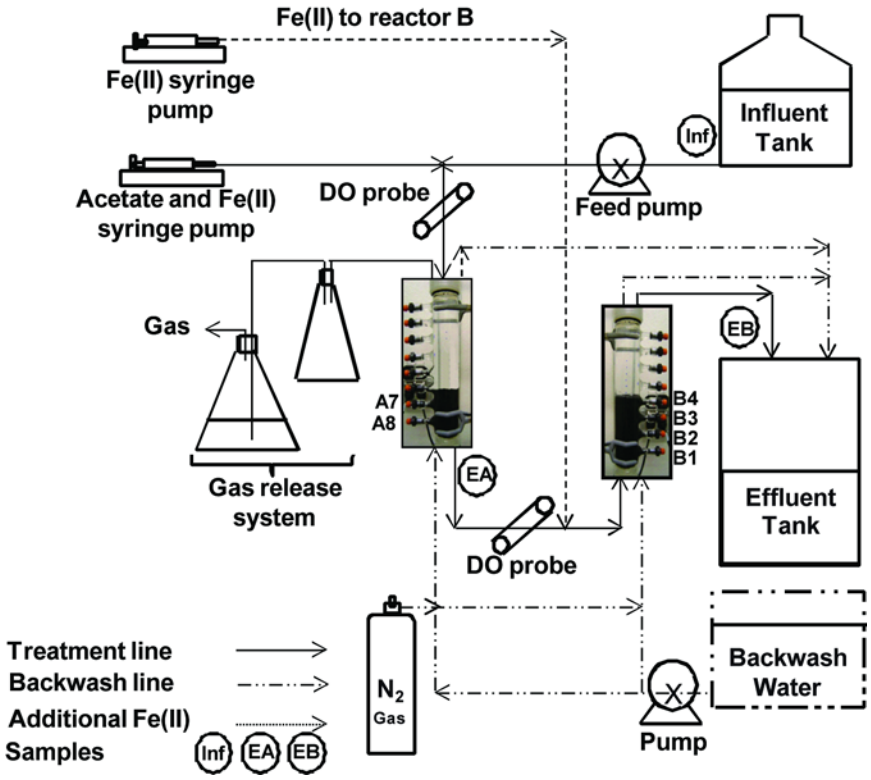

Figure 3.1: *Schematic of the reactor system.*

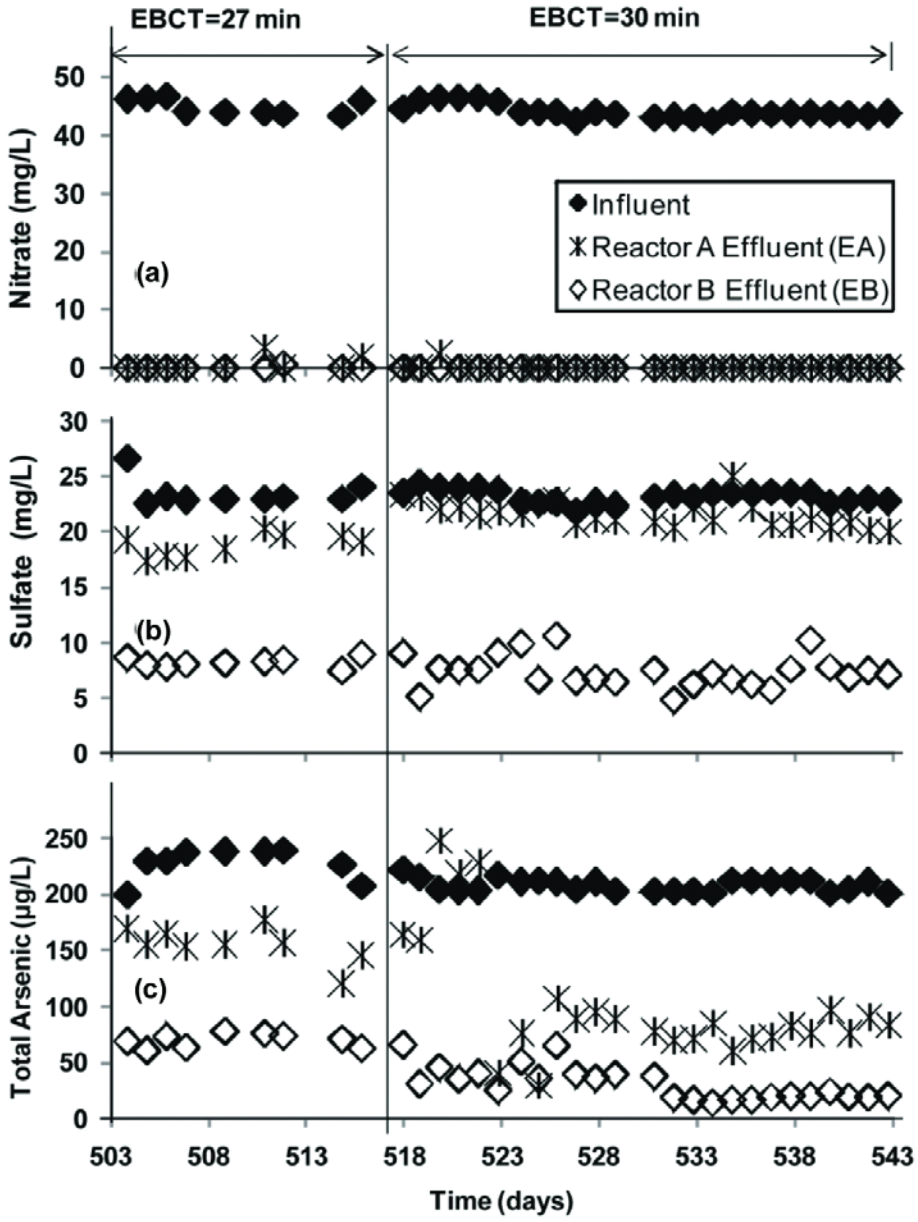

Figure 3.2: *(a) Nitrate, (b) sulfate, and (c) total arsenic concentrations in the influent, the effluent of reactor A (EA), and the effluent of reactor B (EB) versus time of operation. The total EBCT was changed from 27 min to 30 min on day 517 by increasing the EBCT of reactor A from 7 min to 10 min, while the EBCT of reactor B remained at 20 min.*

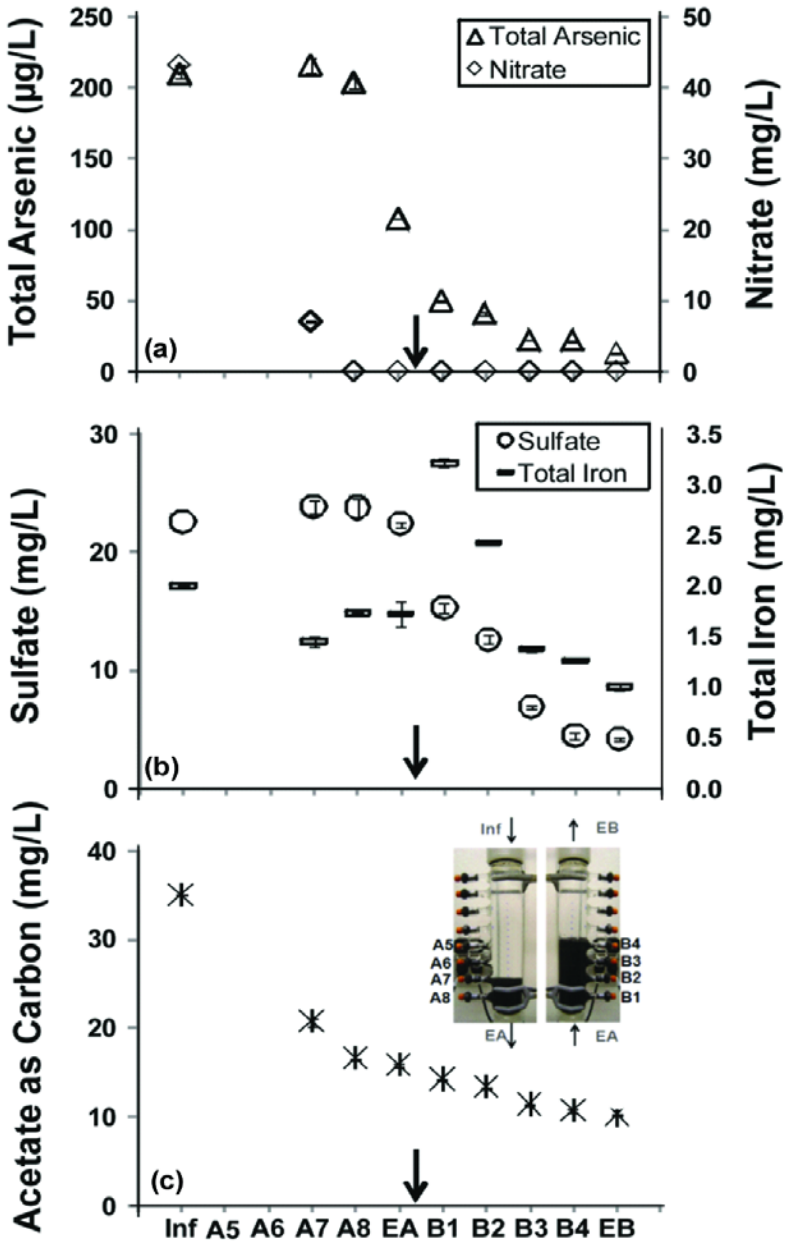

Figure 3.3: *Chemical profiles along the depth of the reactor beds on day 538. Nitrate and total arsenic concentrations (a), sulfate and total iron concentrations (b), and acetate concentrations (c). Inf represents the influent concentrations, A7, A8, and B1-B4 represent the respective sampling ports along the depth of reactors A and B, respectively. EA and EB represent concentrations in the effluents from reactor A and reactor B, respectively. The arrow indicates the location of additional Fe (II) (4 mg/L) addition. Mean (n=3) values are reported with the error bars representing one standard deviation from the mean.*

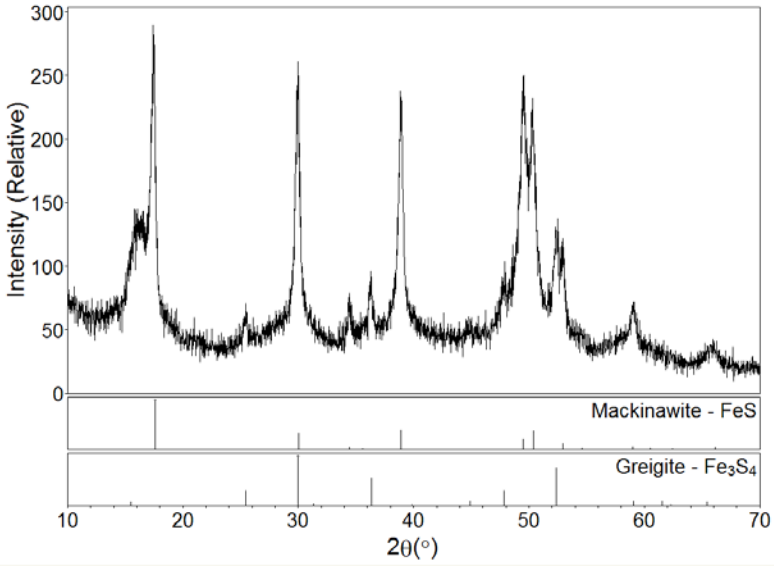

Figure 3.4: *X-ray Diffraction pattern of solids collected from reactor B on day 503. The intensity is reported as counts per second (CPS) along the two-theta range of 10 to 70 degrees. Characteristic patterns of mackinawite and greigite are shown for comparison, powder diffraction files 04-003-6935 and 00-016-0713, respectively.*

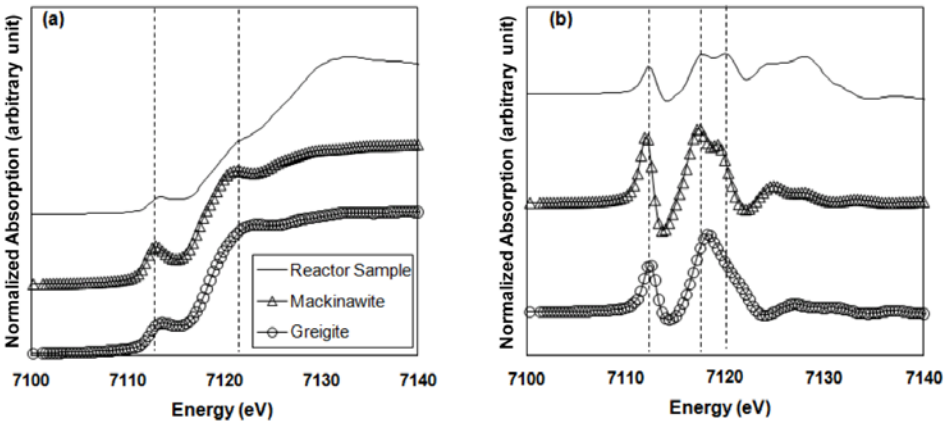

Figure 3.5: *X-ray absorption near edge structure spectrum (a) and its first derivative (b) of the solid sample collected on day 503 along with those of model compounds mackinawite and greigite. The reactor sample has the first derivative with a singlet at 7112 eV and a doublet between 7118 and 7120 eV characteristic of mackinawite. This comparison suggests that the solid sample collected from reactor B is mainly composed of mackinawite rather than greigite.*

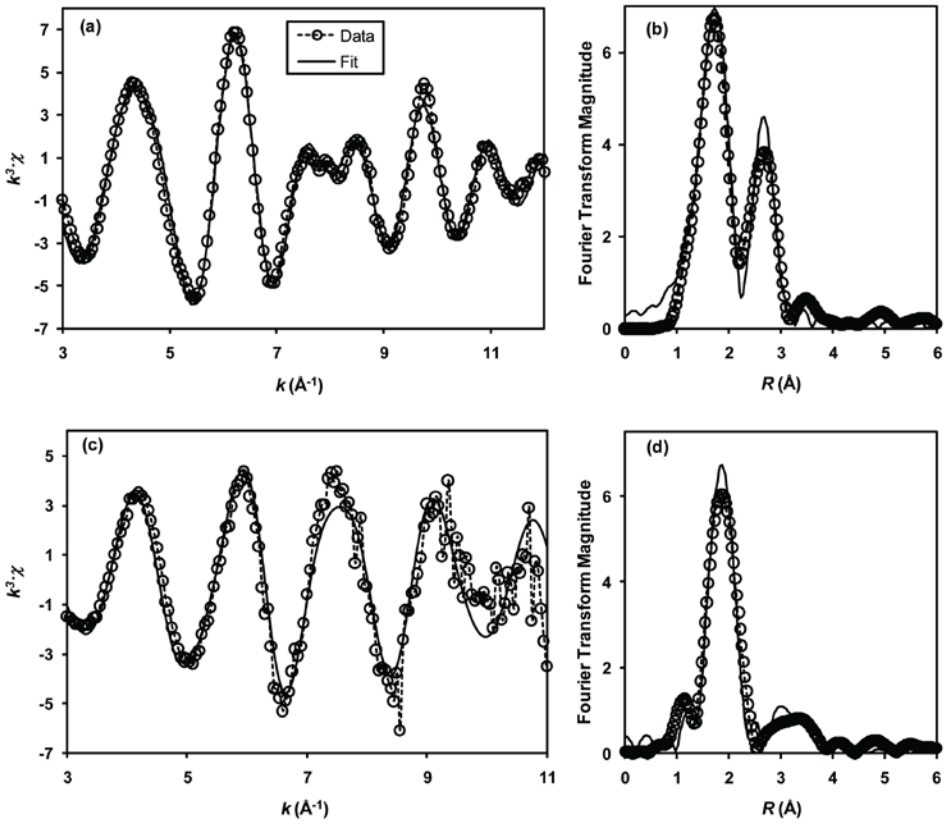

Figure 3.6: *K-edge EXAFS fitting results for Fe in the k-space (a), R-space (b) and for As in the k-space (c) and R-space (d) for the solids collected from reactor B on day 503.*

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Chapter 4

Role of Sulfate and Arsenate Reducing Bacteria in a Biofilm Reactor System Used to Remove Nitrate and Arsenic from Drinking Water

Running Title: SRB and DARB in nitrate and arsenic removing bioreactors

4.1 Abstract

Biological sulfate and arsenate reduction and subsequent sequestration of arsenic can be utilized for arsenic removal from drinking water sources in an engineered system. To optimize bioreactor performance and contaminant removal, it is crucial to understand the structure and activity of the microbial community in such bioreactor systems. This research investigated microbial community structure, spatial distribution of sulfate reducing bacteria (SRB) and dissimilatory arsenate reducing bacteria (DARB), and the activity of SRB and DARB in a system consisting of two biofilm reactors in series that simultaneously removed nitrate and arsenic from a simulated groundwater. Glacial acetic acid was used as the sole electron donor. Compared to average influent levels of 50 mg/L, 22 mg/L, and 300 µg/L, the effluent contained less than 0.2 mg/L NO_3^-, less than 10 mg/L SO_4^{2-}, and less than 30 µg/L As. Bacterial 16S rRNA gene and the dissimilatory (bi)-sulfite reductase (*dsrAB*) gene sequence analyses

indicated a predominance of SRB related to the *Desulfatirhabdium-Desulfobacterium-Desulfococcus-Desulfonema-Desulfosarcina* assemblage. The dissimilatory arsenate reductase (*arrA*) gene sequence analyses indicated the presence of two major DARB populations with a predominance of DARB related to *Geobacter uraniireducens.* Besides SRB and DARB, nitrate and iron reducing bacteria were also detected. Quantitative PCR indicated the presence of SRB and DARB throughout the reactor system, while reverse transcriptase quantitative PCR indicated maximum *dsrAB* activity in the center of the reactor system. The activity of *arrA* increased in the flow direction and declined again after attaining a maximum level in the middle of the second reactor. The activity of SRB and DARB corresponded well with reactor performance.

4.2 Introduction

The presence of arsenic in drinking water sources has resulted in serious health threats to millions of people (3). Arsenate (As(V)) and arsenite (As(III)) species are the most abundant forms of arsenic in oxidizing and reducing natural environments, respectively (11). At near-neutral pH, As(III) species are more mobile compared to As(V) species, which exist as anions at circumneutral pH and exhibit higher affinity for iron or aluminum hydroxides (11). While biologically mediated iron(III) reduction (13, 17) or As(V) reduction (24, 38) can mobilize arsenic from natural rocks and sediments, biological sulfate reduction and subsequent precipitation of sulfides may re-immobilize released arsenic (21, 34). Many sulfate reducing prokaryotes (SRP) are able to reduce and tolerate the toxicity of metals and metalloids, and withstand high concentrations of sulfides

(8). Because of their diversity, ubiquity, and ability to reduce and resist the toxicity of environmental contaminants, SRP have been utilized for bioremediation and contaminants removal in engineered systems that promote biological sulfate reduction (32).

Biological sulfate reduction results in the production of sulfides, which react with heavy metals (19) and metalloids including arsenic (5, 21) to generate sulfide solids that exhibit low solubility (20, 34). Given that As(III) reacts with sulfides (S(-II)) resulting in the formation of arsenic sulfides, such as orpiment (As_2S_3) (33) and realgar (AsS) (34), arsenic removal can be promoted by the generation of As(III) through biological As(V) reduction in an engineered system.

Understanding the microbial community structure and abundance and activity of key microbial populations is crucial to optimize and achieve sustained contaminant removal with an engineered bioreactor system. Highly conserved functional genes, such as the dissimilatory (bi)sulfite reductase (*dsrAB*) gene (41, 46) and the dissimilatory arsenate reductase gene (*arrA*) (31) have served as effective targets for the identification and quantification of the abundance and activity of sulfate and arsenate reducing microbial populations in a variety of environments (23, 25, 40).

The objective of the current study was to elucidate the microbial community structure and assess the abundance and activity of sulfate reducing bacteria (SRB) and dissimilatory arsenate reducing bacteria (DARB) in a bench-scale biofilm reactor system that simultaneously removed nitrate and arsenic from a

simulated groundwater. To better understand the system, microbial data were linked to reactor performance and operational parameters.

4.3 Materials and Methods

Reactor System and Operation. Synthetic groundwater containing arsenic (As(V)) and nitrate was fed to a biologically active carbon (BAC) reactor system consisting of two identical glass columns (4.9 cm inner diameter, 26 cm height) in series (reactor A followed by reactor B) packed with BAC particles (Chapter 3 and Upadhyaya et al., 2010). The BAC particles were collected from a bench-scale and a pilot-scale perchlorate and nitrate removing bioreactor. The bench-scale perchlorate and nitrate removing bioreactor received inocula from a previous perchlorate removing bioreactor and a GAC filter operated at a full-scale drinking water treatment plant in Ann Arbor, Michigan (27). Prior to day 50, the reactors in the current study were operated at 18 °C with an empty bed contact time (EBCT) of 20 min for each reactor (total EBCT 40 min). The operational temperature was raised to 24 ^{o}C on day 50. A syringe pump (Harvard apparatus, Holliston, MA) was used to deliver 35 mg/L acetic acid as C to reactor A as described in Upadhyaya et al. (45). Dissolved oxygen (DO) in the influent was maintained at less than 1 mg/L by sparging the synthetic groundwater with N_2 gas. Initially, 10 mg/L Fe(II) ($FeCl_2.4H_2O$) acidified to a final concentration of 0.02 N HCl was loaded to reactor B using a syringe pump. Reactor A was backwashed every 48 h with a mixed flow of deoxygenated de-ionized (DDI) water (50 mL/min) and N_2 gas to completely fluidize the filter bed for 2 min followed by a flow of DDI water (500 mL/min) for 2 min. Reactor B was

backwashed on day 246 to collect solids deposited in the reactor using the backwashing protocol described above.

On day 121, iron loading was switched to reactor A and 10 mg/L iron(II) (without HCl acidification) was fed to the system along with the acetic acid. This resulted in gradual accumulation of iron hydroxides in the upper part of reactor A (see below). On day 144, the upper part of reactor A was cleaned and the system was operated without iron addition. Iron addition to reactor A was resumed on day 160, i.e., 1 mg/L Fe(II) was added along with the acetic acid. Iron loading was changed again on day 266 when 2 mg/L Fe(II) was added to the system along with the acetic acid. On day 300, the EBCT of reactor A was changed to 15 min (total EBCT 35 min).

Liquid Sample Collection and Chemical Analyses. Water samples from the influent tank (Inf), the effluent from reactor A (EA), and the effluent from reactor B (EB) were collected every 24 h. In addition, liquid samples were collected from the sampling ports along the depth of each reactor on day 300 of reactor operation. With a syringe, the samples were filtered through 0.22 μm filters (Fisher, Pittsburgh, PA). Water samples for total arsenic and total iron were acidified to a final concentration of 0.02 N HCl. The samples were stored at 4 ^{o}C until analyses. Samples for arsenic speciation were acidified to a final concentration of 0.02 N HCl and analyzed within 24 h using a Dionex AS4A-SC column (Dionex, Sunnyvale, CA) combined with ICP-MS (PerkinElmer, Waltham, MA). ACS reagent grade 1.5 mM oxalic acid was used as the eluent at a flow

rate of 2.5 mL/min. Both As(V) and As(III) were detectable at a level of 2.5 µg/L As.

Online WTW multi340 meters fitted with CellOx325 sensors (detection limit 0.01 mg/L) in WTW D201 flow cells (Weilheim, Germany) were used to measure DO levels in the inlet and outlet of reactor A. Acetate, nitrate, nitrite, chloride, and sulfate concentrations were determined in an ion chromatography system using Dionex AS-14 columns (Dionex, Sunnyvale, CA). The eluent contained a mixture of ACS reagent grade Na_2CO_3 (3.5 mM) and $NaHCO_3$ (1 mM). The detection limits for the anions were determined to be 0.2 mg/L for each. Total arsenic and total iron concentrations were measured using ion coupled plasma mass spectrometry (ICP-MS) with detection limits of 2 µg/L As_T and 0.1 mg/L Fe_T, respectively.

Biomass Collection and Nucleic Acids Extraction. Biomass profile samples were collected on days 125, 227, and 300 by collecting BAC particles from the sampling ports along the depth of the reactors. Samples were flash-frozen, and stored at -80^{o}C until processing. Genomic DNA was extracted following a phenol-chloroform extraction protocol (44) with slight modification. Briefly, 15 to 20 BAC particles were mixed with 500 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8.0)), 1 mL phenol-chloroform isoamyl alcohol (25:24:1), 50 µL of 20% sodium dodecyl sulfate, and 0.5 g zirconium beads. The mixture was bead-beaten for 2 min, centrifuged at 12,000 x g for 20 min, and transferred to a phase-lock gel (5-prime, Gaithersburg, MD). After extraction with an equal volume of phenol-chloroform-isoamyl alcohol and centrifugation, the aqueous

phase was transferred to a fresh phase-lock gel and mixed with 700 µL chloroform. The aqueous phase was transferred to a clean microcentrifuge tube and nucleic acids were precipitated with 3 M ammonium acetate (0.1 vol) and isopropanol (0.6 vol) at -20 ^{o}C for 4 h. After centrifugation, the precipitated DNA was rinsed with 70% ethanol, dried, and re-suspended in nuclease-free water. DNA was quantified using a NanoDrop ND1000 (NanoDrop Technology, Wilmington, DE) and stored at -20 ^{o}C.

From the flash-frozen biomass samples collected on day 300, total RNA was isolated following a low pH hot phenol chloroform extraction protocol (6). Contaminating DNA was digested using RNase-free Turbo DNase (Ambion Inc., Austin, TX) at 37 ºC for 30 min. The purified RNA was transferred to a new tube and quantified using a Nanodrop ND-1000. RNA quality was evaluated using Experion Automated Electrophoresis unit (Life Science, CA). The effectiveness of DNase treatment was evaluated by PCR. The purified RNA extracts were stored at -80 ^{o}C.

PCR Amplification and Construction of Clone Libraries. To elucidate the microbial community and SRB and DARB populations present in the system, three separate clone libraries of the 16S rRNA gene, *dsrAB* gene, and *arrA* gene generated from the DNA extracts corresponding to biomass samples collected on days 125, 227, and 300, respectively. PCR amplifications were performed on a Mastercycler thermocycler (Eppendorf International, Hamburg, Germany).

PCR amplification of approximately 1.5 kbp bacterial 16S rRNA gene was performed on DNA extracts from day 125 using primers 8F (5'-AGAGTTTGATCC TGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3') as described by Richardson et al. (36) except that Ex Taq polymerase (Takara Bio Inc, Shiga, Japan) replaced AmpliTaq polymerase.

DNA extracts from day 227 were used to amplify the *dsrAB* gene (~1.9 kbp) by PCR. Approximately 1.9 kbp *dsrAB* gene was amplified in triplicate using $DSR1F_{mix}$ and $DSR4R_{mix}$ (equimolar mixture of all primer variants) (22). Each 25 µL PCR reaction mixture included 500 nM forward and reverse primers, 3 mM $MgCl_2$, 0.4 µg/µL bovine serum albumin (Invitrogen Inc., Carlsbad, CA), 12.5 µL of HotStarTaq Mastermix (QIAGEN Inc., Valencia, CA), and 10 ng DNA template. PCR thermal conditions were adopted from Kjeldsen et al. (22).

An approximately 628 bp fragment of the *arrA* gene was amplified from the genomic DNA extracted from the biomass samples collected on day 300. A nested PCR approach was adopted as suggested by Song et al. (40). Two separate initial PCR amplifications were performed using the primers described by Song et al. (40). The first initial PCR amplification utilized primers AS1F (5'-CGAAGTTCGTCCCGATHACNTGG-3') and AS1R (5'-GGGGTGCGGTCYTTNA RYTC-3'). The second initial PCR was performed with AS2F (5'-GTCCCNATBA SNTGGGANRARGCNMT-3') and AS2R (5'-ATANGCCCARTGNCCYT GNG-3'), respectively. Each 25 µL initial PCR reaction mixture included 400 nM forward and reverse primers, 1 mM $MgCl_2$, 12.5 µL of HotStarTaq Mastermix (QIAGEN Inc., Valencia, CA), and 25 ng DNA template. The nested PCR utilized primers

AS2F and AS1R and PCR products from the initial PCR were used as the template. Each 25 µL PCR reaction mixture for the nested PCR included 600 nM forward and reverse primers, 1 mM $MgCl_2$, 12.5 µL HotStarTaq Mastermix (QIAGEN Inc., Valencia, CA), and 1 µL PCR products from the initial PCR amplifications. PCR thermal cycles were adopted from Song et al. (40).

After PCR amplifications, the PCR products were purified using a MinElute Gel Extraction Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's instructions. The gel-purified PCR products of the 16S rRNA gene, the *dsrAB* gene, and the *arrA* gene were processed separately. The PCR products of each gene corresponding to the samples from the sampling ports in reactors A and B were pooled together after purification using QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA) and cloned into One Shot® TOPO10 Chemically Competent *E. coli* cells using the pCR®4-TOPO cloning kit (Invitrogen Inc., Carlsbad, CA) according to the manufacturer's instructions. The wells in 96-well microplates were inoculated with randomly picked colonies and were sent to the Genomic Center at Washington University (Saint Louis, MO) for sequencing. The clone library of the 16S rRNA gene consisted of four 96 well plates, while one 96-well plate was used for each of the *dsrAB* and the *arrA* gene-based clone libraries.

Phylogenetic Analyses. Phylogenetic relationship of the clones in the clone libraries was determined through the generation of phylogenetic trees of the 16S rRNA, *dsrAB*, and *arrA* gene sequences. The DNA sequences from clone libraries were analyzed and edited using BioEdit (14). Sequences

phylogenetically close to the sequences in this study were obtained using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The 16S rRNA gene sequences were processed using the computer program Mothur (Schloss, 2009). Sequences identified as chimeras by Mothur and verified by using Mallard (4) were excluded from further analyses. Classification of the 16S rRNA gene sequences was based on the RDP taxonomy (47). The aligned sequences were clustered into operational taxonomic units (OTUs) based on a 97% sequence similarity (22). A phylogenetic tree of the identified *Deltaproteobacteria*-like sequences was constructed using 535 nucleotide positions in the 16S rRNA gene sequences starting from the 8F primer end with the software program MEGA (43).

Multiple sequence alignments for the *dsrAB* and *arrA* gene sequences were conducted using ClustalW2 (9). Phylogenetic trees of SRB based on partial *dsrAB* genes and DARB based on partial *arrA* genes were constructed using 648 nucleotide positions and 219 amino acids positions, respectively.

Sequences included in the 16S rRNA gene, *dsrAB* gene and *arrA* gene phylogenetic trees are presented in Appendices 4-A, 4-B, and 4-C, respectively.

Primer design. Two real-time PCR primer sets each specific for a distinct cluster of *arrA* genes within the *arrA* phylogenetic tree were designed using the Genefisher2 program made available by Bielefeld University Bioinformatics Server (http://bibiserv.techfak.uni-bielefeld.de/genefisher2/). The specificities of

the primer sets were evaluated using the Primer Blast function of NCBI (http://www.ncbi.nlm.nih.gov/), while their coverage was evaluated against the clones of interest in the clone library using MEGA (43) (supplementary Table 4-A). The gradient function of a real-time PCR Mastercycler realplex thermocycler (Eppendorf International, Hamburg, Germany) was used to experimentally characterize the specificity of the primer sets. Plasmid DNA extracted from representative clones of the two distinct clusters observed in the phylogenetic tree were used as the target and non-target templates. The target template contained representative sequences based on which the primer sets were designed, while the non-target template contained the sequences representative of the other cluster in the phylogenetic tree.

Quantitative Real Time PCR. Quantitative real time PCR (qPCR) were performed to determine the abundance of the 16S rRNA gene, *dsrAB* gene, and *arrA* gene along the depth of the reactor beds. Bacterial 16S rRNA genes were quantified in the DNA extracts corresponding to the biomass samples collected on day 300 using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3') (35). Each 25 µL PCR reaction contained 12.5 µL QuantiTect SYBR Green Mastermix (QIAGEN Inc., Valencia, CA), 500 nM forward and reverse primers, and DNA template of known concentrations of standards or 30 ng DNA from environmental samples. A triplicate 10-fold dilution series ranging from 10^5 to 10^9 copies/µL of *E. coli* plasmid DNA containing approximately 1.5 kbp fragment of the 16S rRNA gene from *Desulfovibrio vulgaris* was used to generate a standard curve. The PCR thermal cycles

included heating for 2 min at 50 ^{o}C, initial denaturation for 15 min at 95 ^{o}C, 35 cycles of 95 ^{o}C for 15 s, 60 ^{o}C for 30 s, and 72 ^{o}C for 30 s. Melting profiles were collected after the amplification to evaluate the specificity of the amplification.

The abundance of the *dsrAB* gene was quantified using primers DSR1F+ (5'-ACSCACTGGAAGCACGCCGG-3') and DSR-R (5'-GTGGMRCCG TGCAKRTTGG-3') (23). Each 25 µL PCR mixture contained 12.5 µL 2x QuantiTect SYBR Green PCR Master Mix (QIAGEN Inc., Valencia, CA), 1 mM $MgCl_2$, 300 nM forward and reverse primers, and DNA templates of known concentrations of standards or 50 ng DNA template from environmental samples. Amplification cycles were adopted from Kondo et al. (23). Melting profiles were collected after amplification to check the specificity of the amplification. Purified *E. coli* plasmid DNA containing a 221 bp fragment of the *dsrAB* gene of *Desulfovibrio vulgaris* was used to generate a standard curve from triplicates of a 10-fold dilution series ranging from 10^4 to 10^9 copies/µL.

An approximately 187 bp fragment of the *arrA* gene corresponding to cluster II of the *arrA* phylogenetic tree was amplified using primers GArrAF (5'-CCCGCTATCATCCAATCG-3') and GArrAR (5'-GGTCAGGAGCACATGAG-3'). Each 20 µL PCR reaction mixture contained 10 µL QuantiTect SYBR Green PCR Master Mix (QIAGEN Inc., Valencia, CA), 1 mM $MgCl_2$, 300 nM forward and reverse primers, and DNA templates of known concentrations of standards or 10 ng DNA templates from environmental samples. The amplification cycles included initial denaturation at 95 ^{o}C for 15 min followed by 35 cycles of denaturation at 95 ^{o}C for 30 s, annealing at 52 ^{o}C for 30 s, and extension at 72

^{o}C for 1 min. Primers EArrAF (5'-CATCGCTTCTCGCTGTG-3') and EArrAR (5'-GAGGTAGTTGCAG TTTCG-3') were used to amplify an approximately 201 bp fragment of the *arrA* gene corresponding to cluster III. PCR reaction mix remained the same as above except that primers EarrAF and EarrR replaced GarrAF and GarrAR. Thermal cycles were identical to the one presented above except that the annealing temperature was 56 ^{o}C. Purified *E. coli* plasmids containing an approximately 628 bp fragment of the *arrA* genes from clone 62 (representative clone from cluster II) and clone 34 (representative clone from cluster III) of the clone library were used to generate standard curves from triplicates of a 10-fold dilution series for target clones related to cluster II and cluster III, respectively. Melting patterns were collected at the end of qPCR amplifications to evaluate the specificity of the primers used.

Reverse Transcriptase Quantitative Real Time PCR. Reverse transcriptase (RT) qPCR experiments were performed to elucidate the sulfate and arsenate reducing activity along the depth of the reactors using purified RNA extracts corresponding to the biomass samples collected on day 300. Standards of known amount of cDNA copies of the *dsrAB* gene were created following the protocol described by Smith et al. (39) with slight modification. Briefly, the target *dsrAB* gene was amplified from DNA extract of *Desulfovibrio vulgaris* using primers DSR1F+ and DSR-R. The PCR product was purified using QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA) and cloned into One Shot® TOPO10 chemically competent *E. coli* cells using the pCR®4-TOPO cloning kit (Invitrogen Inc., Carlsbad, CA). Transformants were selected on Luria-Bertani

agar plates containing 50 µg/L kanamycine. Colonies were screened for correct orientation by colony PCR with the insert primer DSR-R and vector primer M13F and running the PCR products on a 2% agarose gel. The PCR product that resulted in a band in the gel was PCR purified using QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA). The PCR product was *in vitro* transcribed using MEGAscript T7 Kit (Ambion Inc., Austin, Tx) following the manufacturer's protocol. Contaminating DNA was removed by treatment with Turbo Dnase (Ambion Inc., Austin, TX). RNA transcripts were precipitated with ethanol and cDNA was synthesized using 2-step RT-qPCR kit (Abgene House, UK) following the manufacturer's protocol. A standard series ranging from 10^4 to 10^8 copies of amplicon/µL was generated from the cDNA.

Partial *dsrAB* gene was reverse transcribed from purified RNA extracts of reactor samples (day 227) using a 2-Step RT-qPCR kit (ABgene House, UK) following the manufacturer's protocol. Each 20 µL RT reaction contained 1x cDNA synthesis buffer, 500 nM dNTP mix, 800 nM DSR-R primer, 1 µL RT enhancer, 1 µL Verso enzyme mix, 5 µL RNA template, and Sigma water. The reaction mixtures were incubated at 42 ^{o}C for 30 min and Verso enzyme was inactivated by heating at 95 ^{o}C for 5 min.

To generate standard series for the quantification of *arrA* transcripts, plasmid DNA of clones 62 and 34 were used. Standards for the amplification of *arrA* gene followed the same protocol except that primers GarrAF and GarrAR and EarrAF and EarrAR were used to amplifiy partial *arrA* gene corresponding to clones related to clusters II and III, respectively. Primer M13F was

complemented with primer GarrAR or EarrAR for the evaluation of correct orientation of the *arrA* genes corresponding to clusters II and III, respectively. Reverse transcription of partial *arrA* gene from the reactor samples followed the same protocol described for the RT of *dsrAB* gene except that reverse primers GarrAR and EarrAR were used.

4.4 Results

Reactor Performance. During the period reported herein (day 50 to 310), dissolved oxygen (DO) in the influent to and effluent from reactor A remained less than 1 mg/L and below detection, respectively (data not shown). The pH of the effluents of reactors A and B averaged 7.2±0.2 (mean ± standard deviation). Complete denitrification was observed in reactor A, except during the period from day 125 to 152 when nitrate was detected in the effluent of reactor A (Figure 4.1). Even during this period of reactor upset, nitrate removal in reactor B resulted in complete nitrate removal across the system. Prior to day 50, the reactors were operated at 18 ^{o}C and sulfate reduction was not observed. After adjusting the reactor temperature to 24 ^{o}C on day 50, sulfate reduction slowly increased. Arsenic speciation performed during 50 to 60 days of reactor operation indicated reduction of As(V) to As(III) took place in reactor A (supplementary Table 4-B). With gradual increases in sulfide and As(III) levels across the filter beds, arsenic levels in the effluent from reactors A and B started declining and arsenic concentrations in the final effluent generally remained below 30 µg As/L from day 69 to 122. However, accidental overdosing of acetate occurred on days 118 and 119 (50 mL of concentrated acetate was automatically discharged into the

reactor within 20 min two times) and the reactors frequently experienced no acetate conditions (e.g., days 121, 138, and 142) due to malfunctioning of the syringe pump. During a few of the no acetate events, the tube connecting the acetate containing syringe to the reactor was disconnected resulting in exposure of reactor A to oxygen. After the addition of Fe(II) to reactor A on day 122, reddish brown precipitates were seen in the top part of reactor A which increased progressively with time suggesting possible oxidation of Fe(II) due to oxygen penetration into the reactor. Furthermore, the filter beds were exposed to oxygen for approximately 2 h during biomass sample collection on day 125. These upsets severely impacted sulfate reduction and subsequent arsenic removal as indicated by higher levels of sulfate and arsenic in the effluent from reactors A and B from day 122 to 152 (Figure 4.1). Poor arsenic removal was observed again during day 182 -192 due to low acetate conditions resulting from a malfunctioning of the syringe pump. After day 192, however, reactor performance improved gradually and the final effluent arsenic concentrations remained 25±14 µg As/L from day 199 to 310.

Profile liquid samples collected on day 300 from the sampling ports along the depth of reactors A and B indicated that nitrate was below detection (0.2 mg/L) at and beyond port A6 (Figure 4.2). Although sulfate reduction was limited in the upper part of reactor A, a rapid change in sulfate concentrations was observed between port A6 (18.9±0.2 mg/L) and port A8 (11.8±0.1 mg/L) in reactor A. The rapid sulfate utilization continued up to sampling port B1 (7.8±0.2 mg/L) in reactor B and declined thereafter. Depletion of arsenic and iron levels

followed the trend of sulfate reduction along the flow direction in the reactor beds. The sulfate and arsenic concentrations in the effluent from reactor B were 1.1±0.1 mg SO_4^{2-}/L and 19±1 µg As/L, respectively.

Microbial Community Structure. Out of the 375 16S rRNA gene sequences retrieved from the clone library, 282 sequences were considered for phylogenetic analyses. The other sequences were removed because they were short (<500 bp), contained more than eight homopolymers, or were identified as chimeras. The *Proteobacteria* (57%), *Bacteroidetes* (25%), *Firmicutes* (5%), and *Spirochaetes* (7%) were the major phyla present in the system. Within the *Proteobacteria*, the *Betaproteobacteria* and *Deltaproteobacteria* represented 36% and 19% of the clones, respectively (Figure 4.3).

Based on the 16S rRNA gene sequences, the major genera identified under the *Betaproteobacteria* were *Zoogloea* and *Azospira* with a relative abundance of 13% and 12%, respectively (see supplementary Table 4-C). Clones closely related to SRB shared 12% relative abundance, while clones associated with the iron reducing bacteria of the *Geobacter* genus had a relative abundance of 6%. Clones closely related to members of fermentative bacteria from the genera *Cloacibacterium* and *Treponema* were found at a relative abundance of 15% and 6%, respectively. The rarefaction curve (see supplementary Figure 4-A) did not attain a plateau indicating the limitation of the 16S rRNA clone library to reveal the complete diversity of the microbial community.

Phylogenetic Analysis of *Deltaproteobacteria*. Sequence analyses of the partial 16S rRNA gene of the 54 clones that grouped within the *Deltaprotebacteria* yielded four distinct clusters (Figure 4.4). Cluster I consisted of 29 clones (54%) closely related to uncultured SRB. While an environmental clone (accession # GU472645), obtained from a low sulfate meromictic lake, was the closest relative of this cluster with a sequence identity of 93-98%, *Desulfatirhabdium butyrativorans* strain HB1 was the closest cultured relative with a sequence identity of 85-90%. Cluster II contained 19 clones closely related to the *Geobacteracea; Geobacter metallireducens* being the closest previously described cultured relative with a sequence identity of 90-91%. Interestingly, a clone identified in arsenic containing Bengal Delta sediments (Islam et al., 2004) was 87-90% identical to the 16S rRNA gene sequences in this cluster. Cluster III included three clones that represented an uncultured group of *Deltaproteobacteria*. Finally, four clones were grouped under cluster IV, which comprised several *Desulfovibrio* strains. *Desulfovibrio putealis* shared 96 to 100% sequence identity with the sequences in this cluster.

Phylogenetic Affiliation of the *dsrAB* Gene Sequences. The *dsrAB* gene-based clone library prepared from the biomass samples collected on day 227 resulted in successful sequencing of 85 clones. Analyses of the sequences revealed four distinct clusters of clones closely related to previously described SRB (Figure 4.5). Clones closely related to the *Desulfobacterium-Desulfococcus-Desulfonema-Desulfosarcina* assemblage were grouped under cluster II and represented the largest group of SRB (81% of the sequences).

While the closest relative to these sequences were uncultured bacteria (accession #s AB263672 and AB263656) with 78 to 90 % sequence identity, *Desulfonema limicola* was the closest cultured relative with 75-78% sequence identity. Cluster III contained 10 clones closely related to the previously described cultured bacterium *Desulfovibrio magneticus* with a sequence identity of 79-83*%.* An uncultured bacterium from an anaerobic bioreactor was the closest relative of this group (accession # AY929605). Cluster IV included five clones closely related to *previously described Desulfomonile tiedjei (64–78% sequence identity)*, while the closest relative was an uncultured bacterium clone (AY929602) with sequence identity ranging from 67 to 81%. Finally, Group I constituted only one clone distantly related to the Gram positive bacterium *Pelotomaculum propionicicu*m (AB154391), which was the closest relative with a sequence identity of 56%.

Phylogenetic Affiliation of the ArrA Amino Acid Sequences. Sequence data were retrieved for 58 clones out of the 96 clones included in the *arrA* gene-based clone library prepared from the biomass sample collected on day 300. The DNA sequences were translated into protein sequences using MEGA (65). Only 50 unambiguous amino acid sequences were used to build a phylogenetic tree. Analyses of the sequences revealed three phylogenetically distinct clusters (Figure 4.6). Cluster II included 36 (72%) of the sequences, which were closely related to *Geobacter uraniireducens* Rf4. The amino acid sequences were 81-94% identical to *G. uraniireducens* Rf4 except for clone 37, which had a 65% sequence identity. Cluster III contained 13 sequences distantly related to

Alkalilimnicola ehrlichii, which exhibited 66-68% amino acid sequence identity with the sequences in this cluster. Cluster I contained only one clone, which was closely related to a group of uncultured bacteria from Chesapeake Bay sediments (40).

Spatial Distribution and Activity of the *dsrAB* Gene. The abundance and activity of SRB were estimated by quantifying the copy number of the *dsrAB* gene (relative to total DNA) and *dsrAB* transcripts (relative to total RNA) along the depth of the reactors A and B. The relative abundance of the *dsrAB* gene normalized using total DNA varied between $3.7x10^2$ and $1.7x10^4$, suggesting that SRB were relatively uniformly distributed along the beds of the two reactors (Figure 4.7). In contrast, the maximum abundance of *dsrAB* transcripts, normalized to the mass of total RNA, was observed towards the lower end of reactor A (Figure 4.7) suggesting that sulfate reducing activity was at its maximum at the middle of the reactor system. As can be seen, the relative abundance of *dsrAB* transcripts declined with distance from this central location.

Spatial Distribution and Activity of the *arrA* Gene. Abundance and activity of *arrA* was monitored by quantifying the number of *arrA* genes and *arrA* transcripts present at different sampling ports along the depth of the reactor beds. On day 300, the *arrA* genes closely related to cluster III outnumbered those related to cluster II throughout the reactor system (Figure 4.8). The relative abundance of the *arrA* genes related to clusters II and III attained a maximum at sampling ports A6 and A5, respectively, and declined in the direction of flow. Interestingly, the relative abundance of *arrA* transcripts, representing *arrA* activity, was below

detection at ports A5 and A6 despite their high relative abundance. Additionally, in contrast to the abundance data, the activity data suggested a predominance of the activity of *arrA* genes related to cluster II. Regardless of the clusters, however, *arrA* activity mapped the trend of *arrA* abundance at and beyond port A7. The abundance of activity of DARB related to both the clusters II and III increased in the direction of flow and declined again after attaining a maximum at port B2 in reactor B.

4.5 Discussion

A mixed microbial community, including close relatives of previously described nitrate, iron(III), and sulfate reducing bacteria was established in the reactor system (supplementary Table 4-C) and resulted in sequential uptake of DO, nitrate, arsenate, and sulfate as the electron acceptors (Figure 4.2). DO is the thermodynamically most favorable electron acceptor for microbial growth (29) and was expected to be consumed in the upper part of reactor A (DO was not monitored along the depth of the reactors). Nitrate reduction was efficient and resulted in nitrate concentration below the detection limit (0.2 mg/L NO_3^-) at sampling port A7 and beyond (Figure 4.2). Though arsenic speciation was not evaluated along the depth of the reactors, arsenate reduction was expected to precede sulfate reduction under standard conditions (29, 30). In fact, arsenite was predominant in the effluent from reactor A (supplementary Table 4-B) indicating arsenate reduction took place in reactor A. Sulfate reduction progressed gradually along the flow direction after nitrate was consumed (Figure 4.2) and arsenic depletion followed the sulfate reduction pattern, as expected.

Even though reduced arsenic can be precipitated as realgar (AsS) (26) or orpiment (As_2S_3) (33), the loss of iron corresponded to sulfate removal suggesting iron sulfide precipitation and concomitant removal of arsenic. This is in agreement with earlier conclusions that faster precipitation of iron sulfides limits precipitation of arsenic sulfides (21, 34). In fact, solids collected from reactor B confirmed the presence of mackinawite (FeS_{1-x}) and greigite (Fe_3S_4) (as reported in Chapter 2 and (45)). Despite complete nitrate removal and significant arsenic removal, arsenic levels in the final effluent were not below the maximum contaminant level of 10 μg As/L.

Reactor upsets were observed from days 125 to 152, and days 182 to 192 of reactor operation (Figure 4.1) due to synergistic effects of no or low acetate levels and exposure to oxygen. In the absence of acetate in the influent, sulfate and arsenic levels increased in the effluent while overall nitrate removal was not impacted. Microorganisms capable of nitrate reduction utilizing arsenite or sulfide as the electron donor have been described (16, 42). Interestingly, some *arrA* gene sequences retrieved from this study suggested the presence of bacteria (cluster III) distantly related to *Alkalilimnicola ehrlichii* strain MLHE-1 (Figure 4.6), which can oxidize arsenite or sulfide using nitrate as the electron acceptor under anoxic conditions, while its sustained growth on acetate using oxygen or nitrate is also possible (16). It is possible that the bacteria identified to be distantly related to *A. ehrlichii* in the current system utilized nitrate and acetate in reactor A during normal reactor operation and oxidized sulfides during no acetate conditions resulting in the release of arsenic adsorbed to the iron

sulfides. The accumulation of iron(III) hydroxides in the upper part of reactor A during days 122 to 143 might have complicated the problem associated with the intermittent acetate feeding. Reduction of iron(III) is thermodynamically favorable compared to sulfate and arsenate reduction (29, 30), which would be consistent with a shift of the arsenate and sulfate reducing zones farther down in the reactors resulting in poor arsenic removal.

The 16S rRNA gene-based clone library did not reveal complete microbial diversity in the system as the rarefaction curve did not attain a plateau (supplemental Figure 4-A) and suggested that additional clones would have revealed more OTUs. In agreement with previous studies (10, 27), *Zoogloea*-like and *Azospira*-like nitrate reducing bacteria were abundant in the system. Acetate supplementation resulted in the predominance of bacteria closely related to previously described SRB from the *Desulfatirhabdium-Desulfobacterium-Desulfococcus-Desulfonema-Desulfosarcina* assemblage (Figure 4.4 and 4.5), which includes SRB that can oxidize electron donors completely to CO_2 (1, 12). Phylogenetic analyses also indicated the presence of close relatives of the *Desulfovibrio* genus, which includes bacteria that cannot utilize acetate as an electron donor (12). However, their sustained autotrophic growth on H_2 or through fermentative metabolism has been reported (32). The presence of *Desulfovibrio*-like clones suggested possible utilization of fermentation products (e.g., H_2 and acetate), which could be generated during the metabolic processes of fermentative bacteria related to genera *Cloacibacterium* and *Treponema* detected in the system. Given that only two members of the *Cloacibacterium*

genus have been isolated to date *(2, 7),* their presence in relatively high abundance in the current system warrants further study.

High abundance of *Geobacter*-like microorganisms, which can utilize iron(III) (28), was also observed. Interestingly, the *arrA*-ba*sed* clone library suggested the dominance of DARB closely related to *G. uraniireducens* (Figure 4.6). Previous studies have also reported significant presence of *Geobacter*-related bacterial populations from arsenic-contaminated sites (15, 18). Given the presence of putative genes for arsenate respiration in the genome of *G. uraniireducens* and its sustained growth on arsenate (15), the predominance of *G. uraniireducens*-like DARB in the current system is not surprising. Additionally, the presence of iron(III) hydroxides during the upset period (day 122 to 143) might have resulted in higher abundance of *Geobacter*-like bacteria given that the 16S rRNA gene-based clone library was generated from the biomass collected on day 125. The ArrA sequences under Cluster III in the phylogenetic tree were distantly related to *A. ehrlichii* strain MLHE-1. Even though *A. ehrlichii* lacks a conventional arsenite oxidase, one of the two homologs of putative respiratory arsenate reductase identified in its genome exhibits both arsenate reductase and arsenite oxidase activities (37). However, considering the comparatively low sequence identity of the clones in cluster III with *A. ehrlichii,* the possibility of the presence of novel uncultured arsenate respiring bacteria cannot be ruled out. Isolation of arsenate reducing bacteria from the current system might provide insight into the possible relationship of the clones with *A. ehrlichii*.

SRB were distributed throughout the reactor system, while their activity attained a maximum value at the center of the reactor system. In general, the activity of *dsrAB* corresponded well with sulfate reduction in between two adjacent sampling ports (Figure 4.7). Given that sulfate reduction was noticed at port A6 and beyond, the detection of *dsrAB* gene at port A5 is likely due to the presence of bacteria that can utilize both nitrate and sulfate depending on their availability. The detection of both *dsrAB* gene and *dsrAB* transcripts at port A6, however, suggests the co-existence of nitrate and sulfate reduction zones, which is consistent with the chemical profile (Figure 4.2). It is highly likely that nitrate and sulfate reducing bacteria colonized the outer and inner part of a biofilm, respectively, given that microorganism co-inhabit a biofilm depending on their metabolic capabilities (48). Rapid depletion of sulfate after port A6 is consistent with the increase in SRB activity after this port, which attained a maximum value at port A8. Slower sulfate reduction observed after port B2 in reactor B corresponds well with the lower relative activity of SRB.

Disagreement between the relative abundance of a gene and its activity was most pronounced in the case of the *arrA* gene. The abundance of the *arrA* gene was highest at ports A5 and A6, where *arrA* activity was not detected (Figure 4.8). Additionally, despite the overall higher abundance of the *arrA* genes related to cluster III, the activity data suggested a higher contribution of *Geobacter*-like bacteria in arsenate reduction in the system. Regardless of the clusters, the activity of *arrA*, however, mapped the pattern of the abundance of *arrA* gene beyond port A6. Again, the presence of *arrA* genes in ports A5 and A6

underscores the possibility of the occurrence of microorganisms that exhibit multiple substrate (electron acceptors) utilization capability, which could utilize nitrate within the first two ports in reactor A where nitrate was available. Even though arsenic speciation was not monitored along the flow direction, the detection and increase of both *dsrAB* and *arrA* activity beyond port A6 (Figures 4.7 and 4.8) suggests the coexistence of arsenate and sulfate reducing zones beyond port A6 in reactor A. Furthermore, the co-existence of *dsrAB* and *arrA* genes within the lower part of reactor A resulted in the removal of approximately 193±1 µg/L As in reactor A (Figure 4.2). This further emphasizes that the co-location of sulfate and arsenate reduction and availability of iron(II) is necessary for arsenic removal in the current system.

Overall, biologically generated sulfides reacted with iron(II) resulting in the precipitation of iron sulfides, which concomitantly removed arsenic through co-precipitation or adsorption mechanisms. The activity of *dsrAB* and *arrA* corresponded well with the chemical profiles in the system.

4.6 Conclusions

This study presented the community structure, and the diversity and abundance of SRB and DARB in a biofilm reactor system that removes arsenic and nitrate simultaneously. Molecular data complemented chemical analyses results. The majority of the SRB identified in this research were complete oxidizers, while *Geobacter-like bacteria* were the dominating DARB. The study indicated a potential for optimizing the system to further lower arsenic

concentration in the final effluent by enhancing sulfate reduction and sulfide production in reactor B. Future research will focus on the evaluation of the effects of optimizing the EBCT of reactor A.

4.7 Tables and Figures

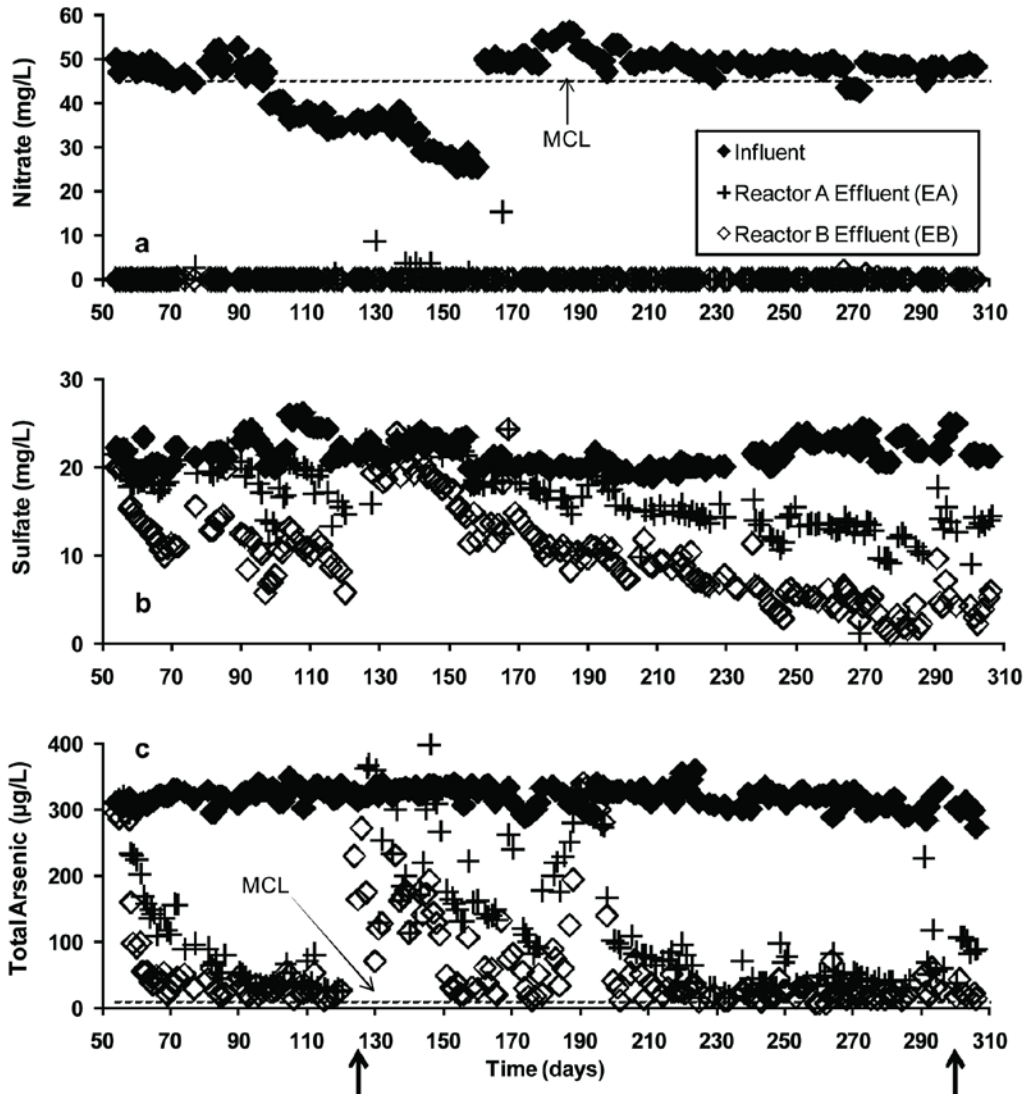


Figure 4.1: *(a) Nitrate, (b) sulfate, and (c) total arsenic concentrations in the influent, the effluent of reactor A (EA), and the effluent of reactor B (EB) versus time of operation. The bold-face up-arrows indicate the days 125 and 300 when biomass samples were collected. Liquid profile samples were also collected on day 300. The total EBCT was 40 min until day 300. On day 300, the EBCT in reactor A was lowered to 15 min (total EBCT 35 min) after collecting liquid and biomass profile samples. The system experienced intermittent acetate feeding and exposure to oxygen during days 122 to 152 and low acetate input during days 182 to 192.*

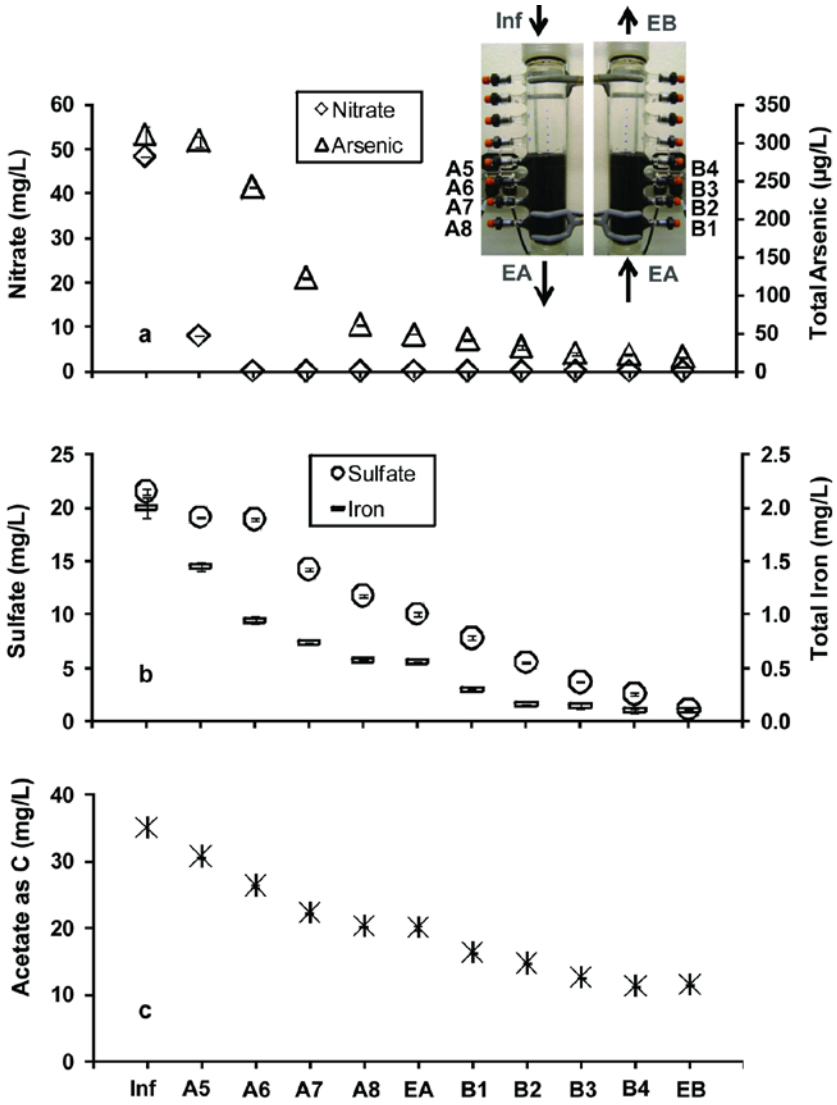


Figure 4.2: *Concentration profiles along the depth of reactor beds on day 300. (a) nitrate and arsenic (b) sulfate and total iron (c) acetate as C. Inf represents the influent concentrations. A5-A8 and B1-B4 represent the respective sampling ports along the depth of reactors A and B, respectively. EA and EB represent concentrations in the effluents from reactor A and reactor B, respectively. Mean values (n=3) are presented with error bars representing one standard deviation from the mean.*

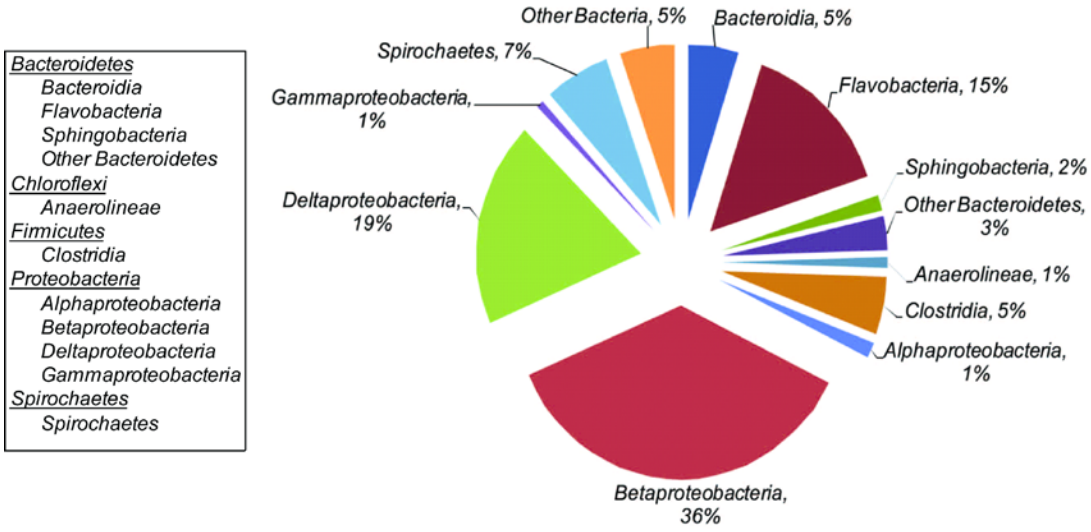


Figure 4.3: *Community composition and relative abundance of clones identified in the 16S rRNA gene clone library generated from biomass collected on day 125.*

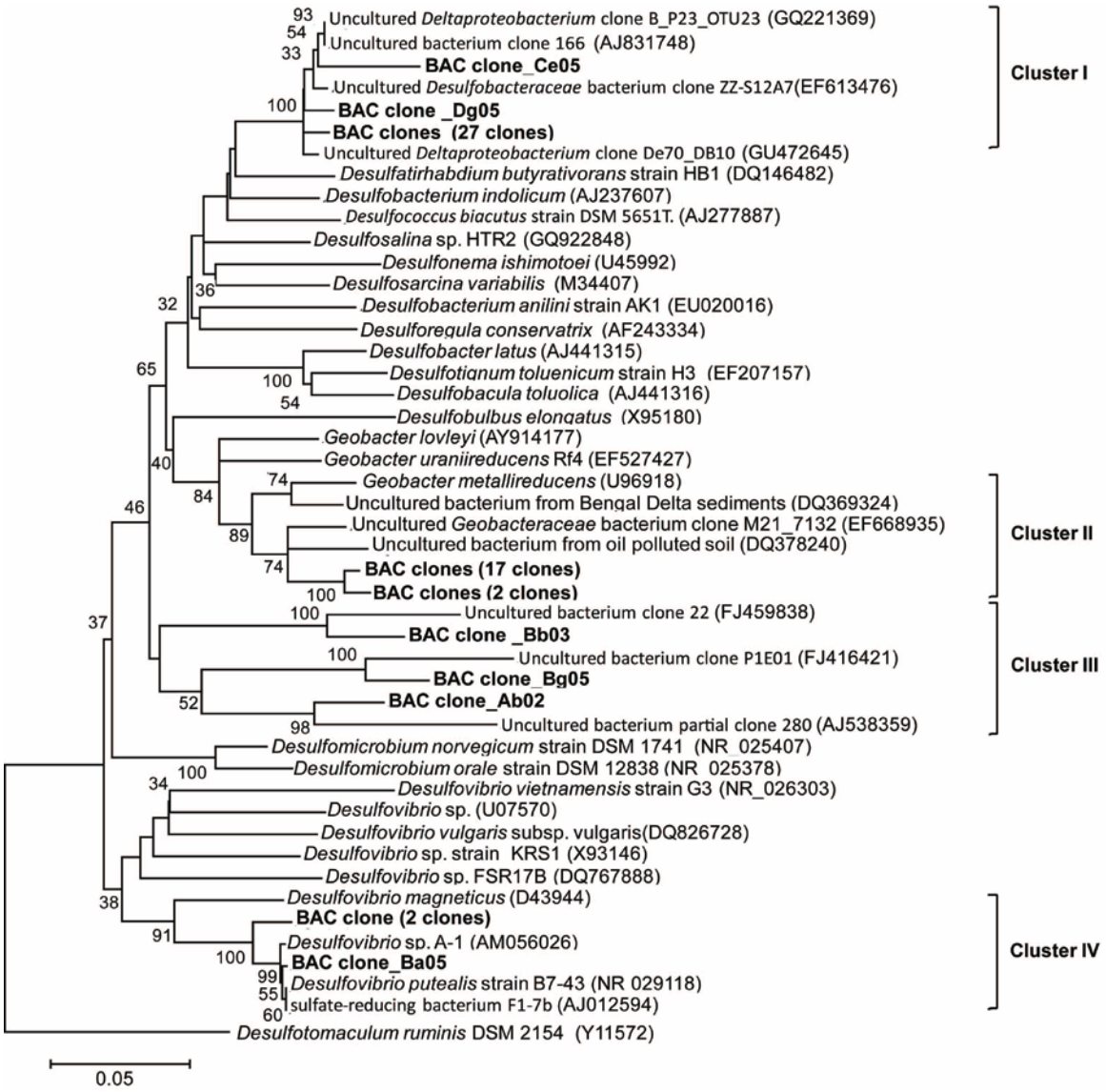


Figure 4.4: *Rooted neighbor-joining distance tree of the clones identified to be closely related to the Deltaproteobacteria based on 533 nucleotide positions of the 16S rRNA genes. The clone library was generated from the DNA extracts from biomass samples collected on day 125. Desulfotomaculum ruminis DSM 2154 was used as the outgroup. The clones from this work are presented in boldface. The bar indicates 5% deviation in sequence. The confidence estimates for the inferred tree topology was obtained by bootstrap re-sampling with 1000 replicates. Percentages of bootstrap support (>30) are indicated at the branch points.*

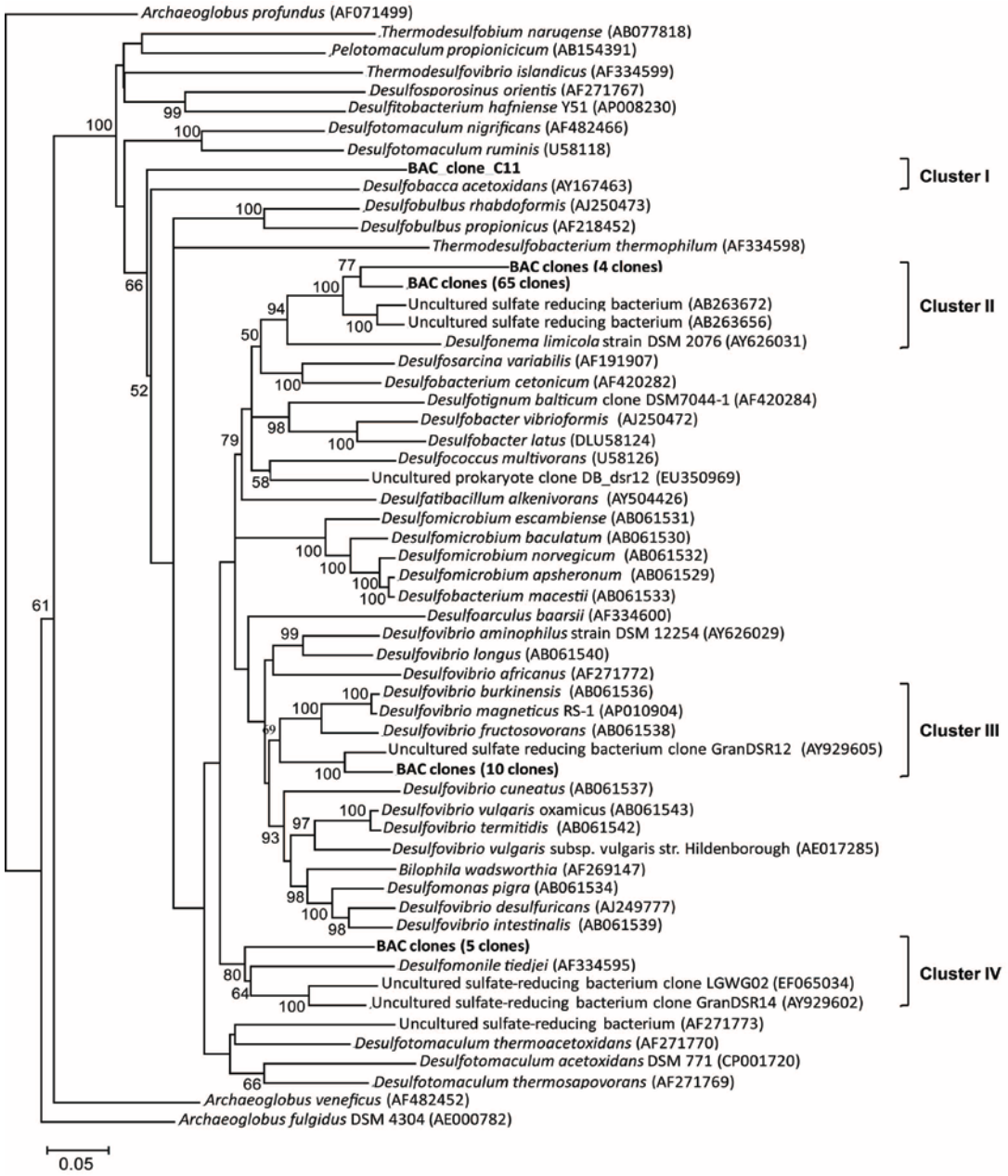


Figure 4.5: *Rooted neighbor-joining distance tree based on 688 nucleotide positions of the dsrAB genes amplified from the DNA extracts of the biomass samples collected on day 227. Archaeoglobus profundus was included as the outgroup. The clones from this work are presented in boldface. The bar indicates 5% deviation in sequence. The confidence estimates for the inferred tree topology was obtained by bootstrap resampling with 1000 replicates. Percentages of bootstrap support (>50) are indicated at the branch points.*

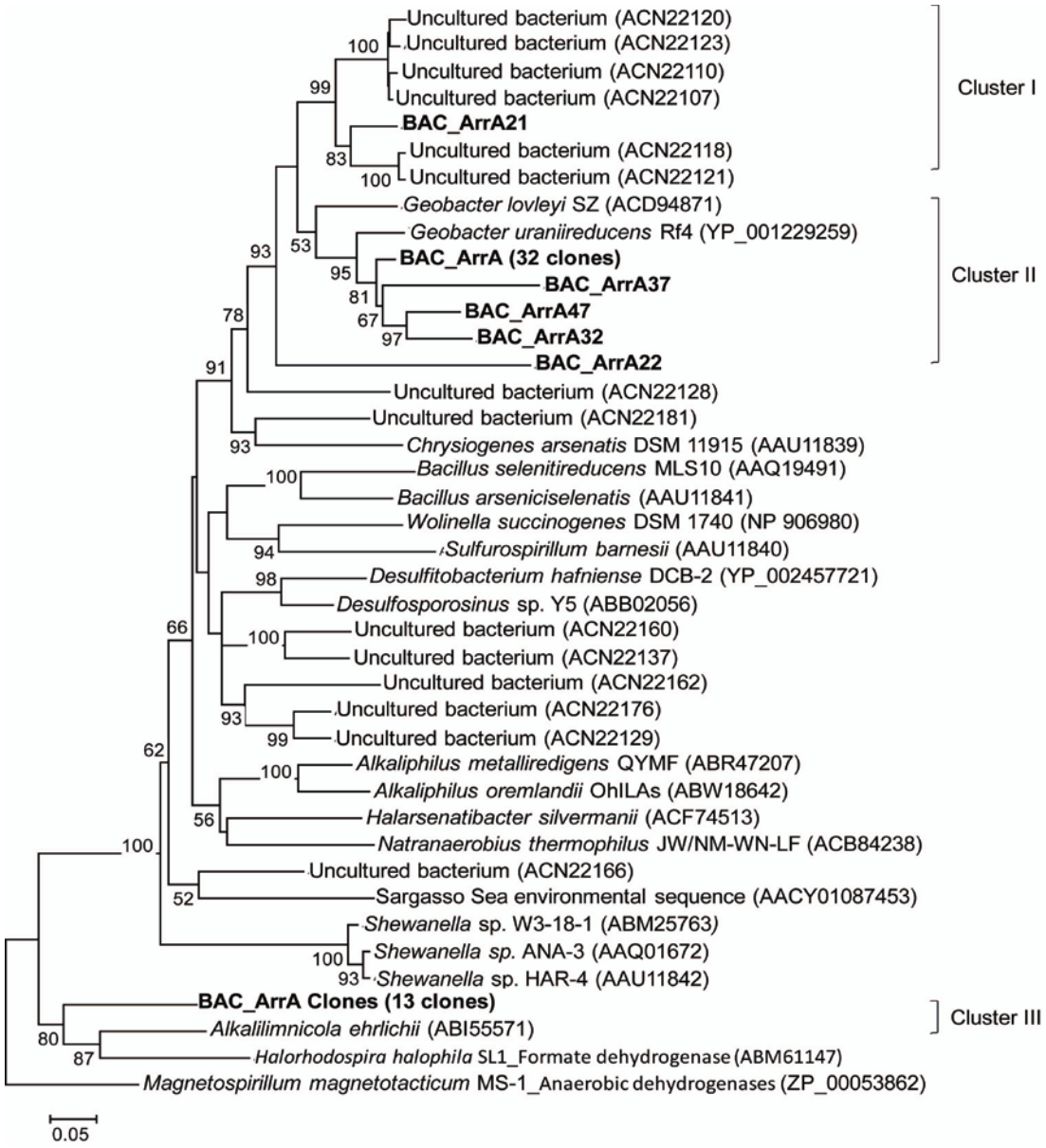


Figure 4.6: *Rooted neighbor-joining distance tree based on 219 amino acid residues of the alpha subunit of arsenate reductase (ArrA) deduced from the ArrA gene sequences retrieved from the clone library generated from biomass samples collected on day 300. Anaerobic dehydrogenase of Magnetospirillum magentotacticum MS-1 was included as the outgroup. Formate dehydrogenase from Halorhodospira halophila SL1 was also included in the tree as few of the sequences were identified to be closely related to this protein and the molybdopterin oxidoreductase from A. ehrlichii. The clones from this work are presented in boldface. The bar indicates 5% deviation in sequence. The confidence estimates were obtained by bootstrap re-sampling with 1000 replicates. Percentages of bootstrap support (>50) are indicated at the branch points.*

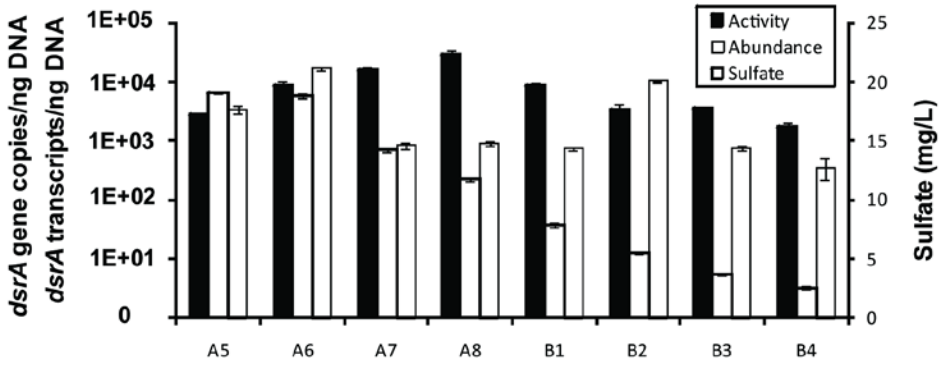


Figure 4.7: *Abundance and activity of the dsrAB gene and dsrAB transcripts along the depth of the reactors on day 300. Abundance is expressed as dsrA gene copies normalized to total DNA. Activity of SRB is presented as the number of dsrA transcripts normalized to total RNA. Mean (n=3) are presented with the error bars representing one standard deviation from the mean.*

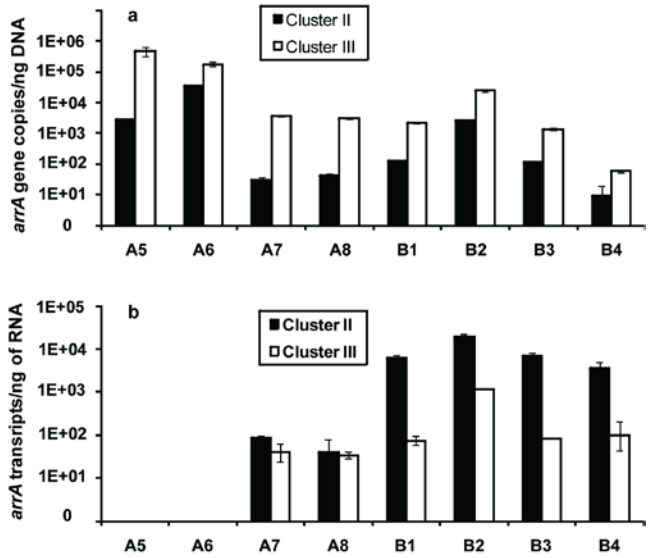


Figure 4.8: *Abundance (a) and activity (b) of arrA genes along the depth of reactors A and B on day 300. Abundance is expressed as arrA gene copies normalizaed to total DNA and activity is presented as arrA transcripts normalized to total RNA. Mean (n=3) is presented with error bars representing one standard deviation from the mean.*

Supporting Materials

Supplementary Table 4-A: *Sequence, coverage, specificity, and annealing temperature for the primers designed in this study.*

Target	For/ Rev	Primer	Sequence (5'-3')	Annealing Temp (^{o}c)	Coverage[1]	Specificity
Cluster II related to G. uraniireducens	F	GArrAF	CCCGCTATCATCCAATCG	52	36/42	No match found in the data base
	R	GArrAR	GGTCAGGAGCACATGAG		35/42	No match found in the data base
Cluster III distantly related to A. ehrlichii	F	EArrAF	CATCGCTTCTCGCTGTG	56	14/16	No match found in the data base
	R	EarrAR	GAGGTAGTTGCAGTTTCG		15/16	No match found in the data base

[1.] *Coverage = number of target clones with perfect match with the primer / number of target clones in the clone library. The denominator in the coverage values are different than the number of clones included in the ArrA phylogenetic tree as only the amino acid sequences matching with the molybdopterin binding super family in the database were included in the phylogenetic tree.*

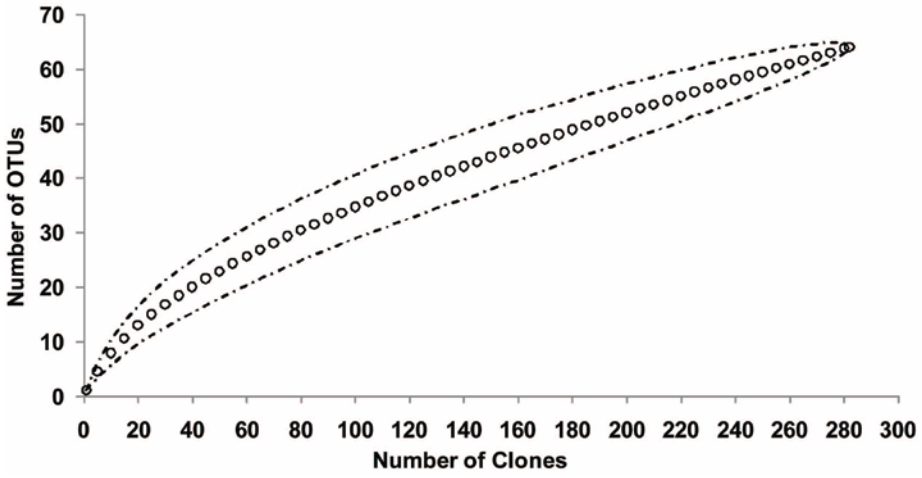
Supplementary Table 4-B: *Arsenate and arsenite concentrations in the influent, effluent of reactor A (EA), and effluent of reactor B (EB)..*

	Concentration (µg/L)								
	Influent			Effluent of reactor A			Effluent of reactor B		
Day	AsT	As(V)	As(III)	AsT	As(V)	As(III)	AsT	As(V)	As(III)
50	302	204	B.D.[1]	301	43	257	287	29	256
54	311	308	B.D.	311	10	298	295	18	276
56	312	312	B.D.	320	19	296	305	18	287
58	317	319	B.D.	293	5	294	286	14	275
60	298	304	B.D.	224	16	203	92	17	75

[1]B.D. - below detection.

Supplementary Table 4-C: *Phylogenetic affiliation and abundance of the clones in the 16S rRNA based clone library generated from the biomass collected on day 125.*

Phylum	Class	Genus	No. of Clones	Relative Abundance (%)
Acidobacteria	*Holophagae*	*Geothrix*	1	0.4
Bacteroidetes	*Bacteroidetes_incertae_sedis*	*Prolixibacter*	2	0.7
	Bacteroidia	*Anaerophaga*	14	5.0
	Flavobacteria	*Cloacibacterium*	41	14.5
		Empedobacter	1	0.4
	Sphingobacteria	*Sediminibacterium*	1	0.4
		Segetibacter	2	0.7
		Terrimonas	1	0.4
	Unclassified Bacteroidetes		6	2.1
Chloroflexi	*Anaerolineae*	*Unclassified Anaerolineaceae*	3	1.1
Firmicutes	*Clostridia*	*Thermohalobacter*	1	0.4
		Geosporobacter	1	0.4
		Anaerovorax	1	0.4
		Sporobacter	3	1.1
		Anaeroarcus	1	0.4
		Anaerosinus	2	0.7
		unclassified_Veillonellaceae	5	1.8
		Thermanaeromonas	1	0.4
Proteobacteria	*Alphaproteobacteria*	*Rhodoblastus*	4	1.4
	Betaproteobacteria	*Inhella*	1	0.4
		Acidovorax	7	2.5
		Pelomonas	4	1.4
		Pseudorhodoferax	1	0.4
		Aquitalea	1	0.4
		Azospira	33	11.7
		Dechloromonas	16	5.7
		Ferribacterium	1	0.4
		unclassified_Rhodocyclaceae	2	0.7
		Zoogloea	36	12.8
	Deltaproteobacteria	*Desulfatirhabdium*	31	11.0
		Desulforegula	1	0.4
		Desulfovibrio	3	1.1
		Geobacter	18	6.4
		Geopsychrobacter	1	0.4
	Gammaproteobacteria	*Modicisalibacter*	1	0.4
		Pseudoxanthomonas	1	0.4
Spirochaetes	*Spirochaetes*	*Treponema*	17	6.0
		Exilispira	1	0.4
SR1	*SR1_genera_incertae_sedis*		2	0.7
Unclassified Bacteria			13	4.6



Supplementary Figure 4-A: *Rarefaction curve (open circles) developed from bacterial 16S rRNA gene sequences retrieved from the clone library. The dotted lines represent the upper and lower 95% confidence levels. An OTU was defined as a group of sequences sharing 97% sequence similarity.*

Appendix 4-A: 16S rRNA sequences

>Seq1 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ag02 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
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TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGACCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq2 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Da12 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAGGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
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GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq3 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ca10 16S ribosomal RNA gene, partial sequence
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AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGACGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq4 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Df12 16S ribosomal RNA gene, partial sequence
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AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
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>Seq5 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Bd06 16S ribosomal RNA gene, partial sequence
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CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
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GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
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>Seq6 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ag10 16S ribosomal RNA gene, partial sequence
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>Seq7 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Dc07 16S ribosomal RNA gene, partial sequence
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GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
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GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq8 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Af07 16S ribosomal RNA gene, partial sequence
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GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
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>Seq9 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Bd04 16S ribosomal RNA gene, partial sequence
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AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
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TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq10 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Cc08 16S ribosomal RNA gene, partial sequence

AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
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GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq11 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Cf12 16S ribosomal RNA gene, partial sequence
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AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCATGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
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GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq12 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ab01 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATAGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq13 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Bf03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
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GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq14 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Df06 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGTTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
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TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC

TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq15 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Bc10 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
GGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATT
GACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq16 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone De02 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAACTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACATTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTG
AGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGCTT
TCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTATTG
ACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq17 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ch07 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq18 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Db07 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq19 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Cc01 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG

TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq20 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ac04 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq21 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Bg03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq22 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Da03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq23 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ad03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq24 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Cf04 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq25 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Cb12 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq26 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone De11 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq27 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ae11 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAAAGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGT
GAGGAATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGC
TTTCGGGTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTAT
TGACGGTACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq28 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Dg05 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCTTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTATCGCGTGGGTAATCTAC
CCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGGTTTC
GGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGCGTAC
CATTAGCTAGCTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGAGAGG
ATGATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGA

ATTTTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGG
GTCGTAAAGCTCTGTCAAGAGGGAAGAAAGTGGGAGATGGTAATACTGTTTTCTATTGACGG
TACCTCTGAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq29 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ab02 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GGGAAGTTAGCAATAACTTTCTAGTGGCGAACGGGCGAGTAACGCGTAGACAACCAACCTT
TTTGTGGGGGACAACACTTCGAAAGGAGTGCTAATACCGCATGAGCTCCAGATGCCGCCTG
GCGTTTGGAGGAAAGGAGCTTCACGGCTTCGCAAAAAGACGGGTCTGCGTCTGATTAGCTA
GTTGGAGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGGTCTGAGAGGATGATCAG
CCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATTTTGCG
CAATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAA
AGCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATTGACGGTACCTCT
GAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq30 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Ce05 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGTACG
AGAAATCCTCTGCTTGCAGGGGAGAGTAATGTGGCGCACGGGTGAGTATCGCGTGGGTAAT
CTACCCTTGAATTCAGGATAACATTTCGAAAGGGGTGCTAATACTGGATAACATCCTGATGG
TTTCGGCCATAAGGATCAAAGATAGCCTCTACATGTAAGCTATAGTTCAGGGATGAGCCCGC
GTACCATTACCTAGTTGGTGGGGTAAGAGCCTACCAAGGCAACGATGGTTAGCTGGTCTGA
GAGGATGATCACCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCACTG
AGGAATTTTGCGCAATGGGGGAAACCCTGACGCATCCACGCCGCGTGAGTGAATAACGCTT
TCGGGTCCTAAAGCTCTGTCACGAGGGAAAAAAGTGGGAGATGGTAAAACTGTTTTCCATTG
CCGGTACCTCTGAAGGAACCACGGGACCAACTCCCGTGCCCACCAGTT

>Seq31 [organism=Desulfatirhabdium] Uncultured Desulfatirhabdium sp. clone Bb03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACAAACGCTGGCGGCGTGTCTTAAGCATGCAAGTCGAGCG
GCAGGCGCAGCAATGCGCTGAGAGCGGCGGACTGGTGAGTAACGCGTGGGTAATCTACCT
TTGGCATGGGGATAGCCACTAGAAATAGTGGGTAATACTGAATACGTTCCCTGGGGGGAGA
TTTCAGGGAAGAAAGGGTGCTACGGCACCGGCCGGAGATGAGCTCGCGTCCCATTAGCTA
GTTGGTGAGGTAACGGCCCACCAAGGCAACGATGGTTAGCTGGTCTGAGAGGATGATCAGC
CACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATTTTGCGC
AATGGGGGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAA
GCTCTGTCAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATTGACGGTACCTCTG
AAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq32 [organism=Geobacter] Uncultured Geobacter sp. clone Cd06 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAGAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTT
TGGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTAC
CATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGG
ATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCG
GGTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGCGGCTAATATCCATGATACTTGACG
GTACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq33 [organism=Geobacter] Uncultured Geobacter sp. clone Da06 16S ribosomal RNA gene, partial sequence
AAGTTTGATCCTGGCTCAAACCCTGTTGGCGGCGTGCCTAACACATGCAAGTCGAACGGATT
TGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGCCTG

ATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTTGG
ACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACCAT
TAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGATG
ATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATT
TTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGT
CGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGGTA
CCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq34 [organism=Geobacter] Uncultured Geobacter sp. clone Be09 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGTTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGAT
GATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TTTTGCGCAATGGGCGAAAGCCTGGCGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGGAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACCCCGTGCCAGCAGCC

>Seq35 [organism=Geobacter] Uncultured Geobacter sp. clone De05 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCATCAGTGGGGAA
TTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGTTAATACCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq36 [organism=Geobacter] Uncultured Geobacter sp. clone Cg02 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACGCATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCACATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCTAGCAGCC

>Seq37 [organism=Geobacter] Uncultured Geobacter sp. clone Cc02 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCGAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTG
CCTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCT
TTGGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATAAGTCTGCGTA
CCATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAG
GATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTC
GGGTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGAC
GGTACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq38 [organism=Geobacter] Uncultured Geobacter sp. clone Bb09 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq39 [organism=Geobacter] Uncultured Geobacter sp. clone Ad07 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq40 [organism=Geobacter] Uncultured Geobacter sp. clone Dg09 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq41 [organism=Geobacter] Uncultured Geobacter sp. clone Ad06 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq42 [organism=Geobacter] Uncultured Geobacter sp. clone Be03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA

ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq43 [organism=Geobacter] Uncultured Geobacter sp. clone Ce07 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq44 [organism=Geobacter] Uncultured Geobacter sp. clone Ag06 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq45 [organism=Geobacter] Uncultured Geobacter sp. clone Ah10 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGCGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq46 [organism=Geobacter] Uncultured Geobacter sp. clone Ae06 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACAGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq47 [organism=Geobacter] Uncultured Geobacter sp. clone Bh11 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC

CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGATTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGAGTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTCGG
GTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGG
TACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq48 [organism=Geobacter] Uncultured Geobacter sp. clone Dg10 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGTTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GGGTTGGGGGGCTTGCTCCTCAATCTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTG
CCTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCATGGGGACT
TTGGTCTTTGCGGGAAAAGGGGGGGACCTTTTGGCCTTCTGTCATCAGATGAGTCTGCGTA
CCATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAG
GATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTC
GGGTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGTTAATACCCATGATACTTGAC
GGTACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq49 [organism=Geobacter] Uncultured Geobacter sp. clone Dg04 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GGGATGGGGAGCTTGCTTCCTATTCTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTG
CCTGACGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGGGACT
TTGGTCTTTGCGGGAAAAGGGGGGGACCTTTTGGCCTTCTGTCGTCAGATGAGTCTGCGTA
CCATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCGACGATGGTTAGCTGGTCTGAGAG
GATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATTTTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAAGGCTTTC
GGGTCGTAAAGCTCTGTCGAGGGGAAAGAAGTGTATTGTGGTTAATACCCATGATACTTGAC
GGTACCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq50 [organism=Desulfovibrio] Uncultured Desulfovibrio sp. clone Ag03 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGACGTGCTTAACACATGCAAGTCGTGCG
AGAAAGGAGACTTCGGTCTCTGAGTAGAGCGGCGCACGGGTGAGTAACGCGTGGATGATCT
ACCCTTGAGTACGGGATAACGGTGCGAAAGCGCCGCTAATACCGAATAACAATCCATTTCAT
CATGGGTTTAAAGCAGGCCTCTGGATGTAAGCTTGCGCTTGAGGATGAGTCCGCGTCCCAT
TAGCTTGTTGGCGGGGTAACGGCCCACCAAGGCTACGGTGGGTAGCTGGTCTGAGAGGAT
GATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCGCAATGGGGGAAACCCTGACGCAGCGACGTCGTGTGAGGGAAGAAGGCTTTCGG
GTCGTAAACCTCTGTCAGAAGGGAAGAAACGTCAGGATTCGAATAGGGTCCTGGCCTGACG
GTACCTTCAAAGGAAGCGCCGGCTAACTCCCGTGCCAGCAGCC

>Seq51 [organism=Desulfovibrio] Uncultured Desulfovibrio sp. clone Ba05 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGACGTGCTTAACACATGCAAGTCGTGCG
AGAAAGGAGGCTTCGGTCTCTGAGTAGAGCGGCGCACGGGTGAGTAACGCGTGGATGATC
TACCCTTGAGTTCGGGATAACGGTGCGAAAGCGCCGCTAATACCGTATAACAATCCATTTCA
TCGTGGGTTCAAAGCAGGCCTCTTCATGAAAGCTTGCGCTTGGGGATGAGTCCGCGTCCCA
TTAGCTTGTTGGCGGGGTAACGGCCCACCAAGGCTACGATGGGTAGCTGGTCTGAGAGGAT
GATCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCGCAATGGGGGAAACCCTGACGCAGCGACGTCGTGTGAGGGAAGAAGGCCTTCGG
GTCGTAAACCTCTGTCAGAAGGGAAGAACATCCGGGAGTCGAACAGCCTCCCGGCCTGACG
GTACCTTCAGAGGAAGCGCCGGCTAACTCCGTGCCAGCAGCC

>Seq52 [organism=Desulfovibrio] Uncultured Desulfovibrio sp. clone Df10 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGATCGAACGCTGGCGACGTGCTTAACACATGCAAGTCGTGCG
AGAAAGGAGACTTCGGTCTCTGAGTAGAGCGGCGCACGGGTGAGTAACGCGTGGATGATCT
ACCCTTGAGTACGGGATAACGGTGCGAAAGCGCCGCTAATACCGAATAACAATCCATTTCAT
CATGGGTTTAAAGCAGGCCTCTGAATGTAAGCTTGCGCTTGAGGATGAGTTCGCGTCCCATT
AGCTTGTTGGCGGGTTAACGGCCCACCAAGGCTACGATGGGTAGCTGGTCTGAGAGGATGA
TCAGCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT
TGCGCAATGGGGGAAACCCTGACGCAGCGACGTCGTGTGAGGGAAGAAGGCTTTCGGGTC
GTAAACCTCTGTCAGAAGGGAAGAAACGTCAGGATTCGAATAGGGTCCTGGCTTGACGGTA
CCCCTAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCC

>Seq53 [organism=Desulforegula] Uncultured Desulforegula sp. clone Bg05 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGACATGCTTTACACATGCAAGTCGAACGG
TAACAGGGAGCTTGCTCCGCTGACGAGTGGCGAACGGGTGAGTAATGCATCGGAACGTACC
GTGTAGTGGGGGATAACGTAGCGAAAGTTACGCTAATACCGCATACGCCCTGAGGGGGAAA
GTGGGGGACCGCAAGGCCTCACGCTATATGAGCGGCCGATGTCGGATTAGCTAGTTGGTA
GGGTAAAGGCCTACCAAGGCGACGATCCGTAGCGGGTCTGAGAGGATGATCAGCCACACT
GGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATTTTGCGCAATGGG
GGAAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAGCTCTGT
CAAGAGGGAAGAATGTAGGAGATGGTAATACTATTTTCTATTGACGGTACCTCTGAAGGAAG
CACCGGCTAACTCCGTGCCAGCAGCC

>Seq54 [organism=Geopsychrobacter] Uncultured Geopsychrobacter sp. clone Dg08 16S ribosomal RNA gene, partial sequence
AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACG
GATTTGAGAAGCTTGCTTCTCAAGTTAGTGGCGCACGGGTGAGTAACGCGTAGATAATCTGC
CTGATGATCTGGGATAACACTTCGAAAGGGGTGCTAATACCGGATAAGCCCACGGAGTCTTT
GGACTTTGCGGGAAAAGGGGGGGACCTTCGGGCCTTCTGTCATCAGATGACTCTGCGTACC
ATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCTACGATGGTTAGCTGGTCTGAGAGGA
TGATCAGCCACACTGGAACCGACACCCGGTCCAAACTCCTACGGGAGGCACCAATGGGGAA
TTTTGCGCAATGGGCGAAAGCCTGACGCAGAACGCCGCGTGAGTGATGAATGCTTTCGGGT
CGTAAAGCTCTGTCTAGGGGAAAGAAGTGTATTGTGGCTAATATCCATGATACTTGACGGTA
CTCCCTATGGAAGCACCGGCTAACTCAGCGCCAGCAGCC

Appendix 4-B: partial *dsrA* gene sequences

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_01
ACCCATTTGGAAACATTGGCGGCATTTGTTGGCGTCAAGGTTACGGCGGTGGCGTTGTCGG
CCGTTATACCGATGATCCCGAGCGTTTCCCTGATGCGCGTGAGTTTCATACCATGCGGGTAA
ACCAGGTTCCCGGCTTTTTTTACACCAGCGAAAAGCTGCGTGCACTGGCGGATATTTGGGA
CAAGTACGGCAGCGGACTTTACAACATGCATGGTTCTACCGGAGACATCATTCTGCTTTGGC
ACCACGACCGAAAACTTTGCAGCCCTGTTTTTGACGCGCTGGGGGAAATCGATTTTTGACCT
CGGCGGTTCCGGTGGCGCCCTGCGGACCTTCGAGCTGTTCGCTGCGGCGAAGCGCGCTG
CGAAAAATCCTGTATCGATGCCATGGATATGATGTATGACCTCACGATGCACTACCAGAACG
AGATGCACCGTCCGGCCTGGCCCTATAAATTCAAAATCAAAATTTCCGGCTGCCCCAACGAT
TTGCGCCGCTGCCTCGGCCCGTTCCGACATGGCCCTGATCGGTACATGGCGTGACGCGAT
CCAGGTGAATCAGGAAGAAGTGCGCAAATATGTAGCGGAAGGCATGAACATGGTTCAAGTC
TGCCGCAAGTGCCCGACCGAAGCT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_02
GCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACAAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCCCGGTCTGACCAATATGCACAGATCCGCCGGTGATATCGTATTTATCGGCAC
CTCCACCCCGCAGCTTGAATAGTTTTTTATGTTCTGAGCCAAATACTGAATCATGATTTCCGC
GGATTTGGCTGCAACCTGCGAACGCCATCGGACTGGCTCGAGACATCCCGCTTGCCCATAG
GTTTGATAGGTTACTTACGCCCTCTGATATGCAATGACGAAGAATGAACATGACAAATTGAG
CCAGAATCGCCTTTGAAACACATTAAATATCAATTTTAATGGTTGCACCAACGTCTGCATATC
GGAATTGCCCGTTGAGAAAGACTGTTAAATATGGCCGAAATGAGTATATCAAACCGTACCGG
AGGATTTAGAATAGAGCTTAATCGAGAGACATATTCCCATTAAGCGAAGATTTGTGATC

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_03
ACCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCAACCTGTGGGAA
CTTCGCGGCAGCAATCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGCCAC
CACCACCCCGCAGCTTGAATAAATTTTTTATGAAATGAACCGTACCCGAAATCTTGATCTCGC
CGGATGCGGCTCCGACCTGCGGATCCCAATGGATTCCCTCCTGGCATTCGACTGCGTTGGT
GAGTGCTATGATACTGCCGCCCCTTGGAACTTAAAGAACCCACCTTATAACGACGAGCTCCT
CCGTCCTCCAGTTCGGGGTTTCACTTTGGCTTTATTTGGCACGCTGCACTCACCGTTGTTGC
GGCCTGCATAATCGCAGATAAAAATGTCAATAATTGGGATCTATCACGAAGATCTCACGGAT
AGGCACGACGCTTATAAACGTGCGGCGCCTACGCAGACGCTGTACACTTCGACGCCCCCTT
TCCGTGACGTGAC

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_04
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCGGCTTGAAGAATTTTTTTATTAACTGACCCATAACCTGAATCATGATCTCG
TCGGATCAGGGTCCAACCTGCGGACCCCATCGGACTGCCGCGGCACATCCCCCTGCCGTG
TGCCTGCTTTGAAACTCAGGCCCCTTGCTCCCCATGAAAATGGTTTAGCAGATACGAGCTGA
AGATGTACTGCTTTAAAAATCTACCTTAACATCTGCCACCTTGTTCTTGTCATCCGCTATGTG
CTCGCTGTTGCGCGGGGTGGCATGGGAGGTAATCGACGCCACAGGAAGATGACGGGTATT
TCGCTGATTTCGCATGTTGTGGGTTCTGCTTATGCCGCGCCCACC

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_05
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAACAAATTTTTTATGAACTGACCCATAACCTGAATCAAGATCTCG
GCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCCGCACATCCCGCTGCGATTA
TGCCTGCTATGATACTCAGGCCCCTTGCTATGCCATGACCATGGATTATCCGGGGACTTGG
GCGGAGTACCGACTTTCCATACCGTTTAAATTCCTCTTCGACAAATGCGTCTCCTGCGCAGA
TAATCCCAAGTGGCCGTTCAGGTTGTACCCCTTATTTCCCCCTGTGTCGCTGGCATGCACGT
CCGGGGTAAACTCGTTCCGGCCTACAGTCTGCATCGATGAGACACGCGAGCGACGAGAGT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_06
ACGCACTGGGAAGCACGGGCGGGTATCGTCGGGCGTTTCTCGGTTACGGGTGGCAGTTGT
TATCGGAAGATACTGGCGATTCAGCCGCAGCAATTTCCCCGAGCGTTCGCCCATTCCTCACA
CGGTTCGCGTCAGCCAGCCGGAGCGGCAAATACTATACCACGAAATACCTCGAAAGACATT
TCGCGACCTCGTGGGAACTTTCGCGGCAGCGGTCTGACCAATATGCACGGATTCCACCGGT
GATATCGTTCCCCATCGGCACCACCACCCCGCAGCTTCGAAGAAAATTTTTATGAAACTCGA
CCCATAACCCTGGAAATTCAGGAATCTTCGGCGGGAATCAGGGCTCCCAACCCTCGCGGAA
CCCCCATTCGGAACTGGCCCTCGGCACATTCCCCGCTTGCGAAATATTGCCTGGCTATTGAA
TACTTCAGGGCCCTTTCGCTATTGCCATGGACCATGGGATTTATCAGGGACGAAACTTGCAC
CGTCCCGCCCTTTTTCCGTACAAATTTTAAATTTCAAATTTTTGACGGGCTGGCCCCAACTGG
CTGCGTGGGCCTCCCATTTGCCCCCGTTCAGACATGTCTTTTTTATCGGGACCCTGGAAAGA
TGATATCCCGTATCGACCAGAAAGCCGTCCAAGCCCTATATCGGCGGCGAGCTGAAACCCA
ATGCAGGCGCACATTCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_07
ACTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACCAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCCTTTTTATCGGCAC
CACCACCCCGCACCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCACGATCTCG
GCGGATCACGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCAAATA
TGCCTGCTATGATACTCACGCCCTTTGCTCTGCCATGACCATGGATTATCAGGACGAACTGC
CCCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCGCAACTGCTGCGTG
GCCTCCATTGCCCGTTGAGACATGTCTTTTATCGGGACCAGAAATGATGATATTTCATATCGA
CCAGAAAGCAGTCCAAGCCTATATCGGCGGCGATCTGACACCCACTGCAAGCGCACATTCC
AGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_08
GGCCACTGGGAAGCACGGCGGTATCGTCGGCGTCTTTCGGTTACGGTGGCGGTGTTATCG
GAAGATACTCGCGATCAGCCGCAGCAATTTCCCCGGCGTTGCCCATTCCTCACACGGTTCG
CGTCAGCCAGCCGGGCGGCAAATACTATACTACGAAATACCTGAAAGACACCCGCGACCCT
GGGAACCTCGCGGCAGCGGTTGACCAATATGCACGGACCCACCGGTGATATCGTTTTTATC
GGCACCACCACCCCGCAGCTTGAAGAAACCTCTATGAACTGACCCATAACCTGAATCAGGAT
CTCGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGC
GAATATGCCTGCTATGATACTCAGGCCCTTTTGCTATGCCATGACCATGGATTATCAGGACG
AACTGCACCGTCCCGCCTTTTCCGTACAAATTTAAATTTCAAATTTTGACGGCTGCCCCAACT
GCTGCGTGGCCTCCATTTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGGGTA
TCCGTATCGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGG
CGCACATTCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_09
CTTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGAAAAGAAGATATCCGTATC
GACCAGAAAGCAGTCCGAGCCTATATTGTAGGTGAGCTGAAACGCAATGCAGGTGCAGATT
CCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_10
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACTAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCTGTATC
GATCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAACCCCCATGCATGCGCACATT
CCCGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_11
ACGCACTGGAAGCACGGCGGTATCGTCGGCGTTCTCGGTTACGGTGGCAGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTATGAACTGACCCATAACCTGAATCAGGATCTCG
GCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAAT
ATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACTG
CACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCGT
GGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATCG
ACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATTC
CGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_12
ACCCATTGGAACACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAAG
ATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAGC
CAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAAC
TTCGCGGCAGCGGTCTGACCAATATGCACGGACCCACCGGTGATATCGTTTTTATCGGCAC
CACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTCG
GCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAAT
ATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACTG
CACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCGT
GGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATCG
ACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATTC
CGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_13
ACTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAGGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCATATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_14
ACTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAGGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCATATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_15
ACGCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGTAC
CACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTCG
GCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAAT
ATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACTG
CACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCGT
GGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATCG
ACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAGTGCAGGCGCACATTC
CGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_16
ACTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGCTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACTTGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_17
ACCCACTGGAAACACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTCTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_18
ACCCACTGGAAACACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_19
ACTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_20
ACTCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_21
ACCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAACACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_22
ACCCACTTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_23
ACCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_24
ACCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_25
ACGCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_26
ACGCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_27
ACGCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_28
GCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATACGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACCAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAGAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAAGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_29
GGCCACTGGAAGCACGGCGGTATCGCCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACACCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCACCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCATCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_3
GGCCACAGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTCGCGACCTGTGGG
AACCTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGG
CACCACCACCCCGCAGCTTGAAGAAATTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGAATGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCACGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_31
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTCGGTTACGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTCTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATTCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTATGAACTGACCCATAACCTGAACCAGGATCTCG
GCGGATCAGGCTCCAACCTGCGGACCCCATCGGAATGCCTCGGCACATCCCGCTGCGAAT
ATGCCTGCTATGATACTCAGGCCCTTTTGCTATGCCATGACCATGGATTATCAGGACGAAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_32
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
AAACAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAAGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_33
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCCGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTACCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_34
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATCCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCCGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_35
GGCCACTGGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGG
AAGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTC
AGCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGG
AACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCCATGATACTCAGGCCCTTTTGCTATGCCATGACCATGGATTATCAGGACGAAA
CTGCACCGTCCCGCCTTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCT
GCGTGGCCTCCATTTGCCCGTTCAGACATGTCTTTTTATCGGGACCTGGAAAGATGATATCC
CGTATCGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCG
CACATTCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_36
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAGCTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCACGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_37
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGCCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGACGGCGAGCTGAAACCCAATGCAGGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_38
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTCATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGGAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_39
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATCTCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGATCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_40
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAAAACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TACGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCACGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_41
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACTACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAGTCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCGCATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_42
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACTATGGATTGTCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_43
GGCCACTGGAAGCACGGCGGCATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGGAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_44
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCGCCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCACGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_45
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCACGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_46
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCACGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_47
GGCCACTGGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGG
AAGTATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGT
CAGCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGG
GAACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCG
GCACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGAT
CTCGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGC
GAATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGA
ACTGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCT
GCGTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGT
ATCGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCAC
ATTCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_48
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGACTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_49
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACTACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_50
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTCGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_51
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCCATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_52
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTATGGTGGCGGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_53
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAAGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_54
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGGCCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_55
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCTGTGTTATCGGAA
GATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCAG
CCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGAA
CTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_56
GGCCACTGGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGG
AAGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTC
AGCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGG
AACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTTGCTATGCCATGACCATGGATTATCAGGACGTA
ACTGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCT
GCGTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGT
ATCGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCAC
ATTCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_57
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTCTTCACACGGTTCGCGTC
AGCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGG
AACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGC
ACCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCT
CGGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGA
ATATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAAC
TGCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGC
GTGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTAT
CGACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACAT
TCCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_58
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_59
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_60
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_61
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_62
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_63
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_64
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_65
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_66
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_67
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_68
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_69
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_70
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_71
GGCCACTGGAAGCACGGCGGTATCGTCGGCGTTTTCGGTTACGGTGGCGGTGTTATCGGA
AGATACTGCGATCAGCCGCAGCAATTCCCCGGCGTTGCCCATTTTCACACGGTTCGCGTCA
GCCAGCCGGGCGGCAAATACTATACCACGAAATACCTGAAAGACATTTGCGACCTGTGGGA
ACTTCGCGGCAGCGGTCTGACCAATATGCACGGATCCACCGGTGATATCGTTTTTATCGGCA
CCACCACCCCGCAGCTTGAAGAAATTTTTTATGAACTGACCCATAACCTGAATCAGGATCTC
GGCGGATCAGGCTCCAACCTGCGGACCCCATCGGACTGCCTCGGCACATCCCGCTGCGAA
TATGCCTGCTATGATACTCAGGCCCTTTGCTATGCCATGACCATGGATTATCAGGACGAACT
GCACCGTCCCGCCTTTCCGTACAAATTTAAATTCAAATTTGACGGCTGCCCCAACTGCTGCG
TGGCCTCCATTGCCCGTTCAGACATGTCTTTTATCGGGACCTGGAAAGATGATATCCGTATC
GACCAGAAAGCCGTCCAAGCCTATATCGGCGGCGAGCTGAAACCCAATGCAGGCGCACATT
CCGGCCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_72
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAAAGATCTTCTTCGAGCTGACCCACAATCACAACCATGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCTCGCTG
CGAATTCCTATGCTACGACACCCACCTGATGTGCCGCGCCTTGATTAATGATTACCATGACA
TGGTGCACCGCCCGCAGTTCCCCTACAAGTTCGATTCAAGTTCTACGGCTGCCCCAACGGC
TGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCAGCACCTGATATGGATGACATCT
AGATCGACCAGACCACTGTGAAGGCTTACGTCAGTGGCGAGATGACTCCCAAACACCGGCG
CCCACC

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_73
ACTCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGGA
CGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTTG
CCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGGA
CATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGGG
CACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACTAGGAC
CTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTGC
GAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACCAGGACAT
GCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGGC
TGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGGCAGGACTACATCA
AGATCAATCAGACCGCTGTGAAGGCTTACTTCGGTGGCGAGATCGCCCCGACCACCCGCAC
CCACTCCAGTCGCGAGT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_74
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAGGCACAACCAGGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCGGCTGATGTGCCACACCTTGACCAATGAATACCAGGAC
ATGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACG
GCTGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGTAAGGACGACAT
CAAGATCGACCAGGCCGCTGTGAAGGCTTACGTCAGTGGCGAGATCGCCCCCAACGCCGG
CGCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_75
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACCAGGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGGATACCAAGACA
TGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGG
CTGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGAAAGGACGACATC
AAGATCGACCAGGCCGCTGTGAAGGCTTACGTCGGTGGCGAGATCGCCCCCAACGCCGGC
GCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_76
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACCAGGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACCAGGACA
TGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGG
CTGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGGAAGGACGACATC
TAGATCGACCAGGCCGCTGTGAAGGCTTACATCGGTGGCGAGATCGCCCCCAACGCCGGC
GCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_77
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACCAGGA
CCTGGGCGGCTCGAGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACCAGGACA
TGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGG
CTGCGTGGCCTCCATCGCCCGCCCCGACTTCTCCGTCATCGGCACCTGGAAGGACGACATC
AAGATCGACCAGGCCGCTGTGAAGGCTTACGTCGGTGGCGAGATCGCCCCCAACGCCGGC
GCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_78
GGCCACTGGAAGCACGGCGGCATCGTTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCG
GACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTTCCACACCGTGCGTC
TTGCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTG
GGACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCT
GGGCACCACCACCCCCCAGCTGGAAGAGATCTTCCTCGAGCTGACCCACAAGCACAACCAG
GACCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGC
TGCGAATTCGCATGCTGCGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACCAGG
ACATGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAA
CGGCTGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGGAAGGACGA
CATCAAGATCGACCAGGCCGCTGTGAAGGCTTACGTCGGTGGCGAGATCGCCCCCAACGC
CGGCGCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_79
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCCCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACCAGGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACCAGGACA
TGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGG
CTGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGGAAGGACGACATC
AAGATCGACCAGGCCGCTGTGAAGGCTTACGTCGGTGGCGAGATCGCCCCCAACGCCGGC
GCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_80
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACCAGGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACCAGGACA
TGCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGG
CTGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGGAAGGACGACATC
AAGATCGACCAGGCCGCTGTGAAGGCTTACGTCGGTGGCGAGATCGCCCCCAACGCCGGC
GCCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_81
GGCCACTGGAAGCACGGCGGCATCGTGGGCGTGTTCGGCTACGGCGGCGGCGTCATCGG
ACGCTACTGCGACCAGCCCGAAAGATTCCCCGGCGTTGCCCACTTCCACACCGTGCGTCTT
GCCCAGCCTTCCGGCCTCTTCTACAAGGCCGACTACCTGGAAGAGCTGTGCGACCTGTGGG
ACATGCGCGGATCCGGCATGACCAACATGCACGGCTCCACCGGAGACATCATCTGGCTGG
GCACCACCACCCCCCAGCTGGAAGAGATCTTCTTCGAGCTGACCCACAAGCACAACCAGGA
CCTGGGCGGCTCGGGTTCCAACCTGCGCACCCCCGCCTGCTGCATGGGCATGTCCCGCTG
CGAATTCGCATGCTACGACACCCAGCTGATGTGCCACACCTTGACCAATGAATACAGGACAT
GCTGCACCGCCCGCAGTTCCCCTACAAGTTCAAGTTCAAGTTCGACGGCTGCCCCAACGGC
TGCGTGGCCTCCATCGCCCGCTCCGACTTCTCCGTCATCGGCACCTGGAAGGACGACATCA
AGATCGACCAGGCCGCTGTGAAGGCTTACGTCGGTGGCGAGATCGCCCCCAACGCCGGCG
CCCACGCCGGTCGCGACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_82
GGCCACTGGAAGCACGGCGGAATCGTTGGAGCCTTCGGGTACGGCGGCGGCATCATCGGT
CGTTACTGCGATCAGCCGACCCTGTTCCCCGGTGTGGCACACTTCCACACCGTGCGCGTGA
ACCAGCCGTCCAGCAAGTATTACACGACGGAATTTCTCCGCGGCCTGTGCAAGCTTTGGAT
GAGCACGGCAGTGGTCTCACCAACATGCACGGCTCCGCGGGTGACATCGTTTTCCTGGGG
ACAACGACCGACCACCTCGAGCCGCTCTTCTTCGACCTGACCCACGAACTGAACCAGGATC
TTGGCGGATCGGGCTCCTACCTCCGTACCCCGGAGTGCTGCCTCGGGAATTCCCGCTGGG
AGTTCGCCTGCGATGATACCGCTGAGATGTGCTACCCCTTCCGGCAACAGGATCGATACGA
CCGTCATCTCACGGCTTTCCCCATCAGCTTCAAGTGCTGGTTCCACGGCTGCGCGAACGAC
TGTGAGCTCTATATCGCTAGTTGCGATATGTCCGACATAGCAAGCAGAACGTATGACAGTGA
TATAGCAGATATCGCGTATGTATGATACAGCTGACAGAGAGCGCACCCCCGACCCCTGACC
GTATATACAGCCGCGACA

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_83
GGAATCGTTGGAGTCTTCGGGTACGGCGGCGGCATCATCGGTCGTTACTGCGATCAGCCGA
CCCTGTTCCCCGGTGTGGCACACTTCCACACCGTGCGCGTGAACCAGCCGTCCAGCAAGTA
TTACACGACGGAATTTCTCCGCGGCCTGTGCAAACTTTGGGATGAGCACGGCAGTGGTCTC
ACCAACATGCACGGCTCCACGGGTGACATCGTTTTCCTGGGGACAACGACCGACCACCTCG
AGCCGCTCTTCTTCGACCTGACCCACGAACTGAACCAGGATCTTGGCGGATCGGGCTCCAA
CCTCCGTACCCCGGAGTGCTGCCTCGGACAGTCCCGCTGTGAATTCGCCTGCTACGATACC
CAGGAACTGTGCTACCAGTTCACCCAGGAGTATCAGGACGAGCTTCATCGCCCGGCCTTCC
CCTACAAGTTCAAGTTCAAGTTTGACGGCTGCCCGAACGGCTGCGTGGCCTCCATCGCTCG
TTCCGACATGTCCGTCATCGGTACCTGGAAAGATGACATTCGCATCGACCAGAAGGCCGTT
GCAGCCTATGTGGGCGGCGAGCTGGCTCCCAACGCCGGTGCCCACTCCAGCCGCAACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_84
GGAATCGTTGGAGTCTTCGGGTACGGCGGCGGCATCATCGGTCGTTACTGCGATCAGCCGA
CCCTGTTCCCCGGTGTGGCACACTTCCACACCGTGCGCGTGAACCAGCCGTCCAGCAAGTA
TTACACGACGGAATTTCTCCGCGGCCTGTGCAAACTTTGGGATGAGCACGGCAGTGGTCTC
ACCAACATGCACGGCTCCACGGGTGACATCGTTTTCCTGGGGACAACGACCGACCACCTCG
AGCCGCTCTTCTTCGACCTGACCCACGAACTGAACCAGGATCTTGGCGGATCGGGCTCCAA
CCTCCGTACCCCGGAGTGCTGCCTCGGACAGTCCCGCTGTGAATTCGCCTGCTACGATACC
CAGGAACTGTGCTACCAGTTCACCCAGGAGTATCAGGACGAGCTTCATCGCCCGGCCTTCC
CCTACAAGTTCAAGTTCAAGTTTGACGGCTGCCCGAACGGCTGCGTGGCCTCCATCGCTCG
TTCCGACATGTCCGTCATCGGTACCTGGAAAGATGACATTCGCATCGACCAGAAGGCCGTT
GCAGCCTATGTGGGCGGCGAGCTGGCTCCCAACGCCGGTGCCCACTCCAGCCGCAACT

>uncultured sulfate reducing bacterium dsrA gene for dissimilatory sulfite reductase alpha subunit, partial cds, BAC_clone_85
GGAATCGTTGGAGTCTTCGGGTACGGCGGCGGCATCATCGGTCGTTACTGCGATCAGCCGA
CCCTGTTCCCTGGTGTGGCACACTTCCACACCGTGCGCGTGAACCAGCCGTCCCGCAAGTA
TTACACGACGGAATTTCTCCGCGGCCTGTGCAAACTTTGGGATGAGCACGGCAGTGGTCTC
ACCAACATGCACGGCTCCACGGGTGACATCGTTTTCCTGGGGACAACGACCGACCACCTCG
AGCCGCTCTTCTTCGACCTGACCCACGAACTGAACCAGGATCTTGGCGGATCGGGCTCCAA
CCTCCGTACCCCGGAGTGCTGCCTCGGACAGTCCCGCTGTGAATTCGCCTGCTACGATACC
CAGGAACTGTGCTACCAGTTCACCCAGGAGTATCAGGACGAGCTTCATCGCCCGGCCTTCC
CCTACAAGTTCAAGTTCAAGTTTGACGGCTGCCCGAACGGCTGCGTGGCCTCCATCGCTCG
TTCCGACATGTCCGTCATCGGTACCTGGAAAGATGACATTCGCATCGACCAGAAGGCCGTT
GCAGCCTATGTGGGCGGCGAGCTGGCTCCCAACGCCGGTGCCCACTCCAGCCGCAACT

Appendix 4-C: partial *arrA* gene sequences

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA01

GTCCCGATGACCTGGGATGAAGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATACCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AATTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA02

GTCCCAATCAGGTGGGATAAAGCACTGGATGCCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTTCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATTG
CTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGCC
GGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTCATG
GAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTACG
GTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCGA
GCTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA05

GTCCCGATTAGCTGGGATGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCGGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAGTTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA03

GTCCCGATTACCTGGGAGAAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AATTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA04

GTCCCGATGACGTGGGACGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACCATTCCCACAGCGC
GATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCG
CGACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCC
AATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTA
TTGCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAA
GCCGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTT
ATGGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCT
ACGGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATAT
CGAGCTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA06

GTCCCGATCAGCTGGGAGGAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCAC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA07

GTCCCTATTACGTGGGAGGAGGCACTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA08

GTCCCGATCACTTGGGATAAGGCTCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGGTGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTATTATTG
CTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGCC
GGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTATG
GAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTACG
GTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCCTCAAGTTCTGGAATATCGA
ACTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA09

GTCCCGATGAGGTGGGACGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCG
CGACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTG
CGCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCA

CTCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTC
CTACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTC
GAGGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAG
ACGCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCC
GATCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCAC
GTCATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTG
AACCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAG
GGCCTCGCAGAGTGGTGGAACATCGAACTAAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA10

GTCCCTATGAGATGGGAGGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
GCTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGTGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTTAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA14
GTCCCAATGAGTTGGGAGGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCCTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGTCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAACTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA17
TGTCCTATGAGATGGGAGGAAGCACTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCATCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACGGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATC
GAGCTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA18
GTCCCGATTAGGTGGGAAAAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCGGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC

CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAACATC
GAATTGAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA19
GTCCCGATTACATGGGACGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGTACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGTGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAGCTCAAGGACCGCACCCA

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA21
GTCCCCATGAGGTGGGAAAAGGCGCTCGATACGGCGGCCGACAAGATGGTCGCGCTACGA
CAGGCCGGCGAACCGCACAAGCTGATGTACATGCGCGGCCGCTACTCCTCGACCTCGACC
GATCTGCTCTACGGCACTCTGCCCAAGGTGTTCGGTACGCCCAACTATTTCTCGCACAGCG
CCATCTGCGCCGAAGCGGAAAAAATGGGGCCGGGCCTGACCCAGGGCTTTTTCGGTTATCG
CGATTACGATCTGGAAAAAACCCAGTGCCTGGTGGTGTGGGGAACCGATCCGCTAGCGTCG
AACCGCATGGTGCCCAATACCATCAACCGCTTTCACGAGATCGTGGCGCGCGGCACGGTCA
TTGCCGTCGACCCCCGGCTTTCCAATTCTGCCGCCAAGGCGCACGAGTGGCTGCCGATCAA
GCCGGGGACTGACGGTGCGCTGGCTGGGGCAGTGGCGCATGTGCTGCTGACCGAAGGATT
GTGGAGCCGTGAATTTGTCGGCGACTTCAAGGATGGCAAGAACCTGTTTGTTGCCGGTCAG
GAAGTCGACGAAGCGGCGTTCGCGGAAAAGGGAACCTTCGGCCTGGTCAAATGGTGGAAC
ATCGAACTAAAAGACCGCACAAG

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA28
GTCCCGATTAGTTGGGAGAAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGTGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCAGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTTACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCGAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGG
GCCTCGCAGAGTGGTGGAACATCGAATTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA30
GTCCCGATGACATGGGACGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACGTGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AATTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA32
GTCCCCATTAGGTGGGATGAGGCGCTGGATCCCCTGGCAGACAAAATGATGGAACTGTGCA
AGAACAACGAACTGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGAC
CTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGAT
CTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCGA
CTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGGTGCGACCCGCTATCATCCAAT
CGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATTG
CTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGTCGATCAAGCC
GGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCCCATGTGCTCCTGACCGAGGGTTTATG
GAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTACG
GTTGACGAGACAGCTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTATGAAAGATCGAA
CTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA33
GTCCCCATCACATGGGAGAAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAATTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA34
GTCCCGATTACTTGGGACGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAGTTGAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA35
GTCCCTATTACGTGGGAGGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGGGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAACTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA37
GTCCCGATTACCTGGGAGAAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA

CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAAGCCCAATGAGTGGCTGCCGATCAA
GCCGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTT
ATGGAGCAAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGC
TACGGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTTCTGGAAT
ATCGAATTAAAAGACCGCACCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA38
GTCCCGATCAGCTGGGACGAAGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGGGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAGCTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA40
GTCCCTATGACCTGGGACGAAGCTCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGTGCCGGAAAAACTGATGTACATGCGTGGCCGCTACCCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAGTTCGTCGGCGATTTCAAGGATGGCAAAAACCTCTTCAAAACCGGCGCCAC
GGTTGATGCAGCGGCCTTTGTGGAAAAACAGACCCACGGCATCGTCAAATACTGGAATCTT
GAATTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA41
GTCCCGATGAGTTGGGAGAAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAATACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AGCTAAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA43
GTCCCGATCAGGTGGGAGGAGGCCCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC

GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAGCATCGAACTAAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA44
GTCCCGATGAGCTGGGACGAGGCACTGGATACCCTGACAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTGCCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AATTGAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA45
GTCCCGATTACGTGGGAAGAGGCTCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAACTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAACATC
GAGTTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA47
GTCCCGATTAGCTGGGAAGAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAGGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGACCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCCATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTCCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCGGAGAAAACAGACCCACGGTATCGTCAAGTTCTGGAAATAT
CGAATTTCAAAGACCCGCAC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA50
GTCCCCATTACGTGGGATAAAGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTAGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC

GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AGCTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA52
GTCCCGATTAGTTGGGAGAAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAGTTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA53
GTCCCGATGAGGTGGGATGAAGCGCTGGATACGGTCGCGGACAAGATGATGGAGCTGCGT
AAGGCCGGAACTCCCGAGAAACTGATGTACATGCGTGGCCGCTACTCCTCAACCGCTACCG
ACCTGCTCTACGGAACGCTCCCCAAGATATACGGAACCGGAAATTATTTCTCCCACAGCGCC
ATCTGCGCCGAAGCCGAGAAGATGGGGCCTGGATATACCCAGGGGTTCTTCGGCTATCGG
GACTATGACCTGGCCAAGACCAGGTGCCTGGTTGTCTGGGGCTGCGACCCGCTCTCTTCCA
ACCGCCAGGTGCCCAACGCCATCTCAAAATTCAGCGATATCCTCGATCGCGGAACGATCAT
AGCAGTTGACCCCCGCATGAGCGCCTCGGTCGCCAAAGCCAACGAATGGCTGCCGATCAA
GCCTGGCGAGGATGGCGCCCTGGCCGCGGCCCTGGCCCATGTGCTGCTGACCGAGGGCT
TCTGGAGCAAGGAGTTCGTCGGCGATTTCAAGGATGGCAAAAACCTCTTCAAAACCGGCGC
CACGGTTGATGCAGCGGCCTTTGTGGAAAAACAGACCCACGGCATCGTCAAATACTGGAAT
CTTGAGTTGAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA54
GTCCCGATTAGTTGGGATAAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGGCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCCCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAGTTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA55
GTCCCTATGAGTTGGGAGAAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCCCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTTAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA57
GTCTCGATCACGTGGGAAGAGGCCCTGGATACCCAGGCAGACAAAATGATGGATCTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGGCGCTACTCTCCTACCTCCACGGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAACGATATCCTCGACCGGGGTACTGTTATTG
CTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGCC
GGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTATG
GAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTACG
GTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCGA
GCTGAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA59
GTCCCGATGACATGGGAGAAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA
TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAACTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA62
GTCCCTATGAGGTGGGAGGAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGTGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTCAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA65
GTCCCCATTAGATGGGATGAGGCGCTGGATACCGTGGCAGACAAAATGATGGAACTGCGCA
AGAGCAACGAGCCGGAAAAACTGATGTACATGCGTGGACTCTACTCTCTTACCTCCACCGAC
CTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCACCAACTACTATTCCCACAGCGCGAT
CTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCCTCGGCTACCGTGA
GTATGACCTGGCCAAGACCAAGTGCCTGGTTGTGTGGGGGTGCGACCCGCTATCATCCAAT
CGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATTG
CTGTTGACCCGAGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGCC
GGGCGAGGACGGCGCCCTGGCCGCCGGTATAGATCATGTGCACCTGACCGAGGGTTTATG
GAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTACG
GTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCGA
ACTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA67
GTCCCGATCAGGTGGGACGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACTTCCACCG
ACCTGTTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG

ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGAACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AGTTGAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA69
GTCCCGATGAGATGGGATGAAGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAGG
CCGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTA
TGGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTA
CGGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATC
GAACTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA73
TGTCCGATGACGTGGGAAAAAGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGACGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCGAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTGAAGGACCGCACCCA

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA75
GTCCCGATCAGATGGGACGAGGCCCTGGATACCCTGGCAGACAAAATGGTGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTGCCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAGTTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTCGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATC
GAATTAAAAGACCGCACCCA

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA76
GTCCCGATCACGTGGGAAGAGGCGCTGCAGACCGTCGCCGACCGGCTCAACACGCTGCGC
GACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTGC
GCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCAC
TCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTCCT
ACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTCGA
GGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAGAC
GCCGAAGACGCGGGTCACCGCCGTCGACGTCCACCTCAACACCACGCTCGCCGCCGCCGA

TCGCGGCCTGCTGATCAAGCCCGGCACCGACGGCGCCCTCGCCCTGGCGATCGCCCACGT
CATCCTCACCGAGGGCCTGTGGGAGCGCTCCTTCGTCGGCGACTTCAAGGACGGCGTGAA
CCGCTTCAAGGCCGGCCAGACGGTCGACCCGGCGAGCTTCGACGAGAAGTGGGTCAAGGG
CCTCGCAGAGTGGTGGAACATCGAGTTAAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA77
GTCCCGATTAGATGGGAAGAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATC
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCCGCCGATCAAG
CCGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTA
TGGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTA
CGGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATC
GAGCTGAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA78
GTCCCGATCAGGTGGGATGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAGGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTGAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA79
GTCCCGATCAGTTGGGAAGAGGCCCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCGA
TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAGG
CCGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTA
TGGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTA
CGGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATC
GAATTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA80
GTCCCCATGAGGTGGGAAGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCTGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCGAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AGCTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA86
GTCCCTATGAGGTGGGAGGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGC
AAGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCG
ACCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTATTCCCACAGCGCG
ATCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGC
GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
ATCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AGTTAAAGGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA89
GTCCCTATCAGCTGGGAAGAGGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
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TCTGCGCCGAGGCCGAAAAGATGGGGCCGGGCTACACCCAGGGATTCTTCGGCTACCGCG
ACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCAA
TCGCCAGGTCCCCAACGCCATTGCCAAGTTCAGCGATATCCTCGACCGGGGTACTGTTATT
GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCTCATGTGCTCCTGACCGAGGGTTTAT
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GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AATTCAAAGACCGCACCCC

>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA91
GTCCCGATCACGTGGGAGGAGGCGCTGCAGACCGTCGCCGACCGGGTCAACACGCTGCG
CGACAAGGGCGAGAGCCATCGCTTCTCGCTGTGCTTCGGCCGCGGCTGGGGCGCCTCCTG
CGCCGGCCTGCTCGGAACCTTCGGTGACCTCTACGGCTCGCCCAACGTGCCGATCGGCCA
CTCGTCGATGTGCTCGGACGGCTCGGTCATGTCCAAGCAGTGCACCGACGGCAACGCCTC
CTACAGCGCCTACGACTATCGAAACTGCAACTACCTCCTGATGTTCGGGGCGAGCTTCCTC
GAGGCCTTCCGGCCCTACAACAACAACATGCAGGTGTGGGGCTACATCCGCGGCGAGAAG
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GATCGCGGCCTGCTGATCAAGCCCGGCACCGACGGTGCCCTCGCCCTGGCGATCGCCCAC
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>arsenate respiratory reductase [uncultured bacterium] _BAC clone_ArrA93
GTCCCGATTAGATGGGAGGAAGCGCTGGATACCCTGGCAGACAAAATGATGGAACTGCGCA
AGAACAACGAGCCGGAAAAACTGATGTACATGCGTGGCCGCTACTCTCCTACCTCCACCGA
CCTGCTCTACGGCACCCTGCCCAAAATTTTCGGCACCCCCAACTACTACTCCCACAGCGCG
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GACTATGACCTGGCCAAGACCAAGTGCCTGGTTGTCTGGGGCTGCGACCCGCTATCATCCA
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GCTGTTGACCCGCGCATGAGCGCTTCGGTAGCAAAAGCCAATGAGTGGCTGCCGATCAAGC
CGGGCGAGGACGGCGCCCTGGCCGCCGGTATAGCCCATGTGCTCCTGACCGAGGGTTTAT
GGAGCAAGGAATTCGTCGGCAGCTTCAAGGATGGCAAAAACCTGTTCAAAACGGGTGCTAC
GGTTGACGAGACAGCTTTTGCAGAGAAACAGACCCACGGTATCGTCAAGTTCTGGAATATCG
AACTAAAAGACCGCACCCC

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Chapter 5

Empty Bed Contact Time Optimization for a Fixed-bed Bioreactor System that Simultaneously Removes Arsenic and Nitrate

5.1 Abstract

A series of terminal electron accepting process (TEAP) zones develops when a contaminated water containing a variety of potential electron acceptors, such as dissolved oxygen (DO), nitrate, iron(III), arsenate, and sulfate, is treated using a fixed-bed bioreactor. Backwashing of such a fixed-bed bioreactor may remove contaminant-laden solid phases from the reactor along with the accumulated biomass. Therefore, it may be advantageous to separate the TEAP zones into multiple bioreactors in order to minimize the production of contaminated sludge. With this objective in mind, a fixed-bed bioreactor system consisting of two biologically active carbon bioreactors in series was operated for biologically mediated nitrate and arsenic removal. The empty bed contact time (EBCT) of the first bioreactor of this two-reactor system was optimized to minimize the volume of arsenic-laden sludge generated during backwashing. The impacts of EBCT changes between 27 and 40 min on sulfate and arsenate reducing populations and on overall reactor performance were evaluated. Lowering the EBCT successively from 40 min to 35, 30, and 27 min shifted the sulfate reduction and arsenic removal zones to the second reactor. Influent nitrate (approximately 50 mg/L NO_3^-) was completely removed during the entire

study period regardless of the EBCTs evaluated. Arsenic was lowered from 200 to 300 µg/L As in the influent to less than 20 µg/L As with an EBCT as low as 30 min. At the lowest EBCT of 27 min, the abundance of sulfate and arsenate reducing bacteria significantly decreased resulting in poor reactor performance. Co-location of sulfate and arsenate reducing activities in the presence of iron(II) and subsequent generation of fresh sulfides were important to accomplish arsenic removal in the system.

5.2 Introduction

A fixed-bed bioreactor comprises a stationary bed of a biofilm attachment medium, such as sand, plastic, or granular activated carbon (GAC). The filter bed provides a surface for microbial growth and minimizes washout of desired microorganisms, especially those that are slow growing, such as sulfate reducing bacteria. A differential redox gradient can be developed across the bed to provide local environments suitable for the growth of microorganisms with varying metabolic capabilities [1]. The diverse microbial consortia that develop can degrade a variety of organic and inorganic contaminants, while utilizing thermodynamically preferred electron acceptor(s), including dissolved oxygen (DO), nitrate, iron(III), sulfate, and a variety of other oxy-anionic contaminants, such as arsenate (As(V)) and uranate (U(VI).

Biologically active carbon (BAC) reactors utilize GAC particles as the support medium. Microorganisms grow in biofilms generated in and on the GAC granules [2] converting the support medium to a bed that couples the adsorption

capacity of GAC with biodegradation [3]. As a result, reactor performance improves [4, 5], while prolonging the life and reducing the regeneration cost of the GAC [6].

Given the apparent advantages of BAC reactors, including the adsorption capacity provided by GAC, which allows removal of inhibitory and slowly biodegrading materials, ample surface area for microorganisms attachment, and rapid acclimation of biomass [4], BAC reactors have gained popularity in water treatment. They have been utilized for the removal of many inorganic contaminants, including perchlorate and nitrate [7], ammonia [8] and bromate [9], and organic contaminants, such as ozonation byproducts [10], synthetic surfactants [5], and trace organics including taste and odor causing compounds [11].

Empty bed contact time (EBCT) is a critical parameter in the design and operation of a fixed-bed bioreactor. EBCT determines whether there is sufficient time for effective diffusion of contaminants into the biofilm and their subsequent utilization by the microorganisms [9]. Minimum EBCT required for contaminant removal depends on many factors, including biotransformation kinetics, adsorption affinity of the contaminants for BAC, and the practical consideration of the targeted treatment standard to be achieved. Increasing the EBCT generally leads to better reactor performance by allowing more time for complete biodegradation, precipitation, and/or adsorption of contaminants. Rhim et al. [3] reported increased biodegradable dissolved organic carbon (BDOC) removal efficiency in a packed bed reactor at an EBCT of 15 min compared to that at 8

min. Wu and Xie [12] observed increased haloacetic acid removal with longer EBCT. Studying the comparative effects of changing the EBCT on the removal of ozonation byproducts through adsorption and biodegradation in a BAC reactor system, Liang [10] reported better removal with increased EBCTs. Increasing the EBCT apparently resulted in better utilization of the adsorption capacity of the BAC rather than improved biodegradation in this case. Operating a fixed-bed reactor system, Lee et al. [13] reported 97% and 60% ammonia-nitrogen removal when the reactors were operated at 60 and 15 min, respectively. However, the reactor size and associated costs of installation and maintenance increase with increasing EBCT making optimization of the reactor system to minimize EBCT without compromising reactor performance a high priority.

Associated with EBCT optimization is the need to establish effective treatment zones within a given reactor system, especially when multiple terminal electron accepting processes (TEAPs) are to be utilized for the treatment of co-contaminants within the same reactor system. For example, in an application of anaerobic fixed-bed bioreactors for the simultaneous removal of nitrate and perchlorate, previous work [7] has shown that considerable biomass can be accumulated in the reactors that requires periodic backwashing in order to maintain optimal reactor performance [14]. Along with the removed biomass, however, other solids formed during treatment also can be removed during backwashing. When these solid phase reaction products include hazardous materials, the potential exists to create an unfavorable solid waste disposal problem. In recognition of this potential, our recent study demonstrated that it

may be preferable to separate high biomass generating TEAPs, such as those that remove DO and nitrate and require frequent backwashing, from TEAPs that may generate hazardous solid waste (e.g., arsenic laden solids) and much less biomass using multiple reactor configurations. Upadhyaya et al. [1] demonstrated that both nitrate and arsenate contaminated water can be effectively treated using two BAC reactors in series. Yet this feasibility study indicated that optimization of the TEAP zones between the two reactors in series was needed to determine if the arsenic solid producing TEAP zone could be shifted to the second reactor. Thus, in addition to minimizing reactor size, EBCT optimization may also be desirable to minimize the generation of backwashed biomass and solids that may require handling as a hazardous solid phase.

In a fixed-bed bioreactor, when a suitable electron donor is present in adequate quantities, microbial populations develop in succession based on the thermodynamic favorability of coupling an electron donor to available terminal electron acceptors in the water to be treated [1]. This results in the development of various TEAP zones along the flow direction with microbial populations of varying metabolic capability and activity. The microbial populations may respond to the changes in operational parameters, such as the influent concentrations and EBCT and impact contaminant removal [7]. Molecular biology tools such as clone library, quantitative PCR, and reverse transcriptase PCR can be utilized to identify and quantify microbial population dynamics and their activity across the filter bed in response to change in an operational parameter [7, 15]. In

combination with the chemical data, such microbial data on population dynamics can be utilized to optimize contaminant removal in an engineered system.

The objective of this study was to assess the impact of EBCT on reactor performance, with the overall goal of maximizing water treatment throughput, while maintaining effective contaminant removal, and if possible to isolate the production of arsenic solid phase reaction products primarily to the second reactor of a two-reactor system. EBCT optimization impacts were assessed by monitoring activity and abundance of key microbial populations and concentrations of the chemical constituents in the final effluent and along the length of the dual BAC column reactor system.

5.3 Materials and Methods

Reactor System and Operation. Two glass columns of 4.9 cm inner diameter and 26 cm height (reactors A and B) were packed with BAC particles collected from bench- and a pilot-scale bioreactors utilized for the removal of nitrate and perchlorate [7]. Reactor A was operated in a downflow mode, while the effluent from reactor A was introduced into reactor B in an upflow fashion. The influent consisted of a synthetic groundwater and contained 300 µg/L arsenic as As(V), 50 mg/L nitrate, and 22 mg/L sulfate (except as noted below) along with other constituents (Table 5.1). Glacial acetic acid (35 mg/L acetate as carbon), serving as the only electron donor, was fed into the influent line of reactor A through a syringe pump (Harvard apparatus, Holliston, MA) along with 2 mg/L Fe(II). In addition to the Fe(II) added to reactor A, up to 4 mg/L Fe(II) was loaded directly

into reactor B (i.e., into the effluent line from reactor A) via a syringe pump to facilitate precipitation of iron sulfide. Dissolved oxygen (DO) in the influent was maintained at less than 1 mg/L by bubbling oxygen-free N_2 gas through the influent for approximately 20 min every 24 h and coverage of the influent tank with a floating cover. Reactor A was backwashed every 2 days with a mixed flow of de-ionized (DI) water (50 mL/min) and N_2 gas to completely fluidize the filter bed for 2 min followed by a flow of N_2 purged DI water (500 mL/min) for 2 min. Reactor B was backwashed on days 247 and 455 to collect the solids deposited in the reactor system following the same protocol. In addition, reactor B was agitated with a flow of N_2 gas and N_2 purged DI water for 2 min on days 369 and 479 to break the aggregated bed material and solids while avoiding the loss of deposited solids. After agitation of the bed material, the solids were allowed to settle for 2 h before resuming reactor operation.

The EBCT of reactor A was varied to assess the impact on total system performance. The two reactors were initially operated with an EBCT of 20 min each, resulting in a total EBCT of 40 min. At this EBCT, sulfate reduction and subsequent arsenic removal started in reactor A and continued into reactor B (as discussed below). To evaluate the possibility of completely shifting the sulfate reducing zone into the second reactor, the EBCT of reactor A was lowered while keeping the EBCT of the second reactor constant at 20 min. Each EBCT condition was evaluated for at least 35 days before a subsequent change to the EBCT was made. On days 300 and 337, the EBCT of reactor A was lowered to 15 min (total EBCT=35 min) and 10 min (total EBCT=30 min), respectively.

Finally, the EBCT of reactor A was lowered to 7 min (total EBCT=27 min) on day 387. From day 428 to day 466, the influent nitrate concentration was maintained at 69.7±1.8 mg/L NO_3^-. Starting on day 448, the influent arsenic concentration was reduced to 200 µg/L As. On day 517, approximately 66% of the BAC in reactor A (17% of the total filter bed) was replaced with BAC from the same stock used for packing the reactors initially that had been stored at 4 ^oC for approximately 17 months. Following this addition of BAC, the EBCT of reactor A was 10 min (total 30 min EBCT).

Liquid Samples Collection and Chemical Analyses. Liquid samples were collected from the influent tank (Inf), the effluent from reactor A (EA), and the effluent from reactor B (EB) every 24 h. Liquid samples were also collected from the sampling ports along the depth of the reactors on days 300, 337, 387, 475, and 538 (referred to as profile samples). Liquid samples were filtered through 0.22 µm filters (Fisher, Pittsburgh, PA), and stored at 4^oC until acetate, sulfate, nitrate, nitrite, chloride, total arsenic, and total iron concentration analyses could be run, typically within 48 h. Samples for total arsenic and total iron were acidified to a final concentration of 0.02 N HCl before storage.

A variety of methods were used to monitor changes in the various constituents in the reactor system. The DO levels in the influent and effluent of reactor A were measured directly in the inlet and outlet lines of reactor A using WTW multi340 meters with CellOx325 sensors in WTW D201 flow cells (Weilheim, Germany). The detection limit for DO was 0.01 mg/L. Anionic species concentrations (i.e., acetate, chloride, nitrite, nitrate, and sulfate) were

determined using an ion chromatography (IC) system (Dionex, Sunnyvale, CA) consisting of an AS-14 (Dionex, Sunnyvale, CA) column with an AG-14 guard column (Dionex, Sunnyvale, CA) and a Dionex DX 100 conductivity detector. The IC eluent contained a mixture of ACS reagent grade 1 mM bicarbonate and 3.5 mM carbonate. The detection limit for each of the anions was 0.2 mg/L. Total arsenic and total iron were measured using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer ALEN DRC-e, Waltham, MA). The detection limit for total arsenic and total iron was 2 µg/L As_T and 0.1 mg/L Fe_T, respectively.

Biomass Collection and Nucleic Acids Extraction. In order to monitor changes in TEAP zone microbial populations, biomass profile samples were collected on days 300, 337, 387, 475, and 538. To accomplish this, several BAC particles were removed from the sampling ports along the depth of the reactors, flash-frozen, and then stored at -80^{o}C until subsequent processing steps were performed. Subsequent steps included quantification of DNA and RNA. Genomic DNA was extracted from the stored biomass samples following a phenol-chloroform extraction protocol (Chapter 4). DNA was quantified using a NanoDrop ND1000 (NanoDrop Technology, Wilmington, DE) and stored at -20 ^{o}C. RNA was isolated from the flash-frozen biomass samples using a hot-phenol-chloroform extraction protocol [16] and was quantified using NanoDrop ND1000 (NanoDrop Technology, Wilmington, DE). RNA quality was evaluated using Experion Automated Electrophoresis unit (Life Science, Ca), and RNA was stored at -80 ^{o}C.

Quantitative Real Time PCR. To determine the amount of sulfate reducing microbial populations present in the bioreactors, the abundance of (bi)sulfite reductase (*dsrAB)* gene from sulfate reducing bacteria (SRB) was quantified by qPCR using primers DSR1F+ (5'-ACSCACTGGAAGCACGCCGG-3') and DSR-R (5'-GTGGMRCCGTGCAKRTTGG-3') [17]. Details of PCR reactions and thermal cycles are given in Chapter 4. Melting temperature profiles were collected to determine the specificity of the amplification. Purified *E. coli* plasmid DNA containing a 221 bp fragment of the *dsrA* gene from *Desulfovibrio vulgaris* was used to generate a standard curve from triplicates of a 10-fold dilution series ranging from 10^4 to 10^9 copies/µL.

Similarly, the abundance of dissimilatory arsenate reducing bacteria (DARB) was determined using qPCR targeting the arsenate respiratory reductase (*arrA*) gene. As described in Chapter 4, two distinct clusters of DARB were present in the reactor system based on a clone library generated from an approximately 628 bp fragment of the *arrA* gene. While cluster II was closely associated with *Geobacter uraniireducens*, cluster III was determined to be only distantly related to *Alkalilimnicola ehrlichii*. The abundance of these two clusters of DARB was evaluated by qPCR experiments using the primer sets GArrAF (5'-CCCGCTATCATCCAATCG-3') and GArrAR (5'-GGTCAGGAGCACATGAG-3') (cluster II) and EArrAF (5'-CATCGCTTCTCGCTGTG-3') and EArrAR (5'-GAGGTAGTTGCAGTTTCG-3') (cluster III). Details of PCR reactions and thermal cycles are provided in Chapter 4. Amplification specificity was verified by collecting melting profiles after the amplification. Standard curves were

generated from triplicates of a 10-fold dilution series of purified *E. coli* plasmids containing an approximately 628 bp fragment of the *arrA* genes of clones 62 (cluster II) and 34 (cluster III), respectively, from the clone library (Chapter 4)

Reverse Transcriptase Quantitative Real Time PCR. Reverse transcriptase (RT) qPCR experiments were performed to elucidate the sulfate reducing bioactivity along the depth of the reactors. Reverse transcription was performed to generate cDNA of the partial *dsrA* transcripts from DNase treated RNA extracts and subsequent PCR amplification were performed as described in Chapter 4.

5.4 Results

Reactor performance. Concentration data were monitored to assess the effectiveness of nitrate and arsenic removal and the stability of reactor performance in terms of removal amounts and final effluent concentrations. These data were also collected to determine if the EBCT could be lowered to change the location of the sulfate reducing TEAP zone without compromising the stability or levels of removal. For the first 300 days, the total EBCT was maintained at 40 min. Except for the initial startup time and during changes to influent concentrations, the reactor performance was generally quite stable. During the time reported here, DO in the influent (inf) and the effluent from reactor A (EA) remained at 0.37±0.37 (mean ± standard deviation) mg/L and below detection, respectively, a stable pH was established in the system, and the pH in the effluents from reactor A and reactor B averaged 7.2±0.2.

In addition to changes in the EBCT, changes in the influent concentrations of nitrate and arsenic were evaluated. The influent concentration of nitrate was increased from approximately 50 to 70 mg/L from days 429 to 466 and the influent concentration of arsenic was lowered from approximately 300 to 200 µg/L starting on day 448. The results of these influent concentration changes are discussed below in the context of the EBCT analysis.

To illustrate the stability of the reactor performance, influent (inf), EA, and EB concentration data for nitrate, sulfate, and arsenate have been converted into the amount removed in each reactor, while the influent concentrations for these compounds are also reported in Figure 5.1. As seen in Figure 5.1, through day 300, complete denitrification was observed in the first reactor, i.e., the influent nitrate was removed to below the detection limit of 0.2 mg/L in the effluent from reactor A. Reactor A also consistently removed 10.8±3.6 mg/L SO_4^{2-} and 243±54 µg/L As. Additional sulfate reduction in reactor B resulted in a stable removal of 7.8±2.3 mg/L SO_4^{2-} but only 26±14 µg/L As, since most arsenic was already removed in reactor A.

To attempt to shift more sulfate reduction and arsenic removal to reactor B, the EBCT of reactor A was lowered to 15 min (total EBCT= 35 min) on day 300. At this EBCT, complete nitrate removal was still achieved in reactor A (Figure 5.1). As desired, the sulfate reduction was shifted more to reactor B with only 4.5±2.3 mg/L sulfate reduced in reactor A. This also shifted some of the arsenic removal to reactor B with only 141±58 µg/L As removed in reactor A during days 301-337. Additional sulfate reduction in reactor B resulted in

16.2±3.9 mg/L SO_4^{2-} and 255±20 µg/L As removal across the system. These average values were calculated excluding the periods for days 315-318 when the influent lacked sulfate and for days 323-327 when the influent contained 14.2±0.3 mg/L SO_4^{2-} (both accidental changes due to operator error). Arsenic removal was also adversely impacted during days 315-318 (Figure 5.1).

Further lowering of the EBCT in reactor A to 10 min (total EBCT= 30 min) on day 337 resulted in a further decrease of sulfate removal in reactor A. During days 337-387, Reactor A removed 2.7±1.4 mg/L SO_4^{2-} and 112±34 µg /L As, while complete denitrification occurred in reactor A. The total sulfate and arsenic removal across the filter beds were 18±4 mg/L SO_4^{2-} and 252±18 µg/L As, respectively.

On day 387, the EBCT of reactor A was lowered to 7 min resulting in a total EBCT of 27 min. Nitrate was still completely removed in reactor A through day 427. Improved reactor performance (22.4±3.6 mg/L SO_4^{2-} and 272±18 µg/L As removal) was observed across the system during this period, while reactor A removed 3.9±1.4 mg/L SO_4^{2-} and 110±22 µg/L As. On day 428, the nitrate concentration was increased by 1/3 and maintained at 69.7±1.8 mg/L NO_3^- through day 466. During this period, denitrification in reactor A was incomplete with 20±6 mg/L NO_3^- leaving reactor A and entering into reactor B. Acetate consumption increased in reactor A (data not shown) due to increased nitrate concentration in the influent. In response to the presence of nitrate, sulfate reduction and arsenic removal declined across both reactors. After returning the influent nitrate concentration to 50 mg/L NO_3^- on day 467, total sulfate reduction

stabilized after day 470 at 16.6±2.1 mg/L SO_4^{2-}, but never fully rebounded to previous removal levels. Given the negative impact of increasing nitrate concentrations on arsenic levels in the final effluent (see Figure 5.1, days 428-450), the influent arsenic concentration was reduced from ~300 µg/L to ~200 µg/L As on day 450. This lowering did not have apparent impact on overall arsenic removal across the system. Given the sensitivity of reactor performance to substantial changes in the level of nitrate, we note that EBCT optimization ideally takes place during relatively stable influent nitrate levels. Nonetheless, the EBCT of 27 min appears to have slightly diminished the ability of the reactor system to lower As concentration values in the effluent, even when the influent concentration of As was lowered by 1/3. This appears to be related to the less complete sulfate reduction achieved across the reactor system at this shorter EBCT.

After the bed material in reactor A was replaced on day 517 (EBCT 30 min), efficient nitrate removal was still observed in reactor A. Sulfate reduction in reactor A remained relatively low for several days as did arsenic removal and removal of both declined until day 522 (data not shown). With time, however, significant arsenic removal was once again observed in reactor A even though overall sulfate reduction remained low in reactor A. From day 523 to 555, 1.91±1.1 mg/L SO_4^{2-} and 124±21 µg/L As removal was observed across reactor A, comparable to that achieved in reactor A during the first test at an EBCT of 30 min from days 337 to 387. After each biomass collection and subsequent lowering of the EBCT on days 300, 337, 387, and 517, sulfate reduction

remained low for a couple of days, probably due to oxygen exposure, even though arsenic removal was not impacted to the same extent.

In general, the EBCT analysis suggested that good arsenic removal could be achieved down to an EBCT of 30 min, and that by decreasing the EBCT in reactor A, most of the sulfate reduction could be shifted to reactor B. However, it was not possible to shift arsenic removal to the same extent, with nearly 50% of arsenic continuously being removed in reactor A, regardless of the EBCT or levels of arsenic or nitrate. This inability to shift arsenic removal primarily to reactor B may, in part, be a result of having sufficient sulfate reduction in reactor A to facilitate arsenic removal, keeping in mind that even 1 mg/L (~10^{-5} M) reduction of sulfate provides excess sulfide relative to the total arsenic of 300 μg/L (~4.0×10^{-6} M).

Chemical Profiles along the Bed Depths. Liquid profile samples were taken to evaluate the impact of EBCT on the TEAP zones within reactors A and B. In particular, we were interested in confirming that changes in the EBCT would shift the active sulfate reducing zone primarily to reactor B. The chemical profiles (Table 5.2) illustrate more directly how the change in the EBCT of reactor A shifts the TEAP zones in both reactors. For example, nitrate was below detection at port A6 in reactor A when the EBCT was 40 min (day 300) and 35 min (day 337). However, 24.7±0.1 mg/L NO_3^- was still measured at this port at the EBCT of 30 min (day 387). When the EBCT was 27 min (day 474), nitrate was below detection at port A8, indicating complete nitrate removal was still possible even

with a 7 min EBCT in reactor A. On day 538 (EBCT 30 min), nitrate was still below detection at port A8 indicating complete nitrate removal in reactor A, which was little impacted by EBCT changes over the course of this study.

Similarly, shifts in the sulfate reducing zone were noted with changes in the EBCT, although the trends are not completely consistent. At the EBCT of 40, 35, and 30 min (day 387), sulfate removals in reactor A were 11.4±0.3, 2.2±0.2, and 5.6±0.2 mg/L SO_4^{2-}, respectively. When the EBCT was 40 min, 35 min, and 30 min (day 387), 2.4±0.3, 0.9±0.5, and 0.2±0.1 mg/L SO_4^{2-}, respectively, were removed within the filter bed before the first sampling port. It is not clear why the least sulfate removal in reactor A occurred for an EBCT of 35 minutes, however, this may be related to the timing of the backwashing cycles compared to our sampling events rather than significant changes caused by EBCT changes. When the EBCT was further lowered to 27 min, sulfate reduction in reactor A (5.6±0.2 mg/L SO_4^{2-}) was not significantly different ($p<0.05$) than that at the first test of the EBCT of 30 min (5.7±0.2 mg/L SO_4^{2-}) started on day 337. However, when the reactor was returned to a 30 min EBCT, the chemical profile samples (Table 5.2) from day 538 indicated that most of the sulfate reduction occurred in reactor B, with ~1 and ~17 mg/L of SO_4^{2-} removed by reactors A and B, respectively. The filter bed prior to the first sampling port (A8) on day 538 did not remove any sulfate, in contrast to the consistent removal observed at the first sampling port during the previous EBCT conditions. One noted difference, however, was that 66% of the BAC had been changed on day 517, and it is

possible that the biofilm was not fully developed in the upper part of the column to support sulfate reduction.

Chemical profile samples also indicated that total arsenic removal did not seem to track the changing TEAP zones for nitrate or sulfate reduction with close to 50% of As removed in reactor A, regardless of the EBCT. Rather the removal of arsenic, while dependent on sulfate reduction and production of sulfide, appears to also depend on other factors (not reported here) related to its removal mechanism by iron sulfide solids (Chapter 3, [1], and Chapter 7)

Overall the chemical profile results confirm that most of the sulfate reduction could be shifted to reactor B by lowering the EBCT, although complete isolation of sulfate reduction and arsenic removal to reactor B could not be achieved, even at the lowest EBCT of 27 min.

Relative Abundance and Activity of Sulfate Reducing Bacteria. Biomass profile samples were collected to evaluate the impact of EBCT on the sulfate reducing populations along the length of reactors A and B (Figure 5.2; note that with decreasing EBCT, the packed-bed height decreases and fewer ports are located within the bed), The abundance of SRB, expressed as the copies of the *dsrA* gene normalized to mass of DNA, indicated that SRB were more or less equally distributed across the BAC filter beds for a given EBCT while the abundance varied across the EBCTs evaluated. For example, the abundance of SRB differed by more than an order of magnitude between the EBCTs of 40 min and 35 min. SRB abundance throughout the reactor system was the least when

the EBCT was maintained at 27 min. After re-adjusting the EBCT in reactor A to 10 min (total EBCT=30 min), enhanced growth of sulfate reducing populations was observed again and SRB were more or less equally distributed throughout the reactor system.

Regardless of the EBCT evaluated, the sulfate reducing activity, expressed as the *dsrA* transcripts normalized to total mass of RNA, attained a maximum value at the centre of the total bed depth (the total filter bed in both reactors) and declined towards both ends of the reactor system from this central location (Figure 5.2). Sulfate reducing activity tracked well with the sulfate concentration profile along the depths of the reactors. In particular, in regions where sulfate concentrations were found to decrease the most, the SRB activity was maximized. For example, when the EBCT was 40 min, port A8 in reactor A showed the maximum SRB activity near the vicinity between A7 and A8 where the maximum gradient in sulfate concentration decrease was observed (Table 5.2, note that the table provides the different sulfate concentrations at each port). Similarly, the SRB activity between port A6 in reactor A and port B2 in reactor B, although relatively high, tapered off from the maximum value in agreement with the general trends of the slightly lower sulfate concentration changes from one port to the next in these regions. When the EBCT was 35 min, SRB activity was mainly centered in the region between ports A8 and B3 with the maximum activity being observed at port B1 in reactor B, again near the maximum sulfate concentration change region. At this EBCT, most of the sulfate removal occurred within the filter bed between ports A8 and B3. Similarly, higher SRB activity was

observed in the filter bed between port A8 in reactor A and B3 in reactor B when the EBCT was 30 min; however, the maximum sulfate reducing activity was shifted to port B2. At this EBCT, again most of the sulfate removal occurred between ports A8 and B3. In contrast, when the EBCT was 27 min, the maximum activity appeared to be in ports B1 and B4 with less activity in between these ports. This different trend at the lowest EBCT suggests that a different SRB population may be responding at B1 under the selective advantage afforded by the decreasing EBCT, while the maximum seen at port B4 is consistent with the general shift in SRB activity to later sampling ports with EBCT decrease. When the EBCT was returned to 30 min, the activity profile of SRB along the depth of reactor followed the general trend of maximum activity close to the centre of the system. As these results show, lowering the EBCT tended to shift the maximum SRB activity increasingly from reactor A to B.

Relative Abundance of Arr*A*. The changes in EBCT also impacted the abundance of arsenate reductase. Out of the two clusters identified in the phylogenetic tree of ArrA (Chapter 4), the abundance of the ArrA from clones distantly related to *A. ehrlichii* (cluster III) was higher regardless of the EBCTs evaluated. Interestingly, relatively lower abundance of DARB was observed throughout the reactor system at the EBCT of 35 and 27 min. Though a consistent trend of the abundance of the ArrA was not observed at the EBCTs evaluated, better arsenic removal was observed when the ArrA was present in significant numbers throughout the reactors with a maximum abundance located towards the early part of the system. For example, the ArrA was more abundant

in ports A5 and A6 during the EBCT of 40 min and 30 min (day 538) (Figure 5.3) when arsenic removal was relatively better. At the EBCTs of 35 and 27 min, lower abundance of the ArrA was observed when arsenic removal was relatively lower.

While it is difficult to attribute any particular cause and effect to the relative abundance numbers at given location points, it is noteworthy that arsenic reducers were present throughout the reactor. Given that arsenate reduction is an essential step for the removal of arsenic by sulfide solid formation, the principal removal pathway in this reactor system [1], the presence of a sufficient population of arsenic reducers is expected to be key to optimal reactor performance. Additional work is needed to characterize the activity of arsenic reducers to determine how they may be responding to changes in reactor conditions and where the most effective arsenate TEAP zones may be located.

5.5 Discussion

The operation of two fixed-bed bioreactors, operated in series, was modified to attempt to promote arsenate and sulfate reduction in the second reactor, while dedicating the first reactor for the reduction of dissolved oxygen (DO) and nitrate. Accordingly, reactor A was expected to exhibit relatively high microbial growth and greater biomass compared to reactor B due to the availability of more thermodynamically favorable electron acceptors (i.e., DO and nitrate). Built on previous experience with a nitrate and perchlorate removing bioreactor [7], the buildup of biomass in reactor A was anticipated to require

backwashing every 48 h. At the same time, due to the limited growth corresponding to sulfate reduction in reactor B, less frequent backwashing (every 3-4 months) was estimated. The generation of sulfides in reactor B was envisaged to (i) provide the needed sulfide for iron sulfide precipitation and sorptive removal of As(III), and (ii) minimize the volume of backwash waste that contains arsenic.

At a total EBCT of 40 min, significant sulfate reduction and consequent sequestration of arsenic from the liquid phase occurred in reactor A. Given that reactor A was backwashed every 48 h, arsenic precipitated or co-precipitated along with the iron sulfides was also removed from reactor A, although this was not confirmed experimentally. To avoid generation and subsequent washout of arsenic containing sludge in reactor A, the EBCT was lowered in an attempt to confine sulfate reduction primarily to reactor B. Lowering the total EBCT to 30 min effectively moved nearly 95% of the sulfate reducing TEAP zone to reactor B, with only 1 mg/L out of 21 mg/L available SO_4^{2-} reduced in reactor A. Yet, this limited amount of sulfate reduction produced sufficient sulfide (i.e., in excess of the molar amount of arsenic) for substantial removal of arsenic in reactor A. Although it is conceivable that an even lower EBCT than those reported here could shift the sulfate reducing zone entirely to reactor B, it may not be feasible to do so while still achieving complete nitrate removal in reactor A. Additional strategies for future work include determining whether changes in the primary electron acceptors (i.e., DO or nitrate) may allow for inhibiting arsenic removal in reactor A, or changing flow rate rather than bed depth to cause wide separation

of TEAP zones. Even with the lack of complete success in shifting arsenic removal entirely to reactor B, the waste generated in reactor A for backwashing may be manageable given that arsenic levels in U.S. soils range from 1 to 40 ppm (parts of arsenic to one million parts of soil) with an average of 5 ppm [18]. This result also points to the need to evaluate a single column reactor system, given the advantages anticipated for the dual column system may not be realized.

As this work has demonstrated, the reactor systems under investigation is capable of sequentially utilizing DO, nitrate, arsenate, and sulfate as the electron acceptors at all the EBCTs evaluated (Table 5.2). Efficient nitrate removal was observed within the upper part of the filter bed in reactor A. Even though arsenate reduction was not continuously monitored, arsenate was expected to be utilized as the next electron acceptor based on thermodynamic data [19, 20] under standard conditions and a pH of 7. Indeed, during days 50-60 of reactor operation (EBCT 40 min), arsenite was the predominant arsenic species in the effluent from reactor A (Chapter 4). The chemical profiles (Table 5.2) and the *dsrAB* activity analyses along the depth of the reactors (Figure 5.2) suggested that sulfate was consumed as the next electron acceptor after complete denitrification. Interestingly, arsenate reducing activity also increased after complete nitrate removal (Chapter 4). Given that biogenically produced sulfides react with arsenite and iron(II) resulting in the formation of arsenic and iron sulfides, [21-23], co-precipitation with and adsorption on iron sulfides or precipitation of arsenic sulfides are expected to be the primary arsenic removal

mechanisms in this reactor system. In fact, in the current system, such phases were found from solids collected from reactor B [1]. In further support of the sulfide based removal processes, when the influent (unintentionally) lacked sulfate during days 315-318, poor arsenic removal was observed (Figure 5.1) indicating that the generation of fresh sulfides in the system is crucial.

The arsenate reductase activity observed on day 300 indicated that arsenate reducing bacteria were active at and beyond port A7 in reactor A (Chapter 4) even though maximum abundance of the *arrA* genes was observed in ports A5 and A6 (Figure 5.3). Given that previously described DARB are not obligate arsenate respirers except strain MLMS-1 [24] and can use other electron acceptors such as DO, nitrate, Fe(III), and sulfate [25], the detection of *arrA* genes in the early part of reactor A suggests the presence of nitrate reducing bacteria that can utilize arsenate as an alternative electron acceptor.

Overall, this study has shown indirectly or directly that changes in EBCT impact the growth and positioning of denitrifying bacteria, SRB, and DARB along the depth of the reactors. The presence of both SRB and DARB in significant numbers and the co-location of sulfate and arsenate reducing activity in the presence of iron(II) are key for arsenic removal in the reactor system.

5.6 Conclusions

Our data show that nitrate and arsenic removal can be achieved under reducing environments utilizing a system consisting of two fixed-bed bioreactors in series and acetic acid as the electron donor. More than 90% arsenic removal

was achieved at a total EBCT as low as 30 min. Lowering the EBCT from 20 min to 10 min in the first reactor shifted the sulfate reduction zone almost entirely and a substantial portion of arsenic removal zone into the second reactor. Elimination of sulfate reduction and subsequent arsenic removal in the first reactor, however, was not achieved. Biomass and liquid profile samples collected showed that effective removal of arsenic was dependent on the presence of both DARB and SRB, and that their co-location in sufficient numbers was necessary for effective arsenic removal. Chemical profile and activity data suggested the presence of bacteria that can utilize multiple electron acceptors. Given the inability to shift all of the arsenic removal to the second reactor, future work should consider the possibility of using a single reactor system for the removal of arsenic with an EBCT greater than 10 min. For the present system and other variations, it will continue to be important to find ways to minimize the volume of arsenic-containing sludge collected during backwashing.

5.7 Tables and Figures

Table 5.1: *Composition of the synthetic groundwater fed to reactor A.*

Chemical	Concentration	Unit
$NaNO_3$	50/70	mg/L as NO_3^-
NaCl	13.1	mg/L as Cl^-
$CaCl_2$	13.1	mg/L as Cl^-
$MgCl_2.6H_2O$	13.1	mg/L as Cl^-
K_2CO_3	6.0	mg/l as CO_3^{2-}
$NaHCO_3$	213.5	mg/L as HCO_3^-
Na_2SO_4	22.4	mg/L as SO_4^{2-}
$Na_2HAsO_4.7H_2O$	0.3/0.2	mg/L as As
H_3PO_4	0.5	mg/L as P
$FeCl_2.4H_2O$[a,b]	6.0	mg/L as Fe^{2+}
CH_3COOH[a]	35.0	mg/L as C

[a] Added as concentrated solution through a syringe pump. The concentrations in the table represent the concentrations after mixing of the concentrated solution and the influent.

[b] In addition to the supplementation of $FeCl_2.4H_2O$ to reactor A, a concentrated solution of $FeCl_2.4H_2O$ was added to reactor B using a syringe pump to provide an additional 4 mg/L as Fe(II) to the system.

Table 5.2: *Chemical concentrations along the depth of the reactor beds.*

EBCT	Nitrate* Concentrations (mg/L)				
	40 min (d 300)	35 min (d 337)	30 min (d 387)	27 min (d 475)	30 min (d 538)
Inf	48.1± 0.1	46.3 ± 0.2	49.0 ± 0.1	44.0 ± 0.1	43.2±0
A5	7.9 ± 0.1				
A6	<0.2	4.2 ± 0.2			
A7	<0.2	<0.2	24.7 ± 0.1		7±0.1
A8	<0.2	<0.2	8.1 ± 0.1	<0.2	<0.2
EA	<0.2	<0.2	<0.2	<0.2	<0.2
B1	<0.2	<0.2	<0.2	<0.2	<0.2
B2	<0.2	<0.2	<0.2	<0.2	<0.2
B3	<0.2	<0.2	<0.2	<0.2	<0.2
B4	<0.2	<0.2	<0.2	<0.2	<0.2
EB	<0.2	<0.2	<0.2	<0.2	<0.2

*The detection limit for nitrate was 0.2 mg/L NO_3^-.

EBCT	Sulfate Concentrations (mg/L)				
	40 min (d 300)	35 min (d 337)	30 min (d 387)	27 min (d 475)	30 min (d 538)
Inf	21.5 ± 0.2	21.9 ± 0.1	29.0 ± 0.1	25.9 ± 0.2	22.5±0.2
A5	19.1 ± 0.1				
A6	18.9 ± 0.2	20.9 ± 0.5			
A7	14.2 ± 0.2	20.7 ± 0.4	28.8 ± 0.1		23.4±0.6
A8	11.8 ± 0.1	20.3 ± 0.1	27.1 ± 0.2	23.5 ± 0.2	23.8±0.8
EA	10.1 ± 0.1	19.7 ± 0.2	23.4 ± 0.2	20.1 ± 0.2	22.4±0.2
B1	7.8 ± 0.2	16.8 ± 0.3	21.2 ± 0.2	12.9 ± 0.1	15.3±0.4
B2	5.5 ± 0.1	15.4 ± 0.4	16.9 ± 0.1	8.5 ± 0.1	12.6±0.3
B3	3.7 ± 0.1	13.2 ± 0.4	12.0 ± 0.1	7.6 ± 0.1	6.9±0.1
B4	2.6 ± 0.1	10.4 ± 0.2	10.0 ± 0.1	7.9 ± 0.1	4.4±0.3
EB	1.1 ± 0.1	8.6 ± 0.3	5.7 ± 0.3	9.9 ± 0.1	4.2±0.1

EBCT	Arsenic Concentrations (µg/L)				
	40 min (d 300)	35 min (d 337)	30 min (d 387)	27 min (d 475)	30 min (d 538)
Inf	309 ± 11.8	291 ± 9.0	300 ± 1.0	196 ± 3.0	209±2.2
A5	302 ± 7.1				
A6	241 ± 1.2	268 ± 7.0			
A7	123 ± 0.4	255 ± 6.8	255 ± 4.4		215±5.6
A8	61 ± 0.3	203 ± 2.8	243 ± 3.6	158 ± 4.0	203±3.2
EA	48 ± 0.5	180 ± 5.1	142 ± 2.1	133 ± 4.0	107±0.3
B1	42 ± 0.7	159 ± 2.1	93 ± 1.2	75 ± 0.5	50±0.5
B2	32 ± 2.1	114 ± 1.1	53 ± 0.5	47 ± 1.2	41±1.2
B3	24 ± 1.2	90 ± 1.0	25 ± 0.1	41 ± 3.7	22±0.1
B4	22 ± 0.7	66 ± 0.9	19 ± 0.3	36 ± 0.1	22±0.6
EB	19 ± 0.7	36 ± 0.2	24 ± 0.5	47 ± 1.1	13±0.3

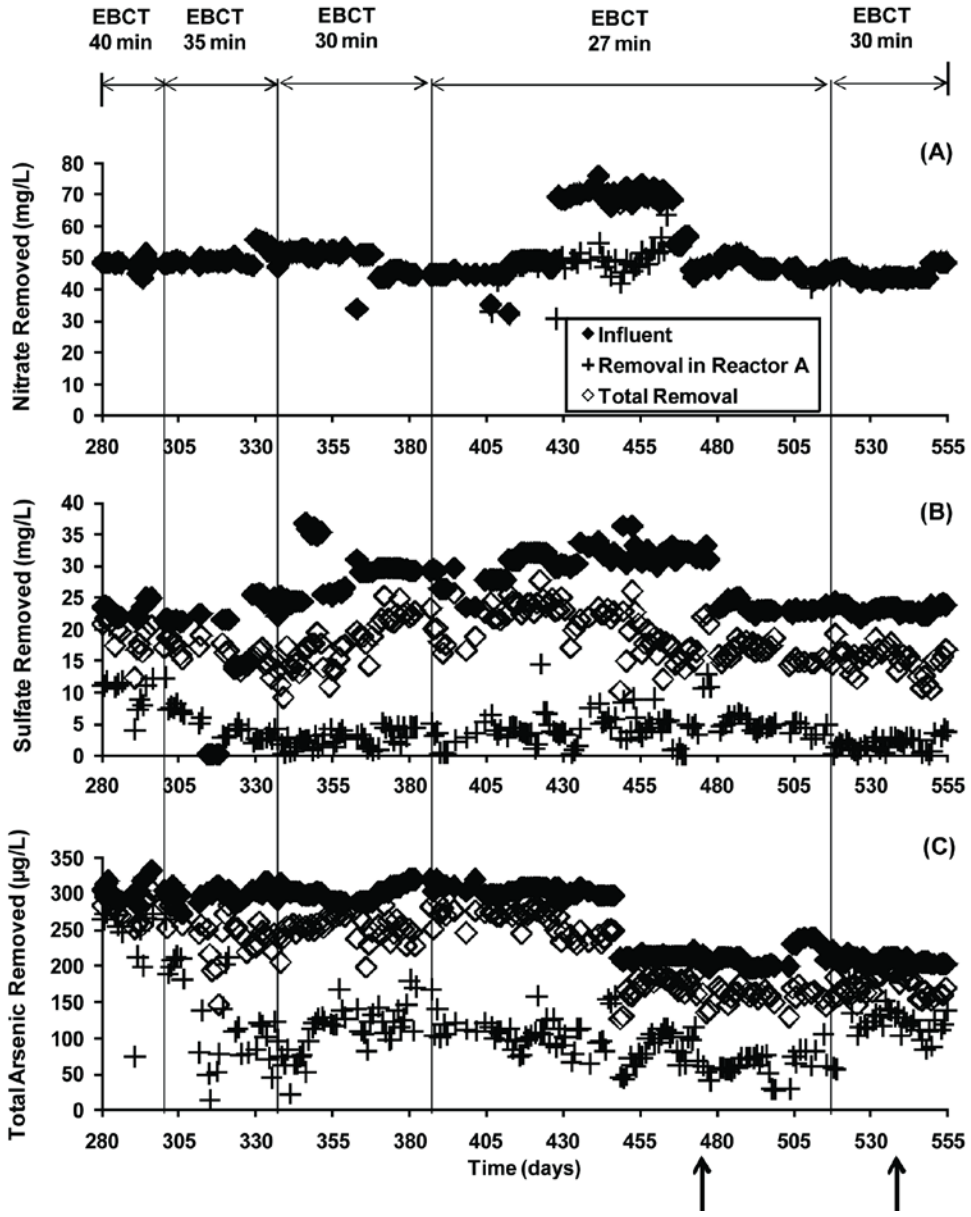

Figure 5.1: *(A) Nitrate, (B) sulfate, and (C) total arsenic removed in reactor A and across the system versus time of operation. Influent concentrations of nitrate, sulfate, and arsenic are also shown. The EBCT of reactor A was changed on day 300, 337, and 387 (marked by vertical lines). The EBCT of reactor B was maintained at 20 min throughout the experiment. On day 517, approximately 66% of the filter bed in reactor A was replaced with BAC particles from the same stock that was used for packing the reactor columns on day 0. Liquid as well as biomass profile samples were collected on the day of EBCT change (except day 517). The arrows indicate day 475 and 538 when additional chemical and biomass profile samples were collected.*

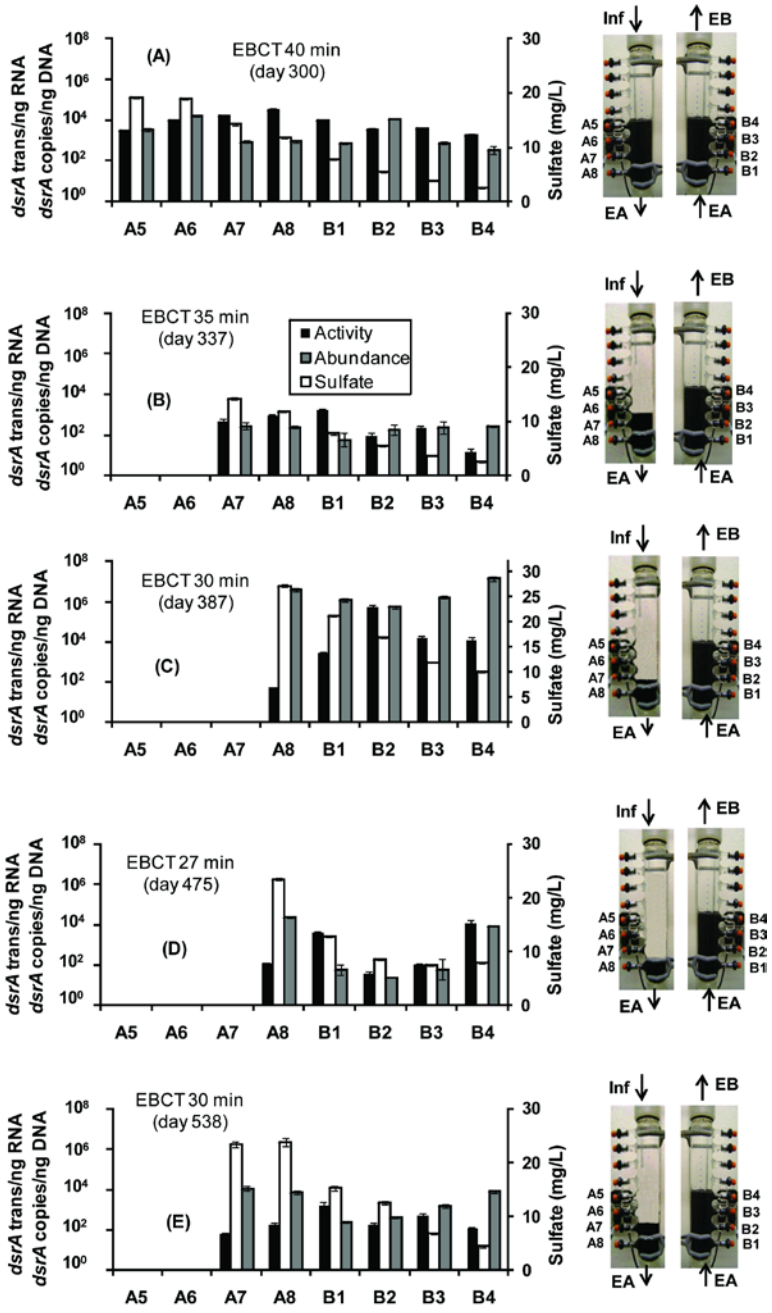

Figure 5.2: *Sulfate concentrations, abundance and activity of dsrAB along the depth of the filter beds on day 300 (A), day 337 (B), day 387 (C), day 475 (D), and day 538 (E). Abundance is expressed as the dsrA gene copies per ng of genomic DNA. The activity is expressed as the dsrA transcripts/ng of total RNA. A5-A8 and B1-B4 refer to the sampling ports along the depth of the reactor beds. Mean of three replicates are presented with error bars representing one standard deviation.*

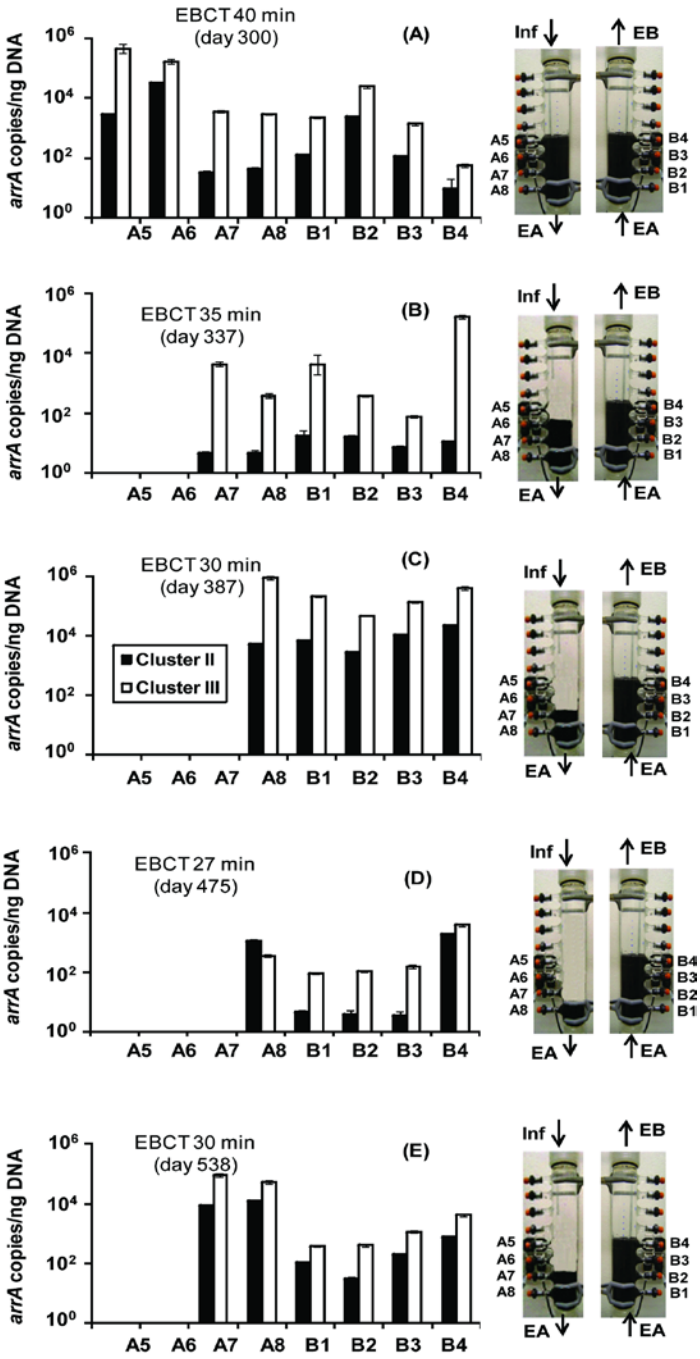

Figure 5.3: *Abundance of the arrA gene along the depth of the reactor beds on day 300 (A), day 337 (B), day 387 (C), day 485 (D), and day 538 (E). A5-A8 and B1-B4 refer to the sampling ports along the depth of the reactor beds. Mean of three replicates are presented with error bars representing one standard deviation.*

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Chapter 6

Effects of Nitrogen Gas-Assisted and Air-Assisted Backwashing on Performance of a Fixed-bed Bioreactor that Simultaneously Removes Nitrate and Arsenic

6.1 Abstract

Contaminant removal under reducing conditions conducive for the growth of denitrifying and sulfate reducing bacteria may require oxygen-free gas (e.g., N_2 gas) during backwashing of a fixed-bed bioreactor. However, replacing N_2 gas with air has practical advantages including ease of operation, and lower cost. A comparative study was conducted to evaluate whether replacing N_2 gas- with air during backwashing would provide equivalent performance in a nitrate and arsenic removing anaerobic bioreactor system that consisted of two biologically active carbon reactors in series. Gas-assisted backwashing, comprised of two minutes of gas injection to fluidize the bed and dislodge biomass and solid phase products, was performed in the first reactor (reactor A) every two days. Regardless of the gas phase used, 50 mg/L NO_3^- was removed within reactor A. In contrast, the final effluent arsenic concentration was between 10 to 20 µg As/L for air-assisted versus below 10 µg As/L when N_2 gas-assisted backwashing was

used. These results indicate that air-assisted backwashing can be implemented but has some impact on the overall effectiveness of arsenic removal.

6.2 Introduction:

Biofiltration has been successfully used in wastewater treatment over the years and is gaining popularity in drinking water treatment as well. In one of the embodiments of the biofiltration processes, fixed-bed bioreactors utilize support material, such as granular activated carbon (GAC) and sand particles for the growth of microorganisms. In a fixed-bed bioreactor, microorganisms accumulate on the support medium (Weber et al., 1978; Wilcox et al., 1983) through biomass growth (Hozalski and Bouwer, 1998) as biofilm or aggregates within the inter-particle spaces (Choi et al., 2007). A GAC system provides a large surface area per unit volume for biofilm growth, and is called a biologically active carbon (BAC) system when colonized by microorganisms (Wilcox et al., 1983). Establishment of a differential redox gradient across the filter bed in a fixed-bed bioreactor provides suitable microenvironments for the growth of a metabolically diverse microbial community that occupies subsequent layers within a biofilm and along the flow direction and ensures multiple contaminant removal in a single system (Upadhyaya et al., 2010). However, head loss increases due to retention of suspended particulates, biologically generated precipitates, and dead biomass, which eventually results in loss of productivity and product quality, and increased process costs. In addition, excessive bio-generation may compromise the biological stability of treated water due to sloughing off of microorganisms from the reactor (Chen et al., 2007). To

minimize these complications, fixed-bed bioreactor systems are routinely backwashed (Brown et al., 2005; Kim and Logan, 2000), usually with a combination of water and air (Amirtharajah, 1993).

Depending on water quality, bed material characteristics (size, density, and shape) (Cleasby et al., 1977), and the ability of microorganisms to be retained in the system (Hozalski and Bouwer, 1998), backwashing may help establish desired microbial populations, avoid proliferation of unwanted filamentous bacteria, and prevent preferential channel formation (Choi et al., 2007). While failure to remove deposited flocs may lead to deterioration of reactor performance as discussed above, over flushing of microorganisms can impact contaminants removal adversely (Brouckaert et al., 2006). Backwashing reduces microbial abundance and has the potential to change the microbial community structure (Kasuga et al., 2007). The studies cited above suggest that the effects of backwashing strategy on microbial community structure and overall reactor performance need to be evaluated for sustained and reliable contaminant removal in a fixed-bed bioreactor.

This study was implemented to evaluate the effects of N_2 gas- and air-assisted backwashing on the performance of a BAC reactor system that simultaneously removes nitrate and arsenic from a synthetic groundwater using acetic acid as the electron donor. Long-term monitoring as well as evaluations of reactor performance immediately after backwash events were carried out. Reactor performance was based on the ability of the system to maintain steady effluent concentrations and effective removal of the targeted contaminants.

6.3 Materials and Methods

Reactor System and Operation. Two biologically active carbon (BAC) reactors (reactors A and B) were operated in series as described by (Upadhyaya et al., 2010). A synthetic groundwater containing arsenic, nitrate, sulfate, and iron (composition given in Table 3.1, and (Upadhyaya et al., 2010)) was fed into Reactor A, operated in a down-flow mode, while the effluent from reactor A (EA) was introduced into reactor B in an up-flow fashion. Glacial acetic acid (35 mg/L acetate as carbon) fed along with 2 mg/L Fe(II) through a syringe pump (Harvard apparatus, Holliston, MA) served as the sole electron donor. To enhance the formation of iron sulfide, reactor B received an additional 4 mg/L Fe(II) (acidified to a final concentration of 0.02 N HCl) directly from the syringe pump until day 599, which was increased to 6 mg/L on day 600. Oxygen-free N_2 gas was bubbled through the influent every 24 h for 20-30 min to maintain dissolved oxygen (DO) less than 1 mg/L, which was further ensured by using a floating cover for the influent tank. Excess biomass and solids accumulated in reactor A were removed by backwashing the reactor every 48 h with a N_2 gas-assisted backwash (NAB) protocol as described below. A mixed flow of deoxygenated de-ionized (DDI) water (50 mL/min) and oxygen-free N_2 gas was passed through reactor A in up-flow mode for 2 min. Then DDI water was forced through the reactor in up-flow fashion at a flow rate of 500 mL/min for 2 min to remove dislodged biomass and solids deposited in reactor A. Reactor B was backwashed approximately every 3-4 months following the same protocol. During the period reported herein, reactor B was backwashed only once on day

632 (see below). Reactors A and B were operated with an empty bed contact time (EBCT) of 10 and 20 min, respectively.

Backwashing Experiment. Prior to the current comparative analysis study of N_2 gas- versus air-assisted backwashing, only NAB cycles were performed every 48 h. For this study, a baseline was established during days 590 to 622, in which reactor A was backwashed with the NAB protocol described above. On day 623 compressed air-assisted backwashing (CAB) was performed following the same protocol as in the NAB protocol except that compressed air replaced N_2 gas. From day 623 to 670, the CAB protocol was continued for backwashing of reactor A. In addition, reactor B was backwashed following the NAB protocol on day 632 to evaluate the impact of the removal of iron sulfides deposited in reactor B.

Liquid Samples Collection and Chemical Analyses. Liquid samples were collected from the influent tank (Inf), the first effluent from reactor A (EA), and the final effluent from reactor B (EB) every 24 h. Reactor performance immediately after the backwash of reactor A with the NAB and CAB protocols was evaluated by collecting effluent samples from both reactors at pre-determined time points after the backwash on day 605 and 623, respectively. In addition, effluent liquid samples and turbidity measurements were collected after the backwash on day 655. Liquid samples were also collected after the backwash of reactor B on day 632. Furthermore, liquid profile samples from the sampling ports along the depth of the reactors were collected on days 606 and 645.

Liquid samples, filtered through 0.22 µm filters (Fisher, Pittsburgh, PA) and stored at 4^{o}C, were measured for concentration of acetate, sulfate, nitrate, nitrite, chloride, total arsenic, and total iron within 48 h. Samples for total arsenic and total iron were acidified to a final concentration of 0.02 N HCl before storing.

Online measurement of DO at the inlet and outlet of reactor A was performed using WTW multi340 meters with CellOx325 sensors in WTW D201 flow cells (Weilheim, Germany). The detection limit for DO was 0.01 mg/L. In an ion chromatography system (Dionex, Sunnyvale, CA), chromatographic separation of acetate, chloride, nitrite, nitrate, and sulfate was achieved using an AS-14 (Dionex, Sunnyvale, CA) column attached with an AG-14 (Dionex, Sunnyvale, CA) guard column. A Dionex DX-100 conductivity detector was used to detect the anions. A mixture of ACS reagent grade 1 mM bicarbonate and 3.5 mM carbonate was used as the elution buffer. The detection limit for each of the anions was 0.2 mg/L. An inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer ALEN DRC-e, Waltham, MA) was used to determine total arsenic and total iron concentrations with a detection limit of 2 µg/L As_T and 0.1 mg/L Fe_T, respectively.

Biomass Collection. After collecting liquid profile samples on day 606, biomass profile samples were collected on the same day. To collect biomass samples from a sampling port, the reactor was drained up to the port and BAC particles were collected and transferred to four 2 mL screw-cap tubes using tweezers. The samples were then flash frozen in liquid nitrogen and stored at -80 ^{o}C. During the sample collection, reactors A and B were exposed to oxygen for

approximately 1 and 2 h, respectively. After sample collection, the bed volume in the reactors was readjusted by adding BAC particles (from the stock kept at 4 ^{o}C, which was initially used for packing the reactors at start-up).

6.4 Results

Reactor Performance. Reactor performance was evaluated during the backwashing study from days 590 to 670 by monitoring concentrations of electron acceptors and contaminants. Regular performance monitoring included determination of concentrations in liquid samples collected every 24 h. Chloride concentrations were monitored as a conservative tracer. Typically, performance was not evaluated immediately after backwashing reactor A. Average influent nitrate, sulfate, and arsenic concentrations were 48.9±1.5 (mean ± standard deviation) mg/L NO_3^-, 22.8±2.1 mg/L SO_4^{2-} and 213±6 µg/L As(V), respectively, during the period reported here. Dissolved oxygen in the influent remained below 1 mg/L at all times. The pH values in the effluent from reactors A and B averaged 7.1±0.2 and 7.0±0.2, respectively. Complete denitrification was observed in reactor A throughout the period despite upsets on day 606 (exposure to oxygen and significant biomass removal) and 619 (exposure to oxygen) (Figure 6.1). During days 590 to 606, arsenic concentrations in EA and EB averaged 26±7 and 9±1 µg/L As, respectively. The corresponding sulfate levels in EA and EB were 15.4±1.4 and 3.6±1.3 mg/L SO_4^{2-}, respectively.

Effluent samples collected immediately after backwashing reactor A following the NAB protocol on day 605 suggested minimal impact on reactor

performance (Figure 6.2). Immediately after backwashing the reactor, a dip in time profile of chloride, acetate, and sulfate was observed, especially in the EA (Figure 6.2). However, arsenic levels in the EA remained higher (mean value calculated for seven sample points was 32±5 µg/L As) than that before the backwash (mean value calculated for two sample points was 19±2 µg/L As). Chloride, acetate, and sulfate levels in the EA approached the concentrations prior to the backwash within 3-4 h. While sulfate levels in the EB mostly remained below detection (0.2 mg/L SO_4^{2-}) before and after the backwash; arsenic levels in the EB (11±3 µg/L As) were close to effluent arsenic concentrations prior to the backwash (10±0 µg/L As).

During biomass collection on day 606, both reactors were exposed to oxygen for 1-2 h. Although reactor A was not disturbed by oxygen exposure, reactor B was negatively impacted as arsenic was released from the solids deposited in the reactor (Figure 6.1). Specifically, arsenic in EA and EB were measured to be 18 and 420 µg/L As, respectively, on day 607. Adverse effects were also noticed on sulfate reduction, especially in reactor B (Figure 6.1). Arsenic removal in reactor A improved with time, while arsenic leaching from reactor B continued (arsenic concentration in EB > arsenic concentration in EA) until day 618. On day 619, the arsenic concentration in the final effluent (12 µg/L As) was equivalent to that from reactor A (13 µg/L As). Accidently, the reactors drained through the gas release system on day 619 and reactor B was again completely exposed to oxygen. The bed material in reactor B exhibited characteristic reddish yellow color of iron(III) hydroxides, presumably due to

oxidation of the deposited iron sulfides. This reverse flow and oxygen exposure of reactor B resulted in poor reactor performance (Figure 6.1) and, as expected, the impact was more pronounced in reactor B. However, the recovery was rapid compared to the earlier upset as arsenic in the final effluent (9 µg/L As) was less than that in the effluent from reactor A (19 µg/L As) on day 624 and then after. From day 624 to 632, while sulfate and arsenic in the EA remained 12.6±0.6 mg/L SO_4^{2-} and 20±7 µg/L As, respectively, 7.0±1.3 mg SO_4^{2-}/L and 12±4 µg/L As were measured in the EB.

Backwashing reactor B following the NAB protocol on day 632 did not impact overall arsenic removal (Figure 6.5), even though sulfate concentrations in the final effluent increased slightly. While arsenic in the EA remained 17±3 µg/L As, 10±1 µg/L As was observed in the final effluent after the backwash compared to that before the backwash (7±1 µg/L As). No dip could be detected in the time profiles of the anionic concentrations since the first data point was after 2 h.

N_2 gas was replaced with compressed air while backwashing reactor A on day 623, which was continued until day 670. Sulfate and arsenic levels in the EA and EB remained 12.5±1.5 mg/L SO_4^{2-} and 36±29 µg/L As, and 6.1±1.3 mg/L SO_4^{2-} and 20±7 µg/L As, respectively (Figure 6.1) during this period, except during the period with 15.4±0.1 mg/L SO_4^{2-} in the influent (days 664-670). During this low influent sulfate period, a correspondingly lower reactor B effluent concentration of 1.8±1 mg/L SO_4^{2-} was resulted.

Effluent samples collected immediately after backwashing reactor A on day 623 following the CAB protocol indicated that the overall reactor performance was re-established immediately after the backwashing even though the effluent from reactor A showed increased arsenic levels (Figure 6.4). A relatively narrower dip (spanning 2-3 h) in the time profile of chloride and sulfate levels in the EA was seen compared to that observed on day 605 using N_2 gas. The dip in the time profile of acetate was longer, however, and acetate concentration in the EA took approximately 6 h to return to near the value prior to the backwash, presumably due to the extended period of acetate consumption from oxygen utilization by aerobic microbial populations. Arsenic concentrations in the EA and EB after the backwash remained 21±4 and 11±2 µg/L As, respectively, compared to their respective levels of 9 and 11 µg/L As before the backwash.

In contrast to the observations from day 623, a prolonged impact on sulfate reduction and arsenic removal in reactor A was observed after the backwashing on day 655 (Figure 6.6). The dip in the time profile of chloride was very narrow; the concentrations in the EA reached that prior to the backwash within 2 h. However, acetate concentration in the EA fluctuated for some time before approaching a stable level after 14 h from the backwash. It also approached a level of near zero for several hours indicating a possible larger impact by aerobic microbial growth at this later stage. Interestingly, only a slight dip was observed in the time profile of sulfate in the EA, which attained a maximum level close to the influent concentration within 2 h from the backwash and gradually declined approaching a steady state at around 14 h. The time

profile of arsenic followed the trend of the sulfate profile. Despite the fluctuations in sulfate and arsenic concentrations in the EA, reactor B dampened the impact and final sulfate and arsenic attained a steady-state level within 3 h. Turbidity in the effluents increased immediately after the backwash (Figure 6.7). However, turbidity in the EA and EB was less than 2 NTU within 6 and 2 h, respectively, from the time of the backwash.

Chemical Profiles along the Bed Depths. Liquid profile samples collected on day 606 and day 645 suggest sequential uptake of the electron acceptors available in the system (Figure 6.3). Nitrate was below detection at sampling port A8 on both day 606 and 645 even though reduction was less complete at the earlier sampling port (A7) on day 606. Lower nitrate concentrations resulted in sulfate reduction, which was observed at port A7 on both the days. After complete removal of nitrate, sulfate reduction progressed along the flow direction in the reactors. Relatively, sulfate reduction was more in reactors A and B on day 645 than on 606, respectively. Both on day 606 and 645, arsenic removal followed the trend of sulfate reduction across the system with the final effluent (EB) concentration of 9 and 13 µg/L As on days 606 and 645, respectively.

6.5 Discussion

Anaerobic fixed-bed bioreactors may perform better and more consistently when backwashing is done with an oxygen-free gas in combination with backwash water. However, replacement of the oxygen-free gas with air would be more cost-effective and operationally easier. This may also be an important

consideration when exploring this treatment process for application in developing countries, where cost, operational complexity, and robustness determine whether a system can be adopted. In this study, we compared N_2 gas-assisted and air-assisted backwashing protocols in a BAC reactor system that consists of two bioreactors in series for simultaneous removal of nitrate and arsenic, which are regulated with a maximum contaminant level (MCL) of 50 mg/L NO_3^- and 10 µg/L As, respectively. The permissible level for arsenic in drinking water in the South East Asian countries, such as Bangladesh and Nepal is 50 µg/L As.

Establishment of diverse microbial populations (Chapter 4) resulted in sequential consumption of DO (not shown), nitrate, arsenate, and sulfate (Figure 6.3). Thermodynamic data suggest utilization of arsenate prior to sulfate reduction (Lovley and Phillips, 1988; Macy et al., 1996) under standard conditions at pH 7, which was reflected in arsenic speciation analyses (data not shown) performed occasionally. Regardless of the use of NAB or CAB protocol for backwashing, sulfate reduction started in the bed material above sampling port A8 in reactor A (Figure 6.3), even though faster sulfate reduction ensued after complete denitrification. This indicated an overlap of terminal electron accepting process (TEAP) zones utilizing nitrate and sulfate as the electron acceptors. Iron depletion along the flow direction followed the trend of sulfate reduction (Figure 6.4), presumably due to the formation of iron sulfides. Arsenic concentrations also followed the trend of sulfate and iron levels, suggesting that arsenic removal occurred through co-precipitation with or adsorption on iron sulfides (Kirk et al., 2010; O'Day et al., 2004) or due to bulk precipitation of

arsenic sulfides (Ledbetter et al., 2007; Newman et al., 1997). In fact, mackinawite (FeS) and greigite (Fe_3S_4) along with arsenic sulfides were detected in the solids collected from reactor B (Upadhyaya et al., 2010).

Regardless of the adoption of the NAB or CAB protocol for backwashing reactor A, arsenic concentrations in the effluent from reactor A immediately after the backwash were higher compared to those prior to the backwash (Figure 6.2 and Figure 6.4) but returned to levels similar to before the backwashing in a short time period. Also, the accumulated and freshly generated iron sulfides in reactor B led to further arsenic removal through adsorption and co-precipitation mechanisms resulting in lower and stable arsenic levels in the final effluent. While the prolonged practice of CAB assisted backwashing impacted sulfate reduction and subsequent arsenic removal in reactor A (Figure 6.6), reactor B compensated for the impact resulting in final effluent arsenic levels of 27±7 µg/L As.

The dip in the concentration time profiles of chloride, sulfate, and acetate, after the backwash on day 606 reflect the dilution effect of the backwashing with the de-oxygenated de-ionized water. As a conservative tracer, the dilution effect observed for chloride matches up reasonably well with that expected for the 490 cm^3 water within the reactor (approximately 49 min) at the influent flow rate of 10 mL/min. The longer duration of the recovery time for sulfate and acetate to return to pre-backwash levels reflect the impact of dilution and the delay in the re-establishment of the reduction processes. In the case of arsenic, the time profile did not show any decrease in arsenic concentration in the EA after the

backwash. It is likely that arsenic adsorbed to the previously deposited iron sulfides was released during the backwash due to abrasion and attrition of the solid particles. A dip in the time profiles of chloride, sulfate, and acetate were not seen after backwashing reactor B following the NAB protocol (Figure 6.5). This observation could be limited by the fact that the first sampling occurred 2 h after the backwash. The increased levels of sulfate in the EB were likely a result of the suppression of sulfate reduction or oxidation of previously deposited iron sulfides perhaps due traces of oxygen entering into the reactor during the preparation prior and after the backwashing.

The sulfate concentration in the EA after backwashing with the CAB protocol on day 623 (Figure 6.4) attained its level prior to the backwash within approximately 2-3 h, but equalization of acetate concentration took longer (approximately 6 h). Even though the DO was not monitored immediately after the backwash, it is highly probable that the DO level in reactor A increased due to the introduction of compressed air. Given that DO is thermodynamically preferred electron acceptor (Lovley and Phillips, 1988), as noted above microbial growth on DO may have resulted in the consumption of acetate. This is consistent with the delay in the achievement of pre-backwash acetate concentration levels. The difference in the time profile of chloride and acetate was more pronounced after prolonged practice of the CAB protocol (Figure 6.6) compared to the first backwashing cycle (Figure 6.4); e.g., chloride reached its pre-wash level within 1 h, while more than 6 h were required to achieve a steady-state acetate concentration. Furthermore, sulfate levels in the EA remained

higher than those prior to the backwash for an extended period compared to chloride, requiring approximately 10 h to return to near pre-wash levels. The oxidation of deposited iron sulfides due to the intermittent intrusion of oxygen may explain some of the increased concentration of sulfate. The presence of aerobic organisms and the low levels of acetate may also have led to the longer period of time before sulfate reduction returned to pre-wash levels.

Arsenic levels were not much impacted by CAB backwashing. It is likely that iron(III) oxy-hydroxides, which are very effective in sequestering arsenic (Farquhar et al., 2002; Gulledge and O'Connor, 1973), were generated in the system due to the oxidation of iron(II), keeping any arsenic sequestered upon oxygen exposure. Visual inspection and solids characterization through XRD (data not shown) did not confirm this. Either the low amount of iron solids generated compared to the biomass collected during backwash or the production of non-crystalline solids could explain the lack of XRD pattern for iron oxides. Given that iron(III) is energetically favorable (Lovley and Phillips, 1988) for microbial growth, it is also possible that iron(III) compounds, if present in the system, would have been rapidly reduced to iron(II) by iron reducing bacteria (Burnol et al.,2007; Papassiopi et al., 2003).

The microbial community in reactor A is expected to be dominated by denitrifying bacteria and many members of this group can utilize DO as an alternative electron acceptor. This might explain the undisturbed performance of reactor A observed after exposure to oxygen on day 606 during biomass sample collection. In contrast, reactor B took a substantially longer time before

stabilizing. A combined effect of the oxidation of iron sulfides, removal of substantial sulfate reducing bacteria (SRB) during sample collection, and slow growth of SRB could have resulted in the observed slight increase of arsenic leaching from reactor B following backwashing events (Figures 6.4 and 6.6). With time of operation, increased population of SRB in reactor B resulted in improved arsenic removal (Figure 6.1).

Given that the microbial community structure may change in response to the backwashing strategy (Kasuga et al., 2007), it is highly likely that a shift in microbial community occurred in the current system due to the shift in backwashing protocol. Intermittent availability of DO and possible generation of iron(III) hydroxides likely enhanced the growth of facultative aerobes/anaerobes and iron reducing bacteria in the system. However, the confirmation of this awaits an analysis of the microbial community structure changes that may have occurred compared to those found prior to this study as illustrated in Chapter 4. Future work will focus on revealing the microbial community structure through pyrosequencing and evaluating the population dynamics through qPCR and RT-qPCR. In addition, a backwashing strategy with a prolonged interval between two backwashes (4 days interval) will be evaluated. This may also allow for increased iron and arsenic solids to be generated in reactor A during the experiment so that X-ray techniques such as, X-ray diffraction, X-ray photoelectron spectroscopy, and X-ray absorption spectroscopy can be used to identify their composition and structure.

6.6 Conclusions

Backwashing of the fixed-bed bioreactor system described in this study did not impact arsenic and nitrate removal when N_2-assisted backwashing was used. Even though arsenic concentration in the final effluent slightly increased after prolonged compressed air-assisted backwashing, arsenic concentrations in the final effluent were below the permissible limit of arsenic in drinking water in the South East Asian countries indicating the viability of this option. Regardless of which backwashing strategy was implemented, nitrate removal was not impacted throughout the experiment. This study showed the feasibility of replacing N_2 by air for backwashing a nitrate and arsenic removing bio-reactor system under reducing environments, one which may be applicable for either developed or developing countries.

6.7 Tables and Figures

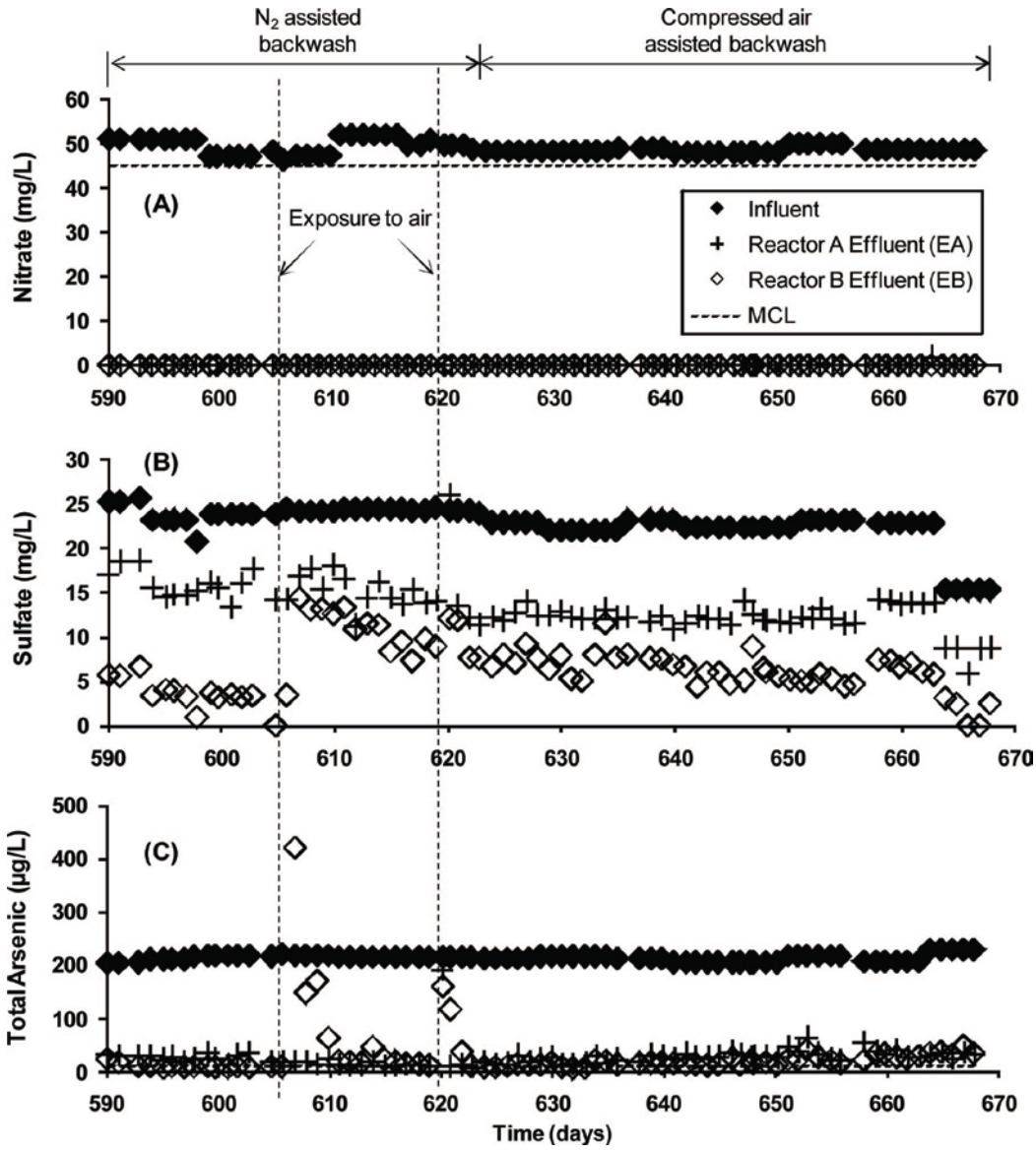

Figure 6.1: *(A) Nitrate, (B) sulfate, and (C) total arsenic concentrations in the influent, the effluent of reactor A (EA), and the effluent of reactor B (EB) versus time of operation. The EBCT was maintained at 30 min throughout the experiment.*

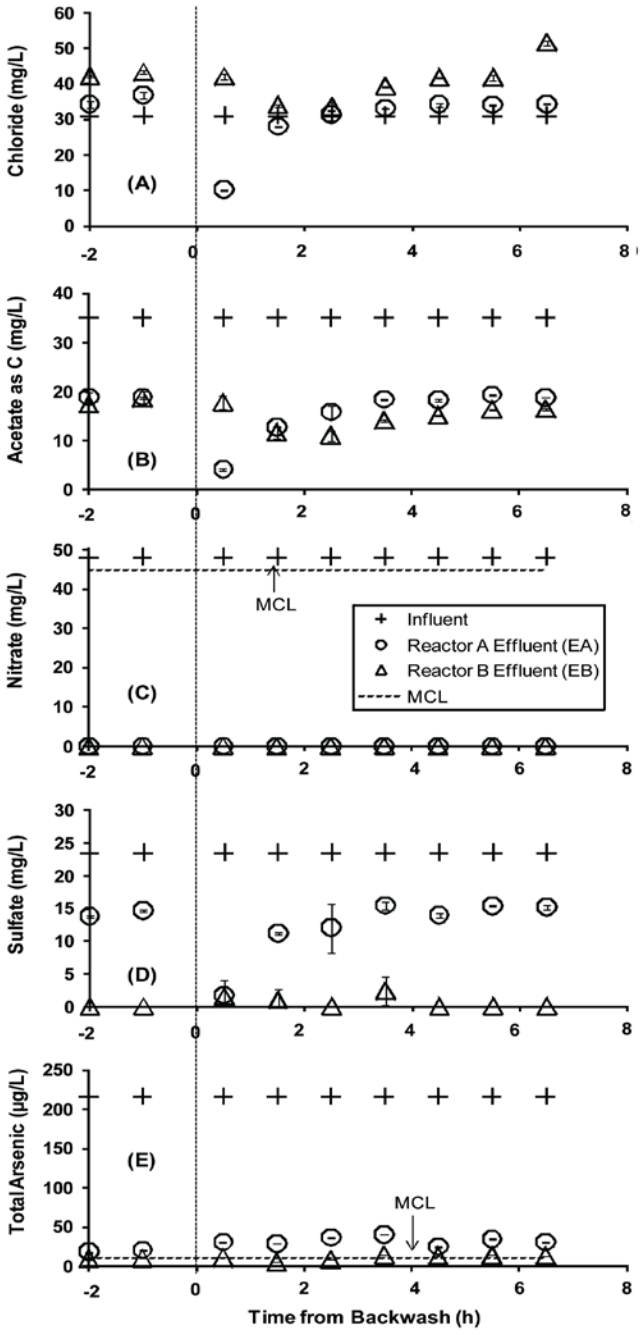

Figure 6.2: *Time profiles of (A) chloride, (B) acetate, (C) nitrate, (D) sulfate, and (E) total arsenic before and after the backwash of reactor A following the NAB protocol on day 605. The vertical line indicates the time of backwash of reactor A. Mean (n=3) values are presented with the error bars representing one standard deviation from the mean.*

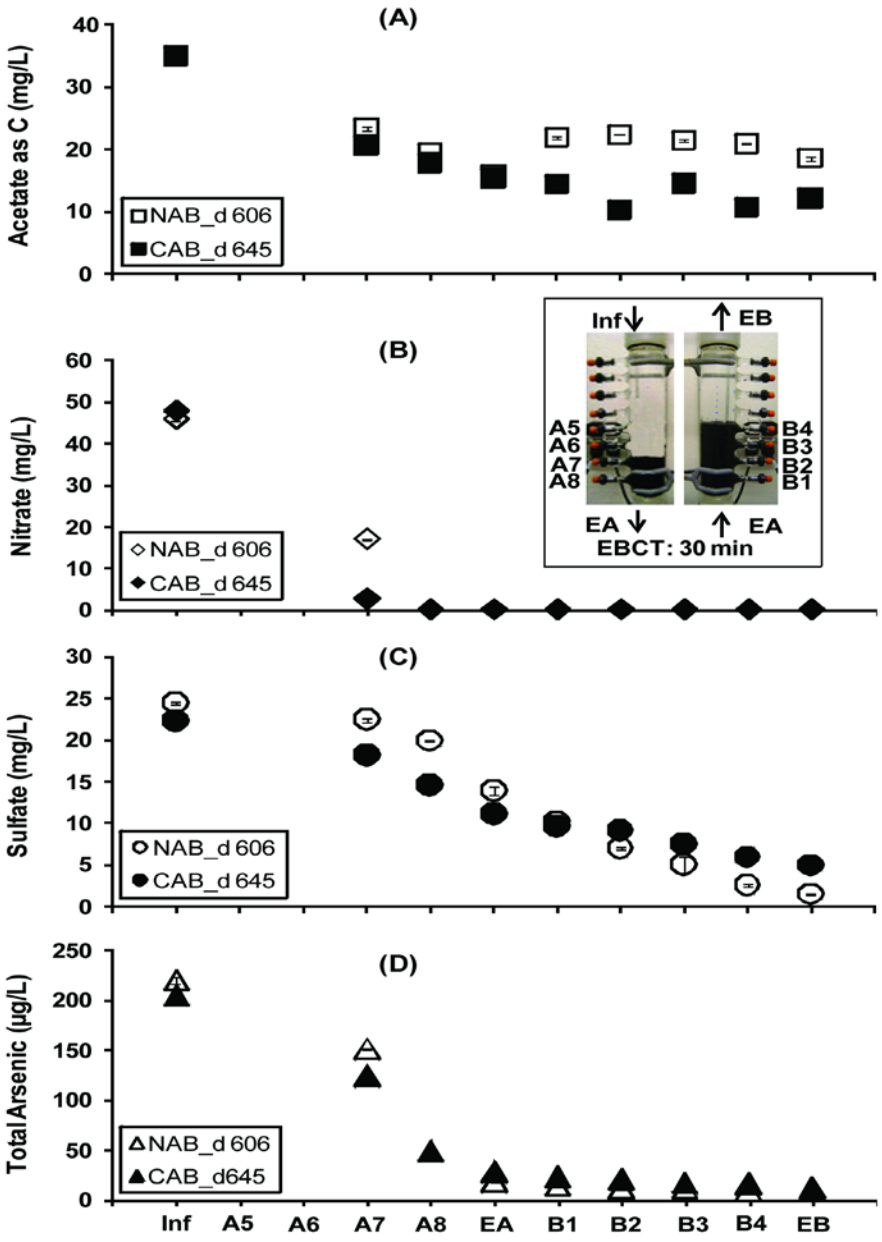

Figure 6.3: *Chemical profiles along the depth of the reactor beds on day 606 and 645. (A) Acetate, (B) nitrate, (C) sulfate, (D) total iron, and (E) total arsenic concentrations. Inf represents the influent concentrations, A7, A8, and B1-B4 represent the respective sampling ports along the depth of reactors A and B, respectively. EA and EB represent concentrations in the effluents from reactor A and reactor B, respectively. Mean (n=3) values are reported with the error bars representing one standard deviation from the mean.*

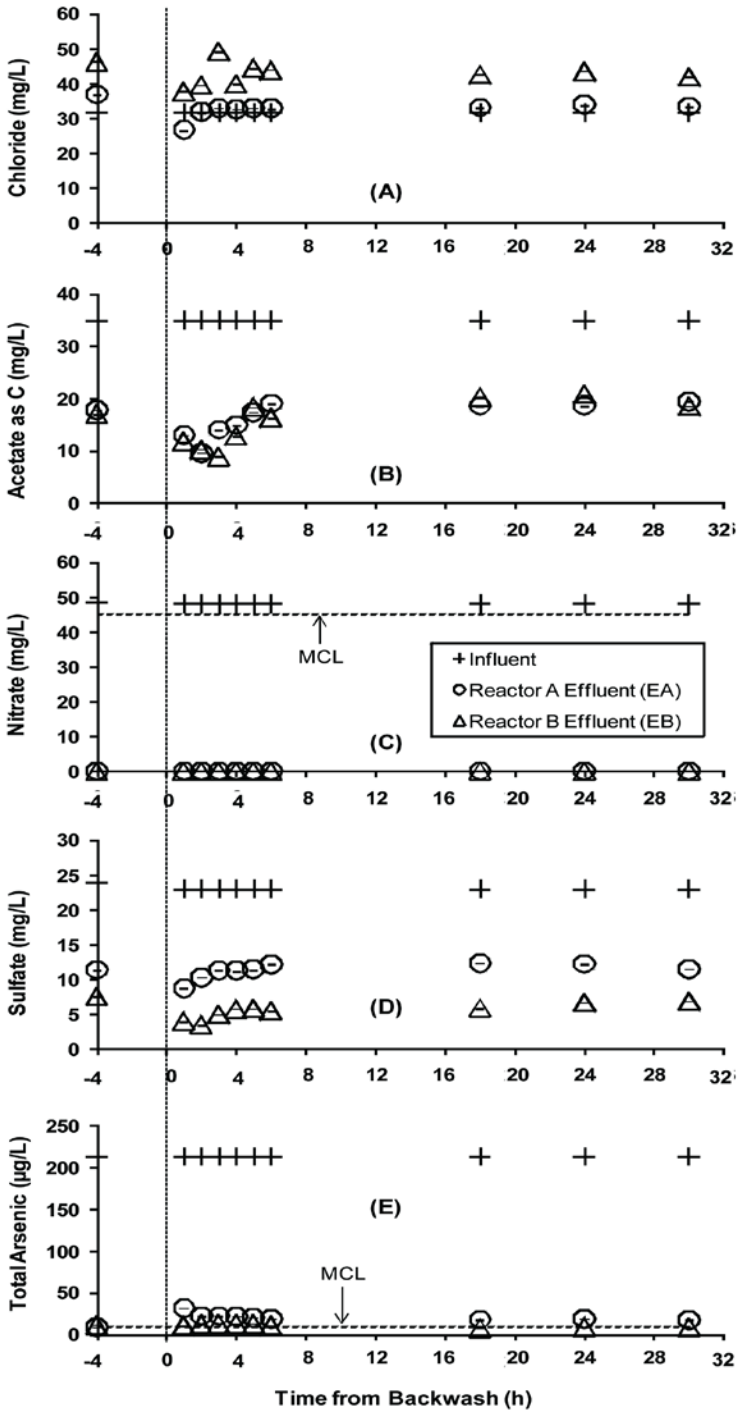

Figure 6.4: *Time profiles of (A) chloride, (B) acetate, (C) nitrate, (D) sulfate, and (E) total arsenic before and after the backwash of reactor A following the CAB protocol on day 623. The vertical line indicates the time of backwash of reactor A. Mean (n=3) values are presented with the error bars representing one standard deviation from the mean.*

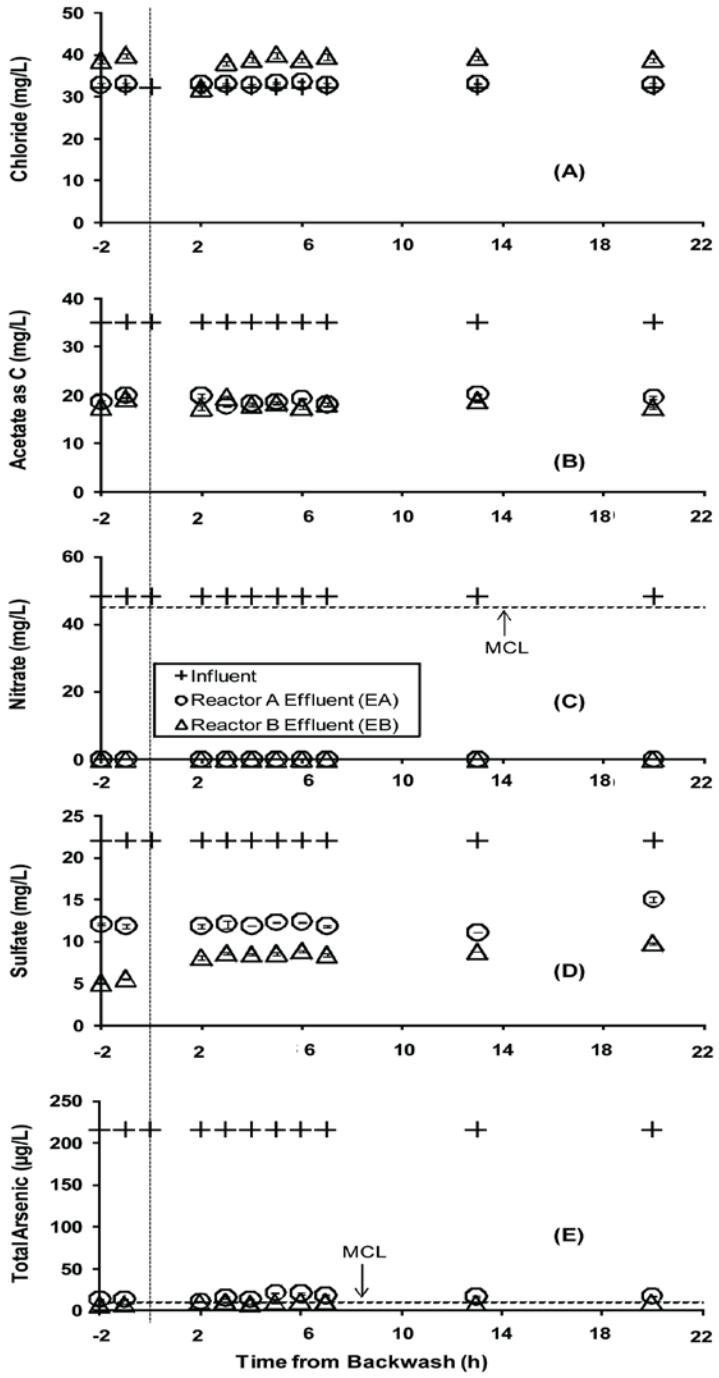

Figure 6.5: *Time profiles of (A) chloride, (B) acetate, (C) nitrate, (D) sulfate, and (E) total arsenic before and after the backwash of reactor B following the NAB protocol on day 632. The vertical line indicates the time of backwash of reactor B. Mean (n=3) values are presented with the error bars representing one standard deviation from the mean.*

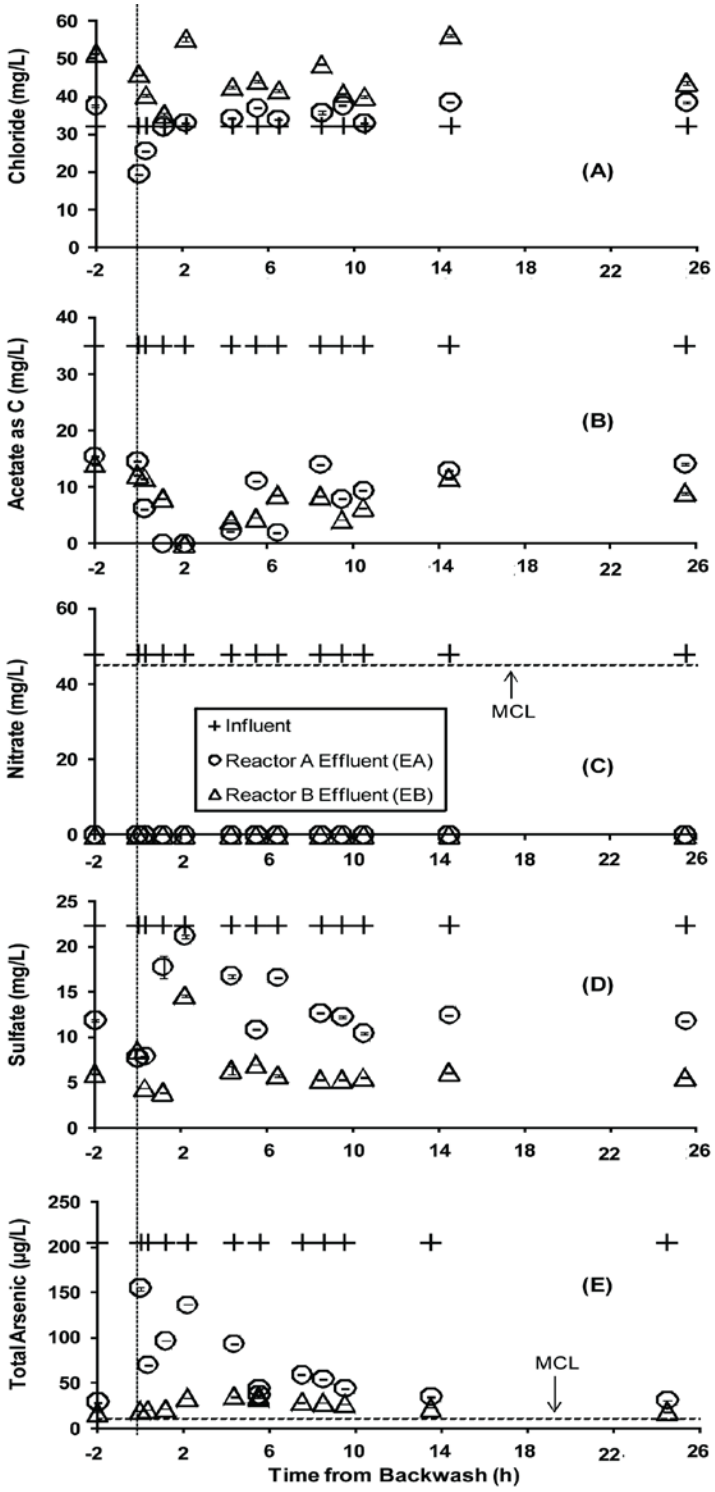

Figure 6.6: *Time profiles of (A) chloride, (B) acetate, (C) nitrate, (D) sulfate, and (E) total arsenic before and after the backwash of reactor A following the CAB protocol on day 655. The vertical line indicates the time of backwash of reactor A. Mean (n=3) values are presented with the error bars representing one standard deviation from the mean.*

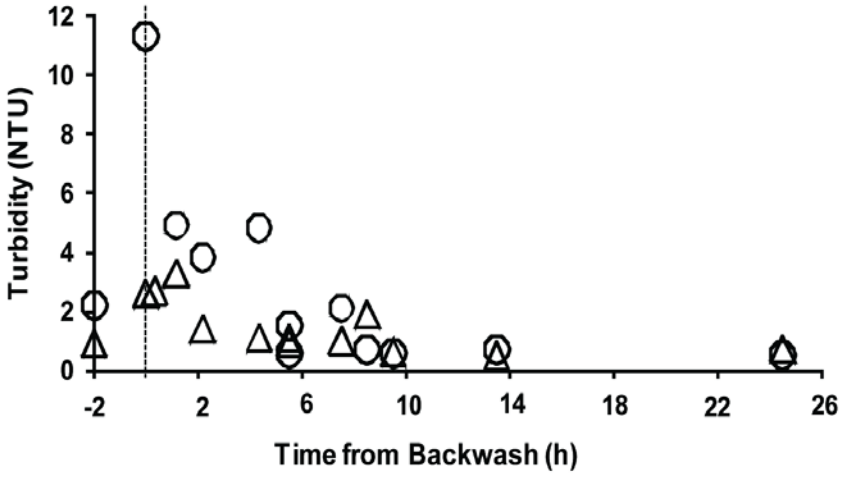

Figure 6.7: *Time profile of turbidity before and after the backwash of reactor A following the CAB protocol on day 655. The vertical line indicates the time of backwash of reactor A*

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Chapter 7

Effects of Phosphorus on Arsenic and Nitrate Removal in a Fixed-Bed Bioreactor System

7.1 Abstract

Phosphorus (P) can be a rate-limiting nutrient in biological drinking water treatment systems and its addition can enhance bioreactor performance. However, aqueous P can react with iron(III) and iron(II) to generate Fe-P solid phases, which may limit the availability of iron if desired for solid phase production for contaminant removal. P was added as a nutrient to a bench-scale biologically active carbon (BAC) reactor system consisting of two reactors operated in series for the simultaneous removal of nitrate and arsenic from a synthetic groundwater using acetic acid as the electron donor. Complete denitrification was observed in reactor A, i.e. nitrate was removed from approximately 50 mg/L NO_3^- in the influent to less than 0.2 mg/L NO_3^- (detection limit) in the effluent from reactor A. At the initial influent P level of 0.5 mg/L, vivianite ($Fe_3(PO_4)_2.8H_2O$) precipitated in reactor A resulting in less available iron for iron sulfide generation, the preferred solid for arsenic removal. Arsenic removal improved after successively lowering P concentrations from 0.5 to 0.2

and 0.1 mg/L P resulting in less than 10 µg/L As in the final effluent. These findings suggest that it is important to evaluate the availability of both P and iron in systems designed for the removal of arsenic utilizing biologically generated iron sulfides.

7.2 Introduction

The use of biological processes in drinking water treatment may provide consistent contaminant removal while reducing the need for the regeneration of sorption matrices or ion exchange resins when adsorptive removal of targeted dissolved species is the primary removal process [1]. In addition, biological treatment offers the possibility of simultaneous removal of two or more contaminants in a single unit without the generation of concentrated waste stream [2]. Many organic and inorganic contaminants can be converted into innocuous compounds with limited additions of chemicals and little or no generation of unwanted byproducts [3]. Despite these advantages, the concern of microbial re-growth in the distribution system has limited the application of biological drinking water treatment processes, especially in the United States, even though it has long been practiced in Europe [4-6]. Biological stability of treated water depends on the microbial community that develops in the treatment and distribution systems [7] and the availability of both organic [8] and inorganic [9, 10] nutrients. Availability of nutrients determines biofilm characteristics [10], which in turn determines the effectiveness of the residual disinfectant in the distribution system [8].

Phosphorus (P) is often a rate-limiting nutrient in drinking water treatment and distribution systems [11-13], and its addition may improve bioreactor performance in biologically mediated water treatment systems by enhancing microbial growth. Miettnen et al. [11] reported increased microbial growth after the addition of as low as 1 µg/L P to water samples collected from surface and groundwater sources in Finland. In a previous study, we reported improved performance in a bench-scale and a pilot-scale biologically active carbon (BAC) reactor by increasing the P concentrations [14]. Similarly, biomass growth and the rate of glucose biodegradation in a BAC reactor was higher in a P-amended system compared to that without P addition [9]. Furthermore, in pilot-scale bio-ceramic filters, the percent removal of organics increased after the addition of 25-50 µg/L PO_4^{3-} as P [13]. Addition of P, however, may not necessarily result in increased microbial growth in environments with carbon limitation. For example, total biomass, estimated as total protein and total carbohydrate, in annular reactors fed with chlorinated drinking water remained comparable regardless of the addition of P (0.03 mg/L P) (Chandy and Angeles, 2001). They reported a significant increase in biofilm biomass when the water was supplemented with both phosphate (0.03 mg/L P) and acetate (0.5 mg/L C).

Conflicting information is reported on the pathogenicity of microbial communities in relation to P concentrations. Polyphosphate, which is a chain of multiple P residues synthesized by the enzyme polyphosphate kinase (PPK) depending on the availability of P [15], in combination with PPK may trigger virulence in several pathogenic bacteria [16]. While Juhna et al. [17] reported

prolonged survival of pathogenic *E. coli* in biofilms with the enrichment of P; activation of a lethal phenotype in *Pseudomonas aeruginosa* was observed with limited P [18]. Torvinen et al. [19] reported enhanced growth of heterotrophic bacteria and decreased culturability (expressed as a ratio of FISH determined and plate-counts determined abundance), of *Mycobacterium avium* with increased phosphorus concentrations. When biofilms grown in annular reactors were exposed to drinking water enriched with 235 µg C/L and 0.5 mg P/L, bacteria related to the *Gammaproteobacteria, a* subclass of *Proteobacteria* that harbors many pathogenic bacteria, increased in number [20]. These studies point to the potential impact of phosphate levels on microbial community structure and the need to characterize microbial community changes with P concentrations that may occur in engineered systems.

Phosphorus availability in an engineered system, however, also depends on the characteristics of the treatment system and treatment steps. For example, the use of poly aluminum chloride or alum during flocculation and subsequent sedimentation may sequester P resulting in dissolved P levels less than 5 µg P/L [9]. Alternatively, phosphorus associated with organic matter may be released in water along with assimilable organic carbon (AOC) [21, 22] by ozone-assisted oxidation of organic matter during disinfection [23]. Furthermore, in a Fe-P system, abiotic reactions may limit P availability. In an oxic environment, precipitation of strengite ($FePO_4.2H_2O$) [24] or adsorption on oxy-hydroxides of iron(III) [25] and aluminum [26, 27] may result in the sequestration of P. In reduced environments, precipitation of vivianite ($Fe_3(PO_4)_2.8H_2O$) [24, 28] may

be observed. In contrast, sorbed P may be released from ferric oxy-hydroxides primarily due to reductive dissolution of Fe(III) phases, especially at lower pH, which prevents re-precipitation of Fe(II) hydroxides [29]. Even if ferrous solids precipitate, i.e., at neutral to basic pH, the resulting compounds such as siderite ($FeCO_3$) are less efficient in adsorbing phosphate [30]. Under sulfate reducing conditions, the reduction or dissolution of less soluble iron solid phases in favor of the formation of less soluble iron sulfides, such as FeS and FeS_2 can lead to phosphorus release to the liquid phase [31, 32]. Given these results and the potential for P limitation or excess to change microbial community structures and solid phase products, the total influent phosphorus levels should be carefully monitored and controlled to ensure optimal bioreactor performance.

In this study, we evaluated the impacts of changing P concentrations on nitrate and arsenic removal in a BAC reactor system. Computer simulations on chemical speciation were also conducted to interpret the reactor performance observed at different P levels.

7.3 Materials and Methods

Reactor System and Operation. Two BAC reactors (reactors A and B) were operated in series [2]. Reactors A and B were packed to 100 and 200 cm^3, respectively, with BAC particles collected from a pilot-scale and a bench-scale nitrate and perchlorate removing bioreactors. The influent flow rate was maintained at 10 mL/min resulting in 10 and 20 min empty bed contact times (EBCTs) in reactors A and B, respectively. The influent contained 200 µg/L

arsenic, 50 mg/L nitrate, 22 mg/L sulfate, and 2 mg/L iron(II) along with other constituents (Table 7.1) and was fed to reactor A in a down-flow mode. The effluent from reactor A (EA) was introduced into reactor B in an up-flow fashion. A syringe pump (Harvard apparatus, Holliston, MA) fed concentrated glacial acetic acid (equivalent to 35 mg/L acetate as carbon final concentration) along with 2 mg/L Fe(II) ($FeCl_2.2H_2O$) to reactor A. Reactor B received an additional 4 mg/L Fe(II) ($FeCl_2.2H_2O$) using a syringe pump to enhance the precipitation of iron sulfides in reactor B. Oxygen-free N_2 gas was bubbled through the influent (80 L) for 40 min to lower the dissolved oxygen (DO) level to below 1 mg/L. Additional purging with oxygen-free N_2 gas was performed every 24 h for 20 min and a floating cover was used to maintain the low influent DO level. Reactor A was backwashed every 48 h with a mixed flow of deoxygenated deionized (DDI) water (50 mL/min) and N_2 gas for 2 min followed by a flow of DDI water (500 mL/min) for 2 min. In general, reactor B was backwashed approximately every 3-4 months. However, reactor B was not backwashed during the period reported herein. Prior to day 557, the influent contained 0.5 mg/L P; this was successively lowered to 0.2 and 0.1 mg P/L on days 557 and 593, respectively. Furthermore, iron(II) added directly to the second reactor was increased to 6 mg/L Fe(II) on day 600 to evaluate if reactor performance could be improved by generating more iron sulfides in reactor B.

Liquid Samples Collection and Chemical Analyses. Liquid samples were collected from the influent tank (Inf), the first effluent from reactor A (EA), and the final effluent from reactor B (EB) every 24 h. In addition, liquid profile samples

were collected on days 538 and 606 from the sampling ports along the depth of the reactors. Liquid samples were filtered through 0.22 µm filters (Fisher, Pittsburgh, PA) and stored at 4^{o}C until analyzed. Samples were analyzed for acetate, sulfate, nitrate, nitrite, chloride, total arsenic, and total iron concentrations typically within 48 h. Samples for total arsenic and total iron were acidified to a final concentration of 0.02 N HCl before storing.

DO in the influent and the effluent from reactor A (EA) was measured using online WTW multi340 meters with CellOx325 sensors in WTW D201 flow cells (Weilheim, Germany). The detection limit for DO was 0.01 mg/L. An AS-14 (Dionex, Sunnyvale, CA) column fitted with an AG-14 guard column (Dionex, Sunnyvale, CA) separated acetate, chloride, nitrite, nitrate, and sulfate chromatographically in an ion chromatography system (Dionex, Sunnyvale, CA) consisting Dionex DX 100 conductivity detector. A mixture of 1 mM bicarbonate and 3.5 mM carbonate prepared from ACS reagent grade sodium bicarbonate and sodium carbonate, respectively, was used to elute the ions from the separation column. The detection limit for each of the anions was 0.2 mg/L. Total arsenic and total iron were measured using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer ALEN DRC-e, Waltham, MA). The detection limit for total arsenic and total iron was 2 µg/L As_T and 0.1 mg/L Fe_T, respectively.

Model Simulation. MINEQL+ version 4.6 [33] was used to evaluate for possible iron solid phase precipitation in the reactor system. Given that biological activities attenuate micro-environments within the reactors and species

concentrations change temporally as well as spatially along the flow direction, MINEQL+ simulations do not necessarily reflect prevailing conditions within micro-environments within biofilms or along the length of the BAC beds [34]. However, simulations were carried using the MINEQL+ titration mode by varying either phosphate (PO_4^{3-}) or hydrogen sulfide (HS^-) for an assumed redox potential (pe) to evaluate the possibility of precipitation of solids, such as green rust (GR) ($Fe_2(OH)_5$), vivianite ($Fe_3(PO_4)_2.8H_2O$), mackinawite (FeS_{1-x}), orpiment (As_2S_3), and realgar (AsS). Simulations were carried out at a pH of 7.2 considering the chemical profile data collected on day 538. Thermodynamic data reported by Gallegos et al. [35] were used and included redox reactions of iron, arsenic, and sulfide in the simulations. A fixed pe+pH method was used for modeling redox reactions since the concentrations of redox couple components (i.e., SO_4^{2-}/HS^-) would be expected to continuously change as a function of microbial sulfate reduction and subsequent reaction of iron(II) with the produced S(-II). The onset of sulfate reduction in natural environments may occur at an Eh of -150 to -200 mV [36]. Accordingly, simulations involving redox reactions under sulfate reducing conditions were performed with fixed pe values ranging from -3.39 to -10 (Table 7.2). Besides the titration mode simulations, single run simulations were also performed to evaluate the possibility of solid precipitation at the influent conditions (influent matrix) and after complete denitrification (at port A8) on day 538 (Table 7.3). For the single run calculations, only arsenate species (i.e., arsenic species in the As(V) oxidation state) with no redox reactions were considered. Sulfides were also not considered in the single run calculations.

7.4 Results

Overall Reactor Performance. The baseline and stability of the reactor performance was evaluated by monitoring the concentrations of nitrate, sulfate, and arsenic in the effluent from reactors A and B compared to their respective influent levels. P levels were changed on days 557 and 593 to assess the effects of P addition on arsenic removal. During the period reported, DO in the influent (inf) and the effluent from reactor A (EA) remained at 0.42±0.28 (mean ± standard deviation) mg/L, while the pH in the effluent from reactors A and B averaged 6.99±0.34 and 6.95±0.27, respectively. Complete nitrate removal (Figure 7.1) was achieved except during days 575 to 581 (denoted as an upset period hereafter) due to low acetate levels (approximately 3.5 mg/L acetate as C) and oxygen exposure (day 574). Prior to lowering the P concentration to 0.2 mg P/L on day 557, sulfate levels in the effluents from reactor A (EA) and reactor B (EB) were measured to be 20.6±0.8 and 7.8±1.3 mg SO_4^{2-}/L, respectively. This result excludes the relative lack of sulfate reduction during days 536 to 539 when acetate concentration in the influent unintentionally remained comparatively lower. As expected, the arsenic concentration time profile followed the trend of sulfate reduction with the arsenic concentration in the EA and EB of 79±10 and 27±11 µg As/L, respectively, prior to day 557.

The P level in the influent was lowered to 0.2 mg P/L on day 557 to evaluate its impact on microbial growth and reactor performance. While decreasing the P had no impact on nitrate reduction, sulfate levels in the effluent from reactors A and B slowly declined after day 557 indicating enhanced sulfate

reduction. Following this trend in increased sulfate reduction, the arsenic concentration in the effluent from reactors A and B also declined. Within 7 days from day 557, sulfate and arsenic in the final effluent achieved concentrations of 4.4±0.7 mg SO_4^{2-}/L and 11±1 µg As/L, respectively.

During days 575 to 581 the acetate feed solution accidently contained approximately 3.5 mg/L acetate as C rather than the intended amount of 35 mg/L. To add to this problem, on day 574 water from reactor B drained into reactor A, caused by a siphoning action through the gas release system of reactor A. The synergistic negative impacts of these two events resulted in poor reactor performance from days 575 to 585. However, once the reactor was reset, recovery of nitrate removal was rapid, while approximately 10 days were required to attain the level of sulfate reduction observed prior to the upset. Exposure to oxygen resulted in leaching of arsenic from reactor B, which continued till day 585 and arsenic in the final effluent attained a stable level after sulfate reduction was re-established on day 585.

Since lowering P level in the influent to 0.2 mg/L P resulted in improved overall reactor performance, the concentration of P was further lowered to 0.1 mg/L P on day 593. Sulfate reduction and subsequent arsenic removal once again improved (Figure 7.1) The effluent from reactors A and B contained 15.5±1.5 and 3.6±1.3 mg/L SO_4^{2-}, respectively, while the corresponding arsenic concentrations averaged 26±7 and 9±1 µg/L As, respectively. On day 600, the Fe(II) concentration added directly to reactor B was increased from 4 mg/L Fe(II)

to 6 mg Fe/L (II), but this did not enhance arsenic removal apparently due to the already low arsenic levels in the EA.

Chemical Profile along the Bed Depths. Liquid profile samples were collected on days 538 and 605 when the influent contained 0.5 and 0.1 mg/L P, respectively, to evaluate the impacts of different P levels on the oxygen, nitrate, and sulfate terminal electron accepting process (TEAP) [37] zones. Chemical analyses indicated a sequential utilization of DO (not shown), nitrate, arsenate, and sulfate as electron acceptors for the oxidation of acetate (Figure 2). Complete denitrification was achieved in reactor A on both days 538 and 605 regardless of P levels in the influent. The effluent from reactor A contained nitrate below the detection level (0.2 mg/L NO_3^-). Sulfate reduction in reactor A was higher with 0.1 mg/L P compared to that with 0.5 mg/L P (Figure 7.2). Accordingly, iron entrapment and subsequent arsenic removal also improved when lower P was added to the influent.

In summary, during this study of the impact of P, nitrate was completely removed from the system in reactor A. Similarly, most of the arsenic removal occurred in reactor A (Figures 7.1 and 7.2), while reactor B provided an additional polishing effect. Low levels of acetate in the influent resulted in poor reactor performance due to the lack of sufficient electron donor to facilitate complete reduction of the various influent electron acceptors present. Oxidation of deposited iron sulfides during the upset period resulted in leaching of arsenic until sufficient sulfate reduction was re-established in the system. Adding a higher concentration of Fe(II) directly to reactor B did not appreciably lower the

final effluent arsenic concentration. Overall, the lowering of phosphate from 0.5 mg/L to 0.1 mg/L P improved reactor performance by enhancing sulfate reduction and arsenic removal, presumably through the enhanced precipitation of iron sulfides and concomitant sorption of As to these solids.

Computer Simulations. To evaluate whether decreasing P concentrations in the influent could enhance the formation of iron sulfides, computer simulations were performed using MINEQL+. For the simulations, denitrification and sulfate reducing conditions, thought to be representative of the conditions in the columns, were assumed. The simulations were run in both titration mode (with variable phosphate or sulfide) or in a single point mode. Based on the single run mode simulations using the influent chemical composition, no solids formed. However, single run simulations conducted with the chemical composition at port A8 on day 538 (except iron being considered as 2 mg/L Fe(II)) without considering redox couples predicted vivianite ($Fe_3(PO_4)_2.8H_2O$) formation. Similarly, in titration simulations with varying concentrations of phosphate under denitrification conditions (no sulfide present), vivianite was found to form when the influent P concentration was $\geq 1.19x10^{-5}$ M (0.368 mg P/L) (Table 2). Titration with varying concentrations of HS^- at $1.61x10^{-5}$ M P (0.5 mg P/L) and $3.58x10^{-5}$ M Fe(II) (2 mg Fe(II)/L), however, suggested the presence of green rust (GR) ($Fe_2(OH)_5$) as the only iron solid up to a pe of -3.73 (Eh -220 mV). Under more reducing conditions of pe between -4.07 (Eh -240) and -8 (Eh -472), co-existence of mackinawite (FeS_{1-x}) and GR was predicted (Table 2), preventing the precipitation of vivianite. In the titrations, vivianite precipitation was predicted

only at pe of -10 (Eh -590) when sulfide levels were quite low, i.e., on the order of $1x10^{-6}$ M (0.3 mg HS^-/L) or lower (data not shown). Realgar (AsS) precipitation was estimated to lower aqueous arsenic levels in the pe range of -6.78 to -10 (Table 2).

7.5 Discussion

The BAC reactor employed in this study relies on the establishment of a microbially mediated differential redox gradient across the filter bed and the generation of iron sulfides. Microorganisms present in the current system utilized the available electron acceptors (i.e., DO, nitrate, arsenate, and sulfate) leading to the generation of segregated TEAP zones along the flow direction (Figure 7.2). Given that microorganisms may co-exist within a biofilm depending on their metabolic capabilities [38, 39], TEAP zones may also overlap at a certain location within the filter bed. In this reactor system, sulfate reduction was observed prior to sampling port A8 in reactor A on day 606 (0.1 mg P/L) where nitrate, the more thermodynamically favorable electron acceptor [40] was still present (Figure 7.2), suggesting the co-existence of nitrate and sulfate reducing TEAP zones. Given that 90% of the arsenic reduction also occurred in reactor A, it is likely that the arsenic TEAP zone overlapped with sulfate and/or nitrate reducing zone. The spatial profile of sulfate reduction and iron depletion from the liquid phase along the flow direction paralleled one another in reactors A and B, suggesting the generation of iron sulfides throughout the system. This is supported by the previously reported presence of mackinawite (a tetragonal iron sulfide, FeS_{1-x}) and greigite (Fe_3S_4) in reactor B in this system [41].

In reducing environments, ferrous arsenate, such as symplesite ($Fe(II)_3(AsO_4)_2 \cdot 8H_2O$) may provide a sink for Fe(II) and As(V) [42], even though dissimilatory arsenate reduction may again release the associated arsenic [43]. In the current system, the arsenic concentration did not decline until sulfate reduction occurred, indicating that ferrous-arsenate solid formation was not likely. In fact, the arsenic spatial profile along the flow direction followed the trend of sulfate reduction and iron depletion, suggesting sequestration of arsenic through the precipitation of arsenic sulfides or adsorption and co-precipitation of arsenic with iron sulfides as previously reported for this system [2]. Therefore, the availability of iron(II) for the generation of iron sulfides appears to be essential for effective arsenic removal in the current system.

The availability of iron, however, may be impacted by the presence of phosphate [24, 28, 44]. Precipitation of iron-phosphate solids, such as strengite ($FePO_4.2H_2O$) [24] or vivianite ($Fe_3(PO_4)_2.8H_2O$) [30] is possible in an Fe-P system in both oxic or reduced conditions, respectively. In the current study, decreasing the phosphate level in the influent on days 557 and 593 resulted in improved arsenic removal (Figure 7.1). The increase in arsenic removal occurred primarily in reactor A. Even though the heterogeneity of microbially established local environments [34] may not be represented in simple thermodynamic modeling of TEAP zones, computer simulation under assumed denitrification conditions and no sulfate reduction predicts vivianite precipitation. Even under sulfate reducing conditions, however, vivianite formation may occur, provided sulfide concentrations remain $\leq 1x10^{-6}$ M HS^- (Table 2). Since flow

characteristics in the system are close to plug-flow and the redox potential sequentially decreases along the flow direction, it is likely that conditions are favorable in the upper part of reactor A for the precipitation of Fe-P solids, such as vivianite, as sulfate reduction was not observed (Figure 7.2). Our efforts to evaluate if vivianite formed in reactor A by X-ray diffraction (XRD) have been inconclusive to date, primarily due to limited amounts of solids collected during backwashing events even after pooling solids from 3-4 successive backwashes. So far, no crystalline solids have been detected by XRD in reactor A, presumably due to the low amount of solid phase inorganic products relative to the large production of biomass.

Interestingly, even though most of the sulfate reduction occurred in reactor B, reactor B did not have much impact on arsenic removal (Figure 7.2). The possible generation of more iron sulfides after increasing the Fe(II) levels in reactor B on day 600 also did not result in apparent improvement of arsenic removal in reactor B. This is more likely due to the fact that most of the arsenic was already removed in reactor A (Figure 7.2). Additionally, the co-location of both dissimilatory arsenate reducing bacteria and sulfate reducing bacteria in sufficient relative abundance probably is necessary for effective arsenic removal.

Changes in P levels may result in a shift in microbial community structure in an engineered system [19, 45]. For example, in both a bench-scale and a pilot-scale nitrate and perchlorate removing bioreactors, we previously reported changes in microbial community structure after increasing the P level in the influent [14]. The population density of perchlorate reducing bacteria related to

Dechloromonas and *Azospira* genera increased in the bench-scale reactor, while *Zoogloea*-like bacteria dominated the pilot-scale reactor after increasing P concentrations. Regardless of the dominant microbial populations, both reactors observed improved nitrate and perchlorate removal after the P addition. As seen in Figure 7.2, both nitrate and sulfate reduction improved after lowering P levels in the influent. The improvement of reactor performance after the decrease in P in the influent might have resulted from a shift in microbial community structure leading to a higher relative abundance of nitrate and sulfate reducing bacteria in the system. However, since microbial community structure was not evaluated during this study, it is premature to draw such a conclusion.

This study showed enhancement of reactor performance related to arsenic removal in particular after lowering the P levels in the influent, which was primarily attributed to the reduction in the formation of Fe-P solids in the nitrate reducing zone of reactor A, allowing more Fe to form iron sulfides in the sulfate reducing zone. Future work will focus on characterizing the solids generated in reactor A. One strategy to generate more solids in reactor A will be to prolong the time interval between two backwash events to allow more solids to accumulate. However, the impact of this less frequent backwashing on biomass accumulation and associated head loss across the reactor will need to be evaluated. Future use of molecular biology tools including pyrosequencing, quantitative PCR, and reverse transcriptase quantitative PCR are expected to assess the potential importance of shifts in microbial community structure and

reactor performance, which may also account for enhanced production of iron sulfide.

7.6 Conclusions

Decreasing the influent P levels led to enhanced removal of arsenic, which was attributed to reduction in the precipitation of vivianite-like iron-phosphate solids (inferred from computer simulations) and concomitant increase in iron sulfide production in reactor A. At the optimal P concentration of 0.1 mg/L as P, the BAC reactor system lowered the influent arsenic concentration of 200 µg/L As to less than 10 µg/L As, the drinking water standard in most countries [46]. The availability of iron for the precipitation of iron sulfides in reactor A was surmised to be crucial for arsenic removal. Regardless of the P concentration, the influent nitrate concentration (50 mg/L NO_3^-) was always lowered to below its detection limit. These data indicate that optimal performance of the BAC reactor system requires consideration of P levels in comparison to the concentration levels of the terminal electron acceptors present in the influent.

7.7 Tables and Figures

Table 7.1: *Composition of the synthetic groundwater fed to reactor A.*

Chemical	Concentration	Unit
$NaNO_3$	50.0	mg/L as NO_3^-
NaCl	13.1	mg/L as Cl^-
$CaCl_2$	13.1	mg/L as Cl^-
$MgCl_2.6H_2O$	13.1	mg/L as Cl^-
K_2CO_3	6.0	mg/l as CO_3^{2-}
$NaHCO_3$	213.5	mg/L as HCO_3^-
Na_2SO_4	22.4	mg/L as SO_4^{2-}
$Na_2HAsO_4.7H_2O$	0.2	mg/L as As
H_3PO_4	0.5/0.2/0.1	mg/L as P
$FeCl_2.4H_2O$[a,b]	6.0/8.0	mg/L as Fe^{2+}
CH_3COOH[a]	35.0	mg/L as C

[a] added as concentrated solution through a syringe pump. Theconcentrations in the table represent the concentrations after mixing of the concentrated solution and the influent.

[b] in addition to the supplementation of $FeCl_2.4H_2O$ to reactor A, a concentrated solution of $FeCl_2.4H_2O$ was added to reactor B using a syringe pump to provide an additional 4 mg/L as Fe(II) to the system.

Table 7.2: *Computer simulation results. The possibility of solids precipitation was evaluated by running titration with HS- ranging from $2X10^{-7}$ to $3X10^{-4}$ M.*

Eh (mV)	pe	Range of HS- concentration (M)			
		Fe2(OH)5	Vivianite	Mackinawite	Realgar
-200	-3.39	$2.0X10^{-7}$ to $3.0X10^{-4}$	--	--	
-209	-3.54	$2.0X10^{-7}$ to $3.0X10^{-4}$	--	--	
-220	-3.73	$2.0X10^{-7}$ to $3.0X10^{-4}$	--	--	
-240	-4.07	$2.0X10^{-7}$ to $3.0X10^{-4}$	--	$1.1X10^{-4}$ to $1.8X10^{-4}$	
-250	-4.24	$2.0X10^{-7}$ to $3.0X10^{-4}$	--	$9.2X10^{-5}$ to $1.7X10^{-4}$	
-300	-5.08	$2.0X10^{-7}$ to $6.1X10^{-5}$	--	$3.7X10^{-5}$ to $1.7X10^{-4}$	
-400	-6.78	$2.0X10^{-7}$ to $3.7X10^{-5}$	--	$1.2X10^{-5}$ to $1.7X10^{-4}$	$2.0X10^{-7}$ to $3.0X10^{-4}$
-472	-8.0	$2.0X10^{-7}$ to $1.9X10^{-5}$	--	$6.3X10^{-6}$ to $1.7X10^{-4}$	$2.0X10^{-7}$ to $3.0X10^{-4}$
-590	-10.0	--	$2.0X10^{-7}$	$6.3X10^{-6}$ to $1.7X10^{-4}$	$2.0X10^{-7}$ to $3.0X10^{-4}$

Table 7.3: *Concentrations of the components included in single run simulations using MINEQL+. Chemical concentrations in the influent and port A8 on day 538 are used for the simulations.*

Component	**Concentration (M)**	
	Influent	**At port A8**
AsO_4^{-3}	$2.71X10^{-6}$	$2.79X10^{-6}$
Ca^{2+}	$1.85X10^{-4}$	$1.85X10^{-4}$
Cl^-	$1.18X10^{-3}$	$1.18X10^{-3}$
Fe^{2+}	$3.58X10^{-5}$	$3.58X10^{-5}$
K^+	$2.00X10^{-4}$	$2.00X10^{-4}$
Mg^{2+}	$1.85X10^{-4}$	$1.85X10^{-4}$
Na^+	$5.08X10^{-3}$	$5.08X10^{-3}$
NO_3^-	$6.97X10^{-4}$	---
PO_4^{3-}	$1.61X10^{-5}$	$1.61X10^{-5}$
SO_4^{2-}	$2.34X10^{-4}$	$2.34x10^{-4}$
CH_3COO^-	$1.46X10^{-3}$	$6.88x10^{-4}$
CO_3^-	$3.60X10^{-3}$	$3.60X10^{-3}$

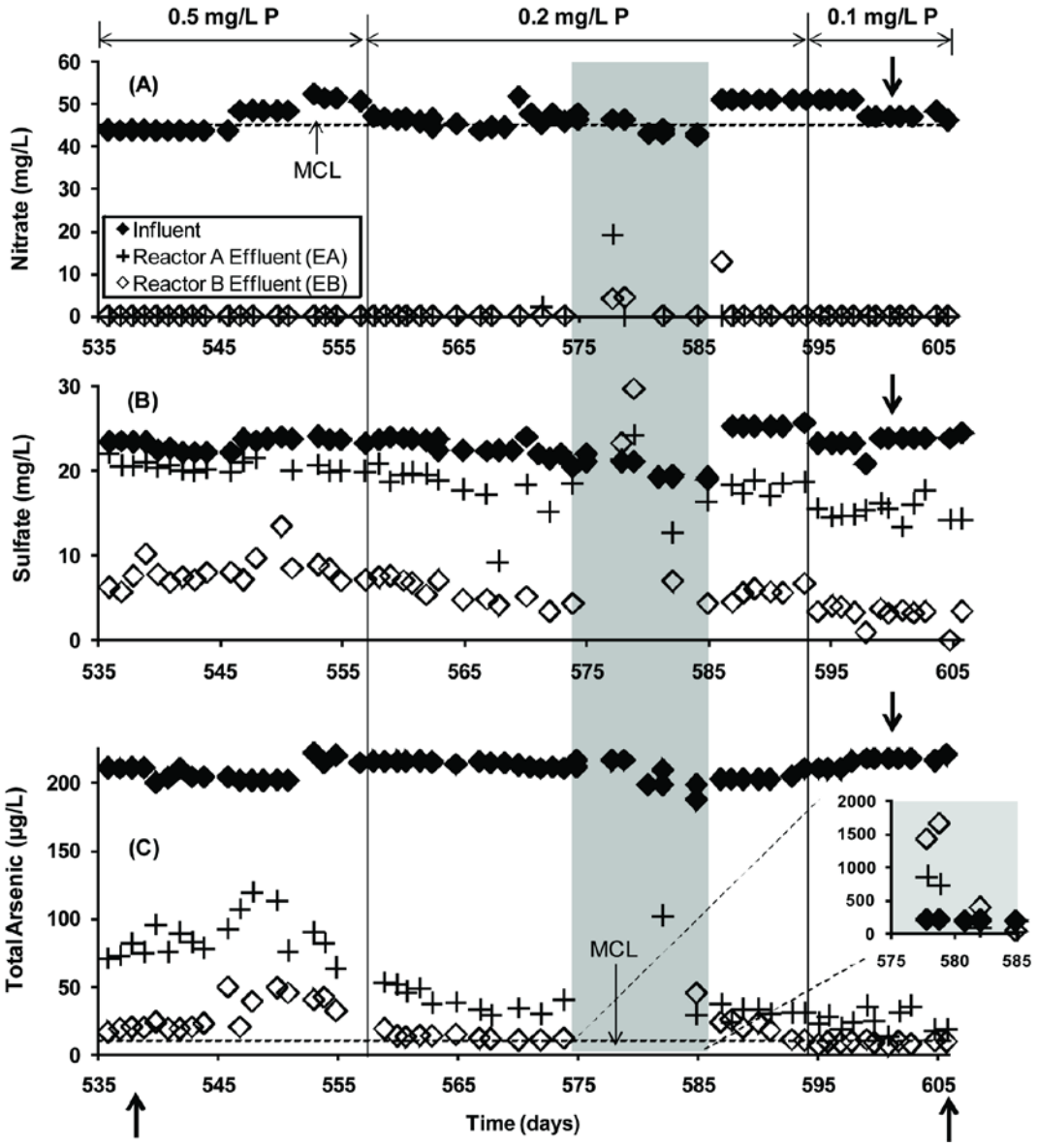

Figure 7.1: *(A) Nitrate, (B) sulfate, and (C) total arsenic concentrations in the influent, the effluent of reactor A (EA), and the effluent of reactor B (EB) versus time of operation. The total EBCT was 30 min. The vertical lines indicate the days when P levels were decreased. The boldface up-arrows indicate day 538 and 606 when profile liquid and biomass samples were collected. The bold face down-arrows indicate day 600 when Fe(II) directly added to reactor B was increased to 6 from 4 mg Fe(II)/L.*

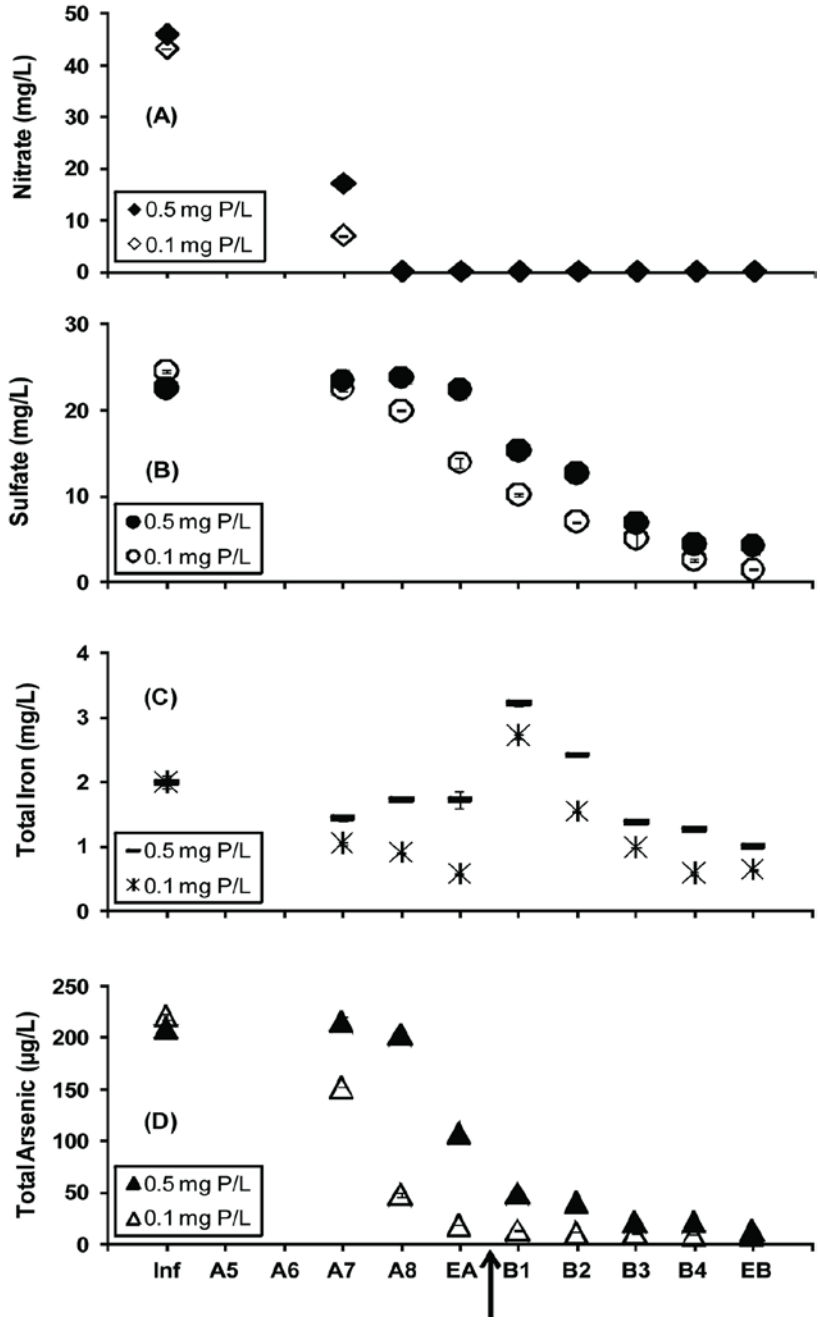

Figure 7.2: *Chemical profiles along the depth of the reactor beds on day 538 and 606. Nitrate concentrations (A), sulfate concentrations (B), total iron concentrations (C,) and total arsenic concentrations (D). Inf represents the influent concentrations, A7, A8, and B1-B4 represent the respective sampling ports along the depth of reactors A and B, respectively. EA and EB represent concentrations in the effluents from reactor A and reactor B, respectively. The arrow indicates the location of additional Fe (II) (4 mg/L) addition. Mean (n=3) values are reported with the error bars representing one standard deviation from the mean.*

Supplemental Table 7-A: Ionic concentrations used for computer simulations. Measured concentrations of total As, acetate, and sulfate at port A8 on day 538 are used for the simulations. Chloride concentrations are presented after achieving electroneutral conditions. The concentrations of other constituents were calculated based on the influent matrix. Single run simulations were conducted in the influent and denitrification conditions. Titration simulations under denitrification conditions were conducted by varying P levels from $1X10^{-7}$ to $2X10^{-5}$ M. Titration simulations under sulfate reducing conditions included HS^- concentrations ranging from $2X10^{-7}$ to $3X10^{-5}$ M.

	Concentration (M)		
Species	Under influent conditions	Under denitrification conditions	Under sulfate reducing conditions
ASO_4^{-3}	$2.71X10^{-6}$	$2.79X10^{-6}$	--
AsO_3^{-3}	--	--	$2.79X10^{-6}$
Ca^{2+}	$1.85X10^{-4}$	$1.85X10^{-4}$	$1.85X10^{-4}$
Cl^-	$1.70X10^{-3}$	$1.70X10^{-3}$	$1.70X10^{-3}$
Fe^{2+}	$3.58X10^{-5}$	$3.58X10^{-5}$	$3.58X10^{-5}$
K^+	$2.00X10^{-4}$	$2.00X10^{-4}$	$2.00X10^{-4}$
Mg^{2+}	$1.85X10^{-4}$	$1.85X10^{-4}$	$1.85X10^{-4}$
Na^+	$5.08X10^{-3}$	$5.08X10^{-3}$	$5.08X10^{-3}$
NO_3^-	$6.97X10^{-4}$	--	--
PO_4^{3-}	$1.61X10^{-5}$	$1.61X10^{-5}$	$1.61X10^{-5}$
SO_4^{2-}	$2.34X10^{-4}$	$2.48X10^{-4}$	--
HS^-	--	--	$2.48X10^{-4}$
CH_3COO^-	$1.46X10^{-3}$	$6.88X10^{-4}$	--
CO_3^-	$3.60X10^{-3}$	$3.60X10^{-3}$	$3.60X10^{-3}$

Appendix A7-1: Tableau- Aqueous Species (Type III)

Aqueous Phases	**e^-**	**H_2O**	**H^+**	**As(III)**	**Ca^{2+}**	**Cl^-**	**CO_3^{2-}**	**Fe^{2+}**	**K^+**	**Mg^{2+}**	**Na^+**	**PO_4^{3-}**	**HS^-**	**Ac^-**	**LOG K**
OH(-1)		1	-1												-14.005
Iron Species															
$Fe(OH)_3$(-1)		3	-3					1							-30.756
$Fe(OH)_2$(aq)		2	-2					1							-19.679
FeOH(+1)		1	-1					1							-6.778
Fe(III)(+3)	-1	0						1							-13.019
FeOH(+2)	-1	1	-1					1							-15.190
$Fe(OH)_2$(+1)	-1	2	-2					1							-20.187
$Fe2(OH)_2$(+4)	-2	2	-2					2							-26.413
$Fe(OH)_3$(aq)	-1	3	-3					1							-23.983
$Fe3(OH)_4$(+5)	-3	4	-4					3							-38.935
$Fe(OH)_4$(-1)	-1	4	-4					1							-32.509
FeOCl(aq)	-1	1	-2			1		1							-15.442
Fe(II)Cl2(aq)						2		1							2.088
Fe(II)Cl(+1)						1		1							26.460
Fe(III)Cl3	-1					3		1							-10.102
Fe(III)Cl(+2)	-1					1		1							-11.609
Fe(III)Cl2(+1)	-1					2		1							-8.745
$Fe(SO_4)_2$(-1)	-17	8	-18					1					2		-74.797
$FeSO_4$(aq)	-8	4	-9					1					1		-33.585
Fe(III)SO_4(+1)	-9	4	-9					1					1		-42.470
$Fe(HS)_2$(aq)								1					2		11.483
Fe(HS)3(-1)								1					3		13.615
Fe(Acetate)(+1)								1							1.4
Fe(HPO4)(aq)			1					1				1			15.975

Aqueous Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
FeH2PO4(+1)			2					1				1			22.273
Fe(HCO3)+			1				1	1							11.429
Sulfur Species															
S(-2)			-1										1		-12.926
H_2S(aq)			1										1		7.041
S_2O_3(-2)	-8	3	-8										2		-29.387
SO_4(-2)	-8	4	-9										1		-33.583
HSO_4(-1)	-8	4	-8										1		-31.588
S_2(-2)	-2	0	-2										2		-9.529
S_3(-2)	-4	0	-3										3		-6.291
S_4(-2)	-6	0	-4										4		-3.281
S_5(-2)	-8	0	-5										5		-0.500
S_6(-2)	-10	0	-6										6		1.441
$H_2S_2O_3$(aq)	-8	3	-6										2		-27.582
HS_2O_3(-1)	-8	3	-7										2		-28.195
HSO_3(-1)	-6	3	-6										1		-30.011
SO_3(-2)	-6	3	-7										1		-37.235
$NaSO_4$(-1)	-8	4	-9										1		13.002
Arsenic Species															
$HAsO_3$(-2)		0	1	1											13.422
H_3AsO_3(aq)		0	3	1											33.665
H_2AsO_3(-1)		0	2	1											24.423
H_4AsO_3(+1)		0	4	1											34.439
AsS(OH)(SH)(-1)		-2	4	1									2		51.594
$As(OH)_2$(SH)(aq)		-1	4	1									1		42.458
$As(OH)_2S$(-1)		-1	3	1									1		37.314
$As(OH)S_2$(-2)		-2	3	1									2		42.462

Aqueous Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
AsS_3(-3)		-3	3	1									3		46.445
HS_3As(-2)		-3	4	1									3		54.335
$As(HS)_4$(-1)		-3	6	1									4		70.586
$(SH)_2As_3S_4$(-1)		-9	14	3									6		174.010
AsO4(-3)	-2	1	-2	1											-6.374
HAsO4(-2)	-2	1	-1	1											5.215
H2AsO4(-1)	-2	1	0	1											11.962
H3AsO4	-2	1	1	1											1.441
Other aqueous species															
CaOH(+1)		1	-1		1										-12.697
MgOH(+1)		1	-1							1					-11.387
CaHCO3+			1		1		1								11.599
CaH2PO4+			2		1							1			20.923
CaHPO4(aq)			1		1							1			15.035
H2CO3(aq)			2				1								16.681
HCO3(-1)			1				1								10.329
MgHCO3(-1)			1				1			1					11.339
NaHCO3(aq)			1				1				1				10.079
FeH2PO4(+1)			2					1				1			22.273
KHPO4(-1)			1						1			1			13.255
MgPO4(-1)										1		1			4.654
MgH2PO4(+1)			2							1		1			21.256
MgHPO4(aq)			1							1		1			15.175
NaHPO4(-1)			1								1	1			13.445
H2PO4(-1)			2									1			19.573

Aqueous Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
HPO4(2-)			1									1			12.375
H3PO4			3									1			21.721
H(Acetate)			1											1	4.757
CaPO4(-1)					1							1			6.46
Ca(Acetate)					1									1	1.18
MgCO3(aqu)							1			1					2.92
NaCO3(-1)							1				1				1.27
K(Acetate)									1					1	-0.196
Mg(Acetate)										1				1	1.27
Na(Acetate)											1			1	-.0180

Appendix A7-2: Tableau - Dissolved Solids (Type V)

Solid Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
Iron Solids															
$Fe(III)Cl_3$(molysite)	-1					3		1							-24.134
FeOOH (goethite)	-1	2	-3					1							-11.089
Fe_3O_4(magnetite)	-2	4	-8					3							-29.806
$Fe_3(OH)_8$	-2	8	-8					3							-33.285
$Fe(OH)_3$(soil)	-1	3	-3					1							-13.587
Fe_2O_3 (maghemite)	-2	3	-6					2							-24.954
Fe_3S_4(Greigite)	-2		-4					3					4		22.022
Wustite (-0.11)		1	-2					0.95							-6.273
$Fe(OH)_3$ (lepidicrocite)	-1	3	-3					1							-53.851
Fe_2O_3(hematite)	-2	3	-6					2							-22.285
$Fe(OH)_3$(c)	-1	3	-3					1							-14.886
Mackinawite			-1					1					1		4.734
$Fe_3(PO_4)_2.8H_2O$ (Vivianite)		8						3				2			36.00
$Fe(OH)_2$		2	-2					1							-11.685
FeS (ppt)			-1					1					1		3.050
$FeSO_4$	-8	4	-9					1					1		-34.090
$FeCO_3$ (Siderite)							1	1							10.24
$Fe_4(OH)_8Cl$	-1	8	-8			1		4							-34.938
$Fe_6(OH)_{12}SO_4$	-10	16	-21					6					1		-81.649
$Fe(OH)_3$(am)	-1	3	-3					1							-14.427
$Fe_2(OH)_5$	-1	5	-5					2							-17.463

Solid Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
Arsenic Solids															
AsS(realgar)	1	-3	5	1									1		54.281
FeAsS (arsenopyrite)	3	-3	5	1				1					1		43.400
AsS	1	-3	5	1									1		-54.69
As_2S_3(am)		-6	9	2									3		112.588
As_4O_6 (ARSENOLITE)		-6	12	4											36.510
As_4O_6 (CLAUDETITE)		-6	12	4											36.628
As_2S_3 (ORPIMENT)		-6	9	2									3		113.903
Other Solids															
CaO (Lime)		1	-2		1										-32.699
Portlandite		2	-2		1										-22.804
$CaHPO_4{:}2H_2O$		2	1		1							1			18.995
Calcite					1		1								8.480
Halite						1					1				45.888
Na_2SO_4	-8	4	-9								2		1		57.755
Sulfur	-2		-1										1		2.203
Huntite					1		4			3					29.968

Appendix A7-3: Tableau - Species not included (Type VI)

Solid Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
Pyrite	-2		-2					1					2		19.024
$FeS_{1.053}$ (pyrrhotite)			-1.1					0.95					1		6.657
Fe(0) metal	2		-2					1							-9.418
FeS_2 (marcasite)	-2		-2					1					2		18.327
FeS (troilite)			-1					1					1		5.541
Fe_2S_3	-2		-3					2					3		27.761
As(0) native	3	-3	6	1											46.258
$Fe_2(SO4)_3$	-26	12	-27					2					3		-80.00
FeSH(+1)								1					1		9.413
O_2 (g)	-4	2	-4												-82.442
H_2S (g)			1										1		8.01
CO_2 (g)		1	-2				1								21.647
Fe(0) metal	2							1							-13.825
FeO		1	-2					1							-11.326
Fe_7S_8 (pyrrhotite)	-2		-8					7					8		52.056
$Fe_3(OH)_7$	-1	7	-7					3							-17.053
Fe_2As	7	-3	6	1				2							23.521
FeAs	5	-3	6	1				1							37.346
$FeAs_2$ (lollingite)	8	-6	12	2				1							87.858
Hydroxylapatite		1	-1		5							3			44.333
Artinite		5	-2				1			2					-9.60
Hydromagnesite		6	-2				4			5					8.766
Periclase		1	-2							1					-21.584
Brucite		2	-2							1					-16.844

Solid Phases	e^-	H_2O	H^+	As(III)	Ca^{2+}	Cl^-	CO_3^{2-}	Fe^{2+}	K^+	Mg^{2+}	Na^+	PO_4^{3-}	HS^-	Ac^-	LOG K
Mg(OH)2 (active)		2	-2							1					-18.794
MgHPO4:3H2O		3	1							1		1			18.175
Nesquehonite		3	0				1			1					4.670
Thermonarite		1	0								2				-0.637
Natron		10	0				1				2				1.311
CaHPO4			1		1							1			19.275
Dolomite (ordered)					1		2			1					17.09
Dolomite (disordered)					1		2			1					16.540
Ca3(PO4)2 (beta)					3							2			28.92
Magnesite							1			1					7.460
Mg3(PO4)2										3		2			23.28
As_4S_4	4	-12	20	4									4		218.78
Fe(III))OCl	-1	1	-2			1									-15.442
$Ca4H(PO4)_3.3H_2O$		3	1		4							3			47.08
Aragonite					1		1								8.30
FeCl3	-1					3		1							-24.134

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Chapter 8

Conclusions and Future Perspectives

8.1 Conclusions

The frequent co-existence of nitrate and arsenic in natural water sources necessitates the development of a single step treatment system for their simultaneous removal. While conventional technologies fail to provide simultaneous removal of these contaminants, advanced technologies, such as reverse osmosis and ion exchange often are cost prohibitive. Furthermore, current technologies for arsenic removal relying on adsorption of arsenic to oxy-hydroxides of iron(III) and aluminum (Gulledge and O'Connor, 1973) are not sustainable as arsenic has the potential to be re-released from the arsenic-laden sludge when disposed under reducing conditions, such as in landfill environments (Ghosh et al., 2006; Sierra-Alvarez et al., 2005). Biological processes may provide attractive alternatives for the simultaneous removal of nitrate and arsenic, as well as additional contaminants.

The goal of this research was to evaluate the potential of a fixed-bed biologically active carbon (BAC) biofilm reactor system for the simultaneous

removal of nitrate and arsenic from drinking water sources utilizing microorganisms originating from a natural groundwater. To accomplish this, three main objectives were pursued: (i) to operate and evaluate the performance of two biofilm reactors in series to produce nitrate and arsenic free drinking water, (ii) to elucidate the mechanisms of arsenic removal in this reactor system, and (iii) to optimize the process parameters, such as empty bed contact time (EBCT), nutrient addition, and backwashing without compromising reactor performance.

Two laboratory-scale BAC reactors were operated in series for approximately 700 days using a synthetic groundwater containing nitrate, arsenate, and sulfate, amended with acetic acid as the electron donor. Operation and monitoring of these bioreactors demonstrated for the first time the potential of biologically mediated simultaneous removal of nitrate and arsenic from drinking water sources under reducing conditions and led to a patent application (UMJ-201-B (UM4430): “System and method for simultaneous biologically mediated removal of contaminants from contaminated water”).

Operation of the two BAC reactors in series, seeded with a microbial inoculum that originated from a natural groundwater and supplemented with acetic acid, resulted in the establishment of a diverse microbial community comprised of nitrate, iron(III), sulfate, and arsenate reducing bacteria (Chapter 4). A redox gradient was established in the system as dissolved oxygen, nitrate, arsenate, and sulfate were sequentially utilized resulting in the development of various terminal electron accepting process (TEAP) zones (Chapter 3). The

exact positioning of the TEAP zones along the bed depths was dependent on the concentration of the electron acceptors. For example, an increase in the influent concentration of nitrate, a thermodynamically preferred electron acceptor compared to sulfate, resulted in the extension of nitrate reducing TEAP zone in the first reactor and a shift of the sulfate reducing TEAP zone towards the end of the reactor system (Chapter 5 and Chapter 7). For most of the operational period, concentrations of nitrate (50 mg/L NO_3^-) and arsenic (200 to 300 µg/L As) in the influent were lowered to below detection (0.2 mg/L NO_3^-) and less than 20 µg As/L, respectively (Chapter 3 and Chapter 4).

To assess the anticipated importance of biogenic sulfate and arsenate reduction for removing arsenic as a solid phase product, molecular biology tools were utilized to study sulfate and arsenate reducing activities along the depth of the filter beds. The sulfate reducing population was dominated by complete oxidizers related to the *Desulfobacterium-Desulfococcus-Desulfonema-Desulfosarcina-Desulforhabdium* assemblage within the *Desulfobacteraceae*. Bacteria closely related to *Geobacter uraniireducens* were the predominant dissimilatory arsenate reducing bacteria (DARB) in the system (Chapter 4). While sulfate reducing bacteria (SRB) and DARB were distributed throughout the reactors, sulfate and arsenate reducing activities increased after complete denitrification and attained their respective maximum levels in the lower part of the first reactor and middle of the second reactor, respectively (Chapter 4). The simultaneous presence of both sulfate and arsenate reducing activities along the length of the reactor was considered essential for optimal arsenic removal as

demonstrated in the study of the effect of EBCT changes on reactor performance (Chapter 5). Enhanced biological sulfate and arsenate reduction resulted in the precipitation of mackinawite (FeS_{1-x}) and greigite (Fe_3S_4) and arsenic removal was attributed to the coprecipitation with or adsorption on iron sulfides or precipitation of arsenic sulfides (Chapter 3). The presence of an electron donor (Chapter 6 and Chapter 7) and fresh generation of iron sulfides (Chapter 5 and Chapter 7) were critical for effective arsenic removal and sustained reactor performance (Chapter 7). Recognizing the possibility of the generation of deleterious gaseous species of nitrate reduction (Ahn et al., 2010) and arsenic transformations (Bright et al., 1994) under anaerobic conditions, it was demonstrated that nitrous oxide (N_2O) and arsine, monomethylarsine, dimethylarsine, and trimethylarsine did not form in the reactor system (Chapter 3).

The reactor system was optimized with respect to the EBCT, carrier gas used for backwashing, and nutrient levels in the influent. The EBCT optimization was motivated by the desire to minimize reactor volume as well as the interest in reducing the volume of arsenic-containing sludge and the sludge collection frequency. Backwashing is necessary in the operation of a fixed-bed bioreactor for sustained contaminant removal (Brown et al., 2005). However, frequent backwashing results in an increased production of contaminants-laden backwash waste (i.e., biomass and precipitated solids). To minimize the arsenic-containing sludge production, the possibility of confining sulfate reduction and subsequent arsenic removal to the second reactor of the two-reactor system without

compromising reactor performance was evaluated by lowering the EBCT of the first reactor (Chapter 5). Microbial populations responded to the changes in the EBCT in the first reactor. For example, the TEAP zone for sulfate reduction shifted towards the second reactor when the EBCT of the first reactor was lowered, suggesting a shift in spatial positioning of SRB along the flow direction. This spatial shifting of TEAP zones corresponded well with reactor performance (Chapter 5). However, while the EBCT of 7 min in the first reactor (total EBCT 27 min) substantially minimized sulfate reduction in this reactor, a complete shift of sulfate reduction to the second reactor was not achieved resulting in considerable arsenic removal in the first reactor. In fact, >90% arsenic removal (influent 200 µg As/L, effluent 10 to 20 µg As/L) was achieved at the optimal EBCT of 10 min in the first reactor (total EBCT 30 min) (Chapter 5), suggesting the need for evaluating an alternative sludge minimization approach. The shifting of TEAP zones along the flow direction during occasional accidental oxygen intrusion suggests the requirement of the optimization of dissolved oxygen levels in the influent.

In general, maintaining reducing conditions in an anaerobic bioreactor that relies on biologically generated sulfides for contaminant removal may require the use of an oxygen-free carrier gas (e.g., N_2) during backwashing of the reactor. However, using compressed air rather than N_2 gas has practical advantages including ease of reactor operation, safety, and lower cost. By comparing reactor performance during backwashing with either compressed air or N_2 gas, it was determined that comparable arsenic removal was achieved, while nitrate removal

was not impacted by the backwashing. Thus, this study suggested the viability of replacing N_2 gas with air during backwashing in a bioreactor removing arsenic under a reducing environment.

While the availability of phosphorus enhances microbial growth and consequently improves reactor performance (Li et al., 2010), its presence in excess may limit the availability of iron(II) for the generation of iron sulfides due to the precipitation of Fe-P solids, such as strengite ($FePO_4.2H_2O$) (Nriagu, 1972a) and vivianite ($Fe_3(PO_4)_2.8H_2O$) (Nriagu, 1972b). This in turn may impact arsenic removal, if iron sulfides are used as the arsenic sequestering solids. While optimizing phosphate levels, it was determined that 0.5 mg/L PO_4^{3-} as P resulted in the precipitation of vivianite (predicted by computer simulations using the software MINIQL+) and limited the availability of iron(II) for the generation of iron sulfides. Enhanced iron availability upon lowering the concentration of phosphate to 0.1 mg/L PO_4^{3-} as P resulted in improved arsenic removal in the system (Chapter 7). This result emphasizes the importance of optimization of P levels in an arsenic removing bioreactor system operated under sulfate reducing conditions.

By utilizing environmental molecular biology methods (microbial community structure analyses, microbial population dynamics, and microbial activity assessment) and environmental chemistry tools (X-ray absorption spectroscopy (XAS), X-ray diffraction (XRD)), and analytical chemical analyses) and correlating the data obtained with reactor performance results, this study has established the mechanistic basis for the effective removal of nitrate and arsenic

using a BAC based water treatment system. Blending engineering practices with scientific knowledge from microbial ecology, environmental chemistry, and material science, findings of this study demonstrated the relationship between operational parameters and reactor performance and how they may be optimized for effective water treatment. The technology developed has the potential to be applied by water utilities in nitrate-contaminated, arsenic-contaminated, or arsenic and nitrate contaminated areas around the world.

8.2 Future Perspectives

The findings in this study demonstrated the potential of utilizing BAC systems for the simultaneous removal of nitrate and arsenic form drinking water sources. To further strengthen the knowledge base of this technology and evaluate practical challenges in its implementation, future work should focus on evaluating biological stability of finished water and stability of arsenic in the arsenic-laden sludge under landfill environments. Starting with batch experiments on the toxicity characteristic leaching test (TCLP) and California waste extraction test (Cal-WET), the stability of the solids during long term exposure needs to be evaluated for typical landfill environmental conditions. The final effluent from the reactor system should be characterized for the presence of microorganisms through total bacterial count, live bacterial count, heterotrophic plate count, and other microbiological methods to evaluate the stability of treated water. In this respect, electron donor optimization experiments may also be performed to minimize the effluent organic carbon and limit the microbial re-growth potential.

For the application of the technology developed in this study in rural arsenic-affected communities in South East Asian countries, the practicality of the present reactor system to be owned, operated, and maintained by local communities needs to be explored. In this respect, the use of GAC as the support medium and acetic acid as the electron donor may present challenges. Therefore, future work should evaluate the possibility of utilizing locally and easily available materials, such as sand or wood chips as a support material for biofilm development. Future efforts to minimize operational costs may also include investigating the potential of locally available alternative electron donor substrates, such as softwood and tree leaves given that such substrates have been successfully utilized for nitrate (Gibert et al., 2008) and sulfate removal (Liamleam and Annachhatre, 2007) in other engineered systems. In addition, the impact of various dissolved oxygen levels in the influent on reactor performance needs to be evaluated. Successful outcomes from these future studies could help in the adoption of this type of treatment process for the removal of arsenic and nitrate from contaminated drinking water sources in developing countries.

8.3 References

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Appendix: Chemical constituents in the influent, effluent from reactor A (EA), and Effluent from reactorB

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
54	7.98	26.4	45.0	49.9	22.2	311	0.1	2.9	7.72	15.5	97.5	0.0	21.5	311	1.0	7.64	17.3	62.8	0.0	19.9	295	7.3
55	-	26.6	42.9	46.9	21.4	311	0.1	3.0	7.75	16.6	50.7	0.0	21.9	314	0.6	7.60	14.9	62.0	0.0	19.9	287	6.2
56	-	22.7	44.4	49.0	21.9	312	0.1	3.0	-	16.8	45.3	0.0	22.1	320	0.5	-	15.1	105.3	0.0	19.8	305	7.4
57	-	30.0	44.2	48.9	21.8	311	0.1	3.3	8.18	19.2	46.5	0.0	22.0	314	0.5	7.32	14.1	282.0	0.0	20.1	295	6.9
58	-	20.2	43.4	49.1	20.3	317	0.1	2.4	7.71	17.8	51.3	0.0	17.9	293	1.4	7.68	16.4	160.9	0.0	15.3	286	11.4
59	6.64	-	36.5	48.8	19.4	312	0.3	3.1	7.13	19.4	56.4	0.0	18.5	234	1.6	7.11	20.2	54.2	0.0	15.6	160	12.5
59	6.59	-	68.6	48.8	19.5	306	0.3	2.6	7.62	19.5	53.5	0.0	18.1	232	1.4	7.38	20.3	55.3	0.0	14.8	99	9.7
60	6.63	38.4	173.8	47.0	19.5	298	1.2	2.4	7.76	18.8	42.6	0.0	18.2	224	1.1	7.56	17.0	55.3	0.0	14.3	92	8.6
61	6.94	36.2	89.0	47.2	19.6	308	0.4	2.4	7.33	13.2	43.3	0.0	18.4	203	0.9	7.12	16.4	56.9	0.0	13.7	99	8.3
62	-	37.4	44.7	48.7	23.3	309	0.1	2.1	-	15.0	45.3	0.0	18.5	169	1.5	-	16.4	56.0	0.0	13.5	56	9.6
63	6.57	45.2	45.2	47.3	19.9	306	1.7	1.9	7.15	16.3	43.9	0.1	18.9	159	1.0	7.13	14.5	56.8	0.0	13.1	55	8.2
64	7.67	45.4	42.2	49.3	20.2	313	0.2	1.7	7.51	17.2	43.3	0.0	18.4	136	1.0	7.25	13.8	48.4	0.0	13.0	40	8.5
65	-	45.2	42.4	47.3	20.3	312	0.1	1.6	-	16.4	41.1	0.0	18.2	149	0.8	-	14.2	49.9	0.0	12.4	47	10.3
65	6.96	41.3	42.3	46.9	20.0	312	0.1	1.9	7.31	16.6	46.3	0.0	18.0	143	1.0	7.12	13.9	48.3	0.0	12.2	36	8.0
66	-	0.0	45.5	48.7	20.4	320	0.2	1.9	-	35.4	39.0	0.0	17.2	110	1.1	-	34.0	52.4	0.0	11.5	50	10.1
67	-	0.0	30.1	46.3	20.1	322	0.1	2.0	-	27.2	40.2	0.0	17.5	136	0.8	-	26.1	53.3	0.0	10.7	40	6.7
68	6.98	11.5	44.2	47.3	19.9	318	0.2	2.1	7.47	15.4	42.5	0.0	18.0	137	1.0	7.48	16.2	55.8	0.0	10.0	54	5.2
69	6.86	-	46.2	45.5	19.5	320	0.1	2.9	7.14	37.4	40.3	0.0	18.3	118	0.8	7.20	38.8	53.6	0.0	10.9	21	9.4
70	6.97	-	-	45.6	20.1	320	0.1	3.4	7.65	15.7	45.8	0.0	20.5	113	0.6	7.26	13.3	57.8	0.0	11.4	27	5.4
71	7.26	-	45.1	44.8	22.2	329	0.0	3.8	7.57	18.1	46.0	0.0	20.3	157	0.2	7.31	18.6	58.4	0.3	11.1	39	5.5
72	7.44	11.1	44.5	45.1	22.2	329	0.0	3.6	7.54	0.0	46.4	0.0	20.9	156	0.3	7.46	0.0	58.9	0.4	11.0	47	3.4
73	-	0.0	44.5	45.6	22.3	330	0.1	3.0	-	0.0	45.1	19.0	21.9	767	0.1	-	0.0	47.2	1.5	21.9	656	2.0
74	6.84	35.7	40.3	46.6	51.3	325	0.1	2.9	6.96	43.8	40.9	0.0	50.3	89	0.6	6.97	42.9	53.2	0.0	47.5	52	12.8
75	7.56	-	41.0	1.0	52.3	324	0.1	2.5	6.84	-	41.0	1.3	50.6	72	0.6	6.87	-	53.7	0.0	47.1	15	11.8

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
76	-	-	41.1	1.3	52.0	330	0.1	1.9	-	-	41.8	0.0	52.0	59	0.5	6.75	-	54.3	0.0	50.5	10	10.1
77	7.12	19.4	40.0	44.7	21.1	316	0.3	1.4	7.20	11.5	40.8	3.0	19.3	97	0.2	7.04	14.6	58.7	0.0	15.6	30	4.7
78	7.38	0.0	41.0	50.0	21.1	329	0.2	1.4	7.51	0.0	41.1	0.0	20.7	198	0.3	7.34	0.0	53.3	0.0	15.7	127	4.4
79	6.77	43.9	61.3	53.5	21.4	303	0.2	1.3	7.21	22.2	41.4	0.5	19.6	142	0.6	7.33	20.9	53.4	0.0	14.6	97	9.3
81	6.66	-	40.2	49.1	21.5	329	0.3	1.2	7.20	22.0	41.9	0.3	19.5	90	0.4	7.22	19.6	56.9	0.0	12.8	45	6.8
82	6.58	-	40.2	48.1	21.6	294	0.2	1.0	7.20	26.1	41.8	0.0	19.1	66	0.4	7.24	19.6	54.8	0.0	12.9	60	6.5
83	7.20	-	40.8	51.8	21.7	295	0.1	0.9	7.26	-	42.2	0.0	19.5	70	0.2	7.30	-	55.4	0.0	14.0	27	4.4
84	6.17	33.9	43.9	52.0	21.8	320	0.1	2.6	7.24	22.5	42.0	0.0	19.9	54	0.2	7.29	23.5	55.5	0.0	14.2	20	6.5
85	6.25	34.8	40.7	49.8	21.6	319	0.1	1.4	6.95	22.9	40.5	0.0	19.9	46	0.4	7.07	23.0	53.4	0.0	14.5	20	9.3
86	7.31	36.9	40.2	47.5	21.0	316	0.1	-	7.46	16.8	42.3	0.0	22.1	80	0.1	7.23	15.1	53.8	0.0	19.8	23	4.0
87	-	30.7	40.0	47.1	22.1	296	0.3	-	7.33	2.8	46.4	0.0	20.9	131	3.0	7.29	1.2	55.7	0.0	14.0	75	3.5
88	7.12	32.8	39.0	47.8	22.1	318	0.1	1.5	7.49	4.0	41.6	0.0	21.0	133	0.4	7.56	3.1	53.0	0.0	12.5	120	4.0
89	-	33.1	36.4	50.0	22.4	317	0.1	0.5	7.35	25.3	40.2	0.0	20.9	65	0.2	7.40	25.9	54.2	0.0	15.2	91	7.9
90	6.53	40.6	36.4	52.6	23.0	307	0.1	0.3	7.24	22.2	40.4	0.0	20.6	53	0.2	7.36	20.5	53.0	0.0	12.5	48	7.3
91	-	42.1	39.4	48.1	24.0	322	0.1	0.5	7.22	21.7	39.0	0.0	18.9	50	0.2	7.20	20.0	53.5	0.0	12.3	34	7.7
92	7.34	31.6	37.4	46.0	23.2	327	0.1	0.8	7.27	22.7	40.9	0.0	19.8	47	0.2	7.20	20.3	54.3	0.0	8.4	24	6.1
93	7.31	34.6	38.3	48.0	24.3	323	0.1	0.4	7.24	23.1	39.7	0.0	19.8	39	0.2	7.21	20.6	53.7	0.0	11.9	18	6.0
94	6.97	32.8	37.9	48.4	22.6	335	0.1	0.4	7.38	4.4	38.2	0.0	18.8	125	0.1	7.48	1.5	51.3	0.0	13.7	132	3.2
95	7.21	35.0	37.3	48.8	23.0	322	0.1	0.9	7.24	19.0	38.5	0.0	18.1	37	0.2	7.29	17.9	51.6	0.0	10.6	35	6.1
96	7.61	36.2	38.3	49.8	22.5	339	0.1	1.2	7.52	17.8	38.8	0.0	17.2	36	0.2	7.09	17.9	51.8	0.0	10.2	20	5.7
97	7.87	32.7	38.0	45.0	20.0	329	0.1	0.4	7.26	22.1	38.5	0.0	14.0	31	0.2	7.14	19.3	53.1	0.0	5.8	26	4.7
98	7.84	33.6	39.4	46.8	21.4	328	0.1	0.6	7.12	24.4	40.2	0.0	11.3	33	0.2	7.87	24.7	54.3	0.0	6.9	31	5.4
99	-	-	38.5	39.7	20.4	331	0.1	0.9	6.92	-	37.8	0.0	13.7	32	0.5	7.21	27.3	59.0	0.0	7.0	24	4.6
100	-	-	38.9	39.9	19.7	332	-	1.2	-	17.1	68.4	0.0	12.8	38	-	-	16.9	52.3	0.0	7.8	26	-
101	-	-	38.9	40.1	20.7	321	0.1	1.0	-	17.8	40.9	0.4	17.7	34	0.2	-	15.9	52.2	0.0	10.8	28	5.4
102	-	-	39.5	40.4	20.8	310	0.1	0.5	7.26	16.7	39.3	0.0	16.6	32	0.1	7.44	17.1	52.8	0.0	10.4	33	4.6

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
103	7.04	39.9	40.0	38.8	22.0	311	0.1	0.4	7.21	19.1	40.2	0.0	16.9	35	0.2	7.36	18.0	54.3	0.0	11.3	0	5.4
104	-	-	44.8	38.0	25.9	330	0.1	0.5	7.24	-	44.8	0.0	20.4	68	0.2	7.26	-	44.8	0.0	13.2	55	5.8
105	6.97	-	45.3	36.2	25.2	349	0.2	0.5	7.19	-	45.5	0.0	20.5	53	0.2	7.20	-	63.6	0.0	12.8	51	6.4
106	-	-	46.6	36.9	25.9	297	0.0	0.6	7.08	-	45.8	0.0	19.8	38	0.2	7.17	-	60.4	0.0	11.6	14	4.8
108	-	-	46.4	36.8	26.1	335	0.1	0.5	7.18	-	46.0	0.0	20.0	34	0.2	7.36	-	59.2	0.0	10.8	22	4.9
109	-	-	45.3	37.3	24.9	301	0.0	0.9	7.34	24.1	-	0.0	19.7	41	0.2	7.30	21.1	-	0.0	11.0	23	5.5
110	-	-	43.3	38.1	24.6	322	0.1	0.3	-	23.4	-	0.0	19.2	72	0.2	-	22.0	-	0.0	10.1	26	4.4
111	-	-	41.1	37.7	24.4	324	0.1	0.4	7.48	22.7	-	0.0	17.1	37	0.1	7.57	20.3	-	0.0	10.6	29	3.6
112	6.75	-	42.4	37.1	24.5	334	0.1	0.5	7.38	20.8	-	0.0	19.0	82	2.3	7.13	20.0	-	0.0	11.7	54	0.2
113	-	-	43.3	35.7	24.4	336	0.1	0.4	-	23.4	-	0.0	19.7	32	0.1	-	21.3	-	0.0	11.1	23	3.9
114	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
115	-	-	42.3	37.9	24.3	326	0.1	1.0	7.27	22.2	40.7	0.0	17.1	30	0.5	7.10	19.7	53.5	0.0	9.8	12	2.4
116	-	-	42.1	33.8	20.7	333	0.1	0.7	-	21.2	40.5	0.0	13.4	26	0.4	-	18.3	53.2	0.0	8.7	17	3.7
117	-	-	41.6	34.2	21.4	344	0.1	0.8	-	20.3	41.2	0.0	15.4	34	0.4	-	18.2	67.0	0.0	9.3	-	-
118	-	-	41.9	34.1	21.1	315	0.0	0.6	-	22.1	40.6	1.2	16.2	24	0.2	-	21.6	55.4	0.0	8.6	19	4.5
119	-	-	41.7	34.6	22.3	317	0.0	0.6	-	22.5	41.2	0.0	15.6	28	0.2	-	20.4	55.1	0.0	8.2	22	4.5
120	-	-	43.9	34.6	21.2	337	0.0	0.7	-	-	42.7	0.0	14.7	28	0.2	-	-	57.3	0.0	5.8	27	4.4
121	-	-	43.9	36.3	21.2	322	0.0	-	-	-	47.8	0.0	0.0	31	12.2	-	-	54.0	0.0	1.1	22	2.7
122	-	-	-	-	-	-	-	1.4	-	-	-	-	-	833	1.4	-	-	-	-	-	140	31.4
124	-	0.0	42.4	35.4	21.2	326	0.0	0.5	-	-	53.1	0.0	21.4	626	3.1	-	-	53.0	0.0	21.7	230	3.1
125	-	0.0	41.5	36.3	22.0	312	0.0	0.9	-	-	52.9	0.0	22.4	657	4.9	-	-	53.2	0.0	21.3	164	2.9
126	-	0.0	41.4	36.0	22.7	319	0.0	0.9	-	0.0	42.5	0.2	23.2	711	2.2	-	0.2	43.4	0.0	22.6	272	2.3
128	-	0.0	40.4	34.4	22.9	318	0.1	1.0	-	-	37.1	0.0	15.9	362	5.1	-	16.3	19.6	0.0	21.2	175	2.3
128	-	0.0	41.4	35.4	22.0	319	0.0	0.5	-	13.4	65.9	0.0	20.9	367	5.5	-	14.3	67.4	0.0	19.3	820	2.9
129	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	-	0.0	50.6	35.2	21.0	334	0.0	0.7	-	0.0	51.5	8.8	21.6	360	2.3	-	3.8	56.7	0.0	19.8	71	0.2

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
131	-	0.0	50.0	37.2	21.2	320	0.0	1.4	-	13.7	66.3	0.0	20.7	130	1.3	-	15.2	67.0	0.0	18.3	120	1.7
132	-	0.0	50.1	36.1	21.1	340	0.1	0.3	-	18.5	68.8	0.0	20.8	254	8.2	-	15.9	67.9	0.0	18.6	128	4.6
134	-	-	-	-	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	-	0.0	40.1	34.1	23.0	319	0.1	0.8	-	0.0	45.3	0.0	23.0	234	0.1	-	0.0	40.0	0.0	23.9	880	0.1
136	-	0.0	42.9	35.8	22.0	329	0.0	1.3	-	18.4	66.1	0.0	22.3	300	6.4	-	19.0	63.2	0.0	18.9	231	4.1
137	-	0.0	47.0	38.2	22.1	330	0.0	0.6	-	12.8	63.1	0.0	22.9	162	2.2	-	8.6	62.7	0.0	20.7	162	2.4
138	-	0.0	48.1	35.1	21.8	330	0.0	0.6	-	18.1	64.1	0.0	23.4	184	2.9	-	17.4	63.7	0.0	20.5	169	2.0
139	-	0.0	47.2	36.4	23.2	333	0.0	0.8	-	28.1	66.1	3.8	23.4	201	5.1	-	21.2	65.2	0.0	20.2	178	2.4
140	-	0.0	48.9	32.5	22.9	329	0.0	-	-	21.7	64.3	2.5	22.8	115	0.2	-	20.8	65.2	0.0	19.3	115	0.0
142	-	0.0	48.4	32.2	24.1	334	0.1	-	-	31.5	66.8	3.8	23.9	129	0.1	-	17.8	64.8	0.0	21.1	611	0.1
143	-	0.0	49.9	33.2	22.2	321	0.0	-	-	21.7	67.2	2.5	23.3	171	5.0	-	20.5	66.8	0.0	20.1	176	4.3
144	-	0.0	47.4	28.9	23.3	322	0.0	-	-	24.2	48.9	0.0	22.9	220	2.7	-	23.3	48.9	0.0	18.9	142	2.4
145	-	-	-	-	-	-	-	-	-	24.1	49.0	0.0	23.0	300	1.8	-	23.4	49.2	0.0	19.1	173	1.6
146	-	0.0	47.9	29.1	23.2	337	0.0	-	-	24.3	56.7	3.8	23.1	397	2.4	-	24.3	49.2	0.0	18.5	194	1.1
147	-	0.0	48.0	29.2	23.2	337	0.0	-	-	24.9	49.9	0.0	22.1	316	1.7	-	23.3	49.2	0.0	18.2	144	1.4
148	-	0.0	-	28.7	22.3	326	0.0	-	-	24.2	42.3	0.0	21.2	310	1.1	-	23.6	49.2	0.0	17.5	129	1.0
149	-	0.0	-	28.6	22.6	333	0.0	-	-	24.7	43.4	0.0	22.3	267	1.1	-	23.4	48.1	0.0	18.0	111	0.8
151	-	0.0	-	27.7	22.0	335	0.0	-	-	22.0	41.8	0.0	22.2	177	1.2	-	24.1	41.6	0.0	17.5	49	1.0
152	-	0.0	42.3	28.0	23.1	333	0.0	-	-	23.0	41.7	0.0	22.1	166	1.2	-	23.2	41.6	0.0	15.5	31	0.9
153	-	0.0	43.1	26.4	22.3	339	0.0	-	-	24.2	42.1	0.0	21.9	159	1.0	-	22.6	42.0	0.0	15.4	30	0.7
154	-	0.0	42.2	25.3	23.2	327	0.0	-	-	24.5	42.0	0.0	21.8	150	0.9	-	23.6	41.9	0.0	14.8	38	0.7
155	-	0.0	43.0	26.3	23.4	331	0.0	-	-	34.5	42.2	0.0	21.4	132	0.9	-	23.6	42.2	0.0	14.4	22	0.6
156	-	0.0	41.7	25.7	19.7	305	0.0	-	-	-	41.7	0.0	17.9	131	0.7	-	20.6	42.1	0.0	11.4	21	0.4
157	-	0.0	37.7	28.8	17.9	330	0.0	-	-	21.4	41.6	1.7	18.5	222	0.7	-	18.5	41.5	0.0	14.9	108	1.5
159	-	0.0	41.6	25.2	19.8	337	0.0	-	-	24.0	41.3	0.0	18.0	160	0.7	-	17.5	33.9	0.0	11.8	32	0.6

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
160	-	0.0	41.6	25.4	20.0	326	0.0	-	-	23.0	41.4	0.0	17.9	162	1.0	-	20.9	41.6	0.0	13.6	29	1.0
161	-	-	41.5	49.8	20.6	336	0.0	-	-	-	-	-	-	154	0.6	-	-	-	-	-	24	0.5
162	-	0.0	40.7	49.9	20.7	334	0.0	-	-	22.6	41.5	0.0	18.3	143	0.6	-	21.0	41.6	0.0	13.7	62	0.4
163	-	0.0	41.2	48.9	21.2	337	0.1	-	-	23.1	41.7	0.0	18.2	137	0.7	-	19.2	36.9	0.0	11.7	39	0.4
164	-	0.0	40.0	49.3	20.1	320	0.0	-	-	20.0	46.3	0.0	20.5	140	1.0	-	-	43.4	0.0	13.5	59	0.6
165	-	0.0	40.2	50.3	21.2	311	0.0	-	-	22.2	41.9	0.0	18.4	148	0.9	-	22.5	41.9	0.0	12.7	23	0.4
166	-	0.0	39.9	49.3	20.2	322	0.0	-	-	21.8	42.2	0.0	12.6	134	0.9	-	23.6	42.0	0.0	18.2	21	0.3
167	-	0.0	39.3	49.4	20.1	321	0.0	-	-	0.0	40.5	15.4	24.3	641	0.3	-	0.0	40.9	23.2	24.3	133	0.3
169	-	0.0	37.9	49.1	19.8	333	0.0	-	-	5.4	39.7	0.0	18.4	262	0.3	-	4.2	39.6	0.0	14.8	74	0.1
170	-	0.0	38.3	49.0	20.1	310	0.0	-	-	6.1	39.6	0.0	18.1	241	0.3	-	5.4	39.4	0.0	14.1	82	0.1
173	-	0.0	38.1	51.0	20.3	292	0.0	-	-	22.5	38.8	0.0	17.5	120	0.7	-	21.3	38.9	0.0	12.8	56	0.2
174	-	0.0	37.9	50.8	20.3	288	0.0	-	-	19.8	38.8	0.0	17.7	111	0.7	-	19.8	39.0	0.0	12.3	30	0.2
175	-	0.0	37.7	50.3	20.2	289	0.0	-	-	26.3	38.9	0.0	17.7	100	0.9	-	21.0	38.9	0.0	12.2	18	0.3
176	-	0.0	38.3	48.9	20.6	300	0.0	-	-	19.1	39.0	0.0	17.1	82	0.9	-	21.1	39.1	0.0	11.4	13	0.3
177	-	0.0	37.9	49.7	20.3	300	0.1	-	-	21.6	39.3	0.0	17.3	93	1.1	-	20.2	39.2	0.0	10.7	19	0.4
178	-	0.0	41.7	48.5	20.1	310	0.0	-	-	-	42.3	0.0	16.4	92	1.1	-	-	42.0	0.0	10.0	52	0.3
179	-	0.0	41.6	54.1	19.7	332	0.1	-	-	12.4	42.7	0.0	17.4	178	0.7	-	11.3	42.8	0.0	11.1	21	0.2
179	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
181	-	-	-	-	-	-	-	-	-	19.0	42.9	0.0	16.5	218	0.6	-	-	-	-	-	56	0.2
182	-	0.0	42.0	53.9	20.0	335	0.1	-	-	24.7	42.7	0.0	16.4	201	0.8	-	20.5	42.9	0.0	10.6	90	0.2
183	-	0.0	41.8	54.2	20.2	329	0.1	-	-	25.6	56.7	0.0	16.5	220	1.3	-	15.1	43.0	0.0	11.2	75	0.2
184	-	-	-	-	-	-	-	-	-	17.6	54.5	0.0	15.6	176	2.4	-	13.7	55.0	0.0	10.4	35	0.2
185	-	0.0	41.4	55.7	19.8	324	0.0	-	-	-	57.9	0.0	14.8	230	5.1	-	11.2	55.3	0.0	8.3	60	0.3
185	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
187	-	0.0	40.9	56.2	19.8	308	0.0	-	-	11.4	53.9	0.0	10.5	251	0.5	-	11.6	53.9	0.0	10.5	126	0.6
188	-	0.0	41.0	55.8	19.8	314	0.0	-	-	14.3	42.3	0.0	16.8	281	0.6	-	11.1	53.8	0.0	9.6	194	0.6

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
190	-	0.0	42.3	52.3	20.1	316	0.0	-	-	10.0	41.4	0.0	18.0	330	0.1	-	6.4	56.9	0.0	9.7	330	1.3
191	-	0.0	41.3	51.9	19.8	298	0.0	-	-	9.3	41.8	0.0	19.1	302	1.5	-	2.8	56.7	0.0	11.1	340	0.1
192	-	0.0	44.5	51.7	21.7	294	0.1	-	-	8.5	44.7	0.0	19.1	329	0.2	-	6.0	59.7	0.0	11.0	336	2.4
194	-	0.0	40.9	50.3	20.8	292	0.0	-	-	8.9	41.4	0.0	18.1	316	0.3	-	3.6	55.7	0.0	10.4	328	1.5
195	-	0.0	40.5	50.2	20.7	340	0.0	-	-	13.0	42.4	0.0	18.5	332	0.5	-	9.0	55.4	0.0	11.2	303	4.2
196	-	0.0	40.2	49.9	20.6	325	0.1	-	-	14.2	41.3	0.0	17.4	278	0.6	-	10.5	41.4	0.0	9.6	301	2.7
197	-	0.0	40.5	49.6	20.4	336	0.0	-	-	13.5	41.4	0.0	17.6	273	0.4	-	10.1	41.6	0.0	10.7	281	1.5
198	-	0.0	45.2	47.1	18.9	323	0.0	-	-	12.4	45.8	0.0	15.8	167	0.9	-	15.7	46.1	0.5	9.2	140	1.0
200	-	0.0	48.3	53.3	19.3	325	0.0	-	-	12.5	45.9	0.0	15.7	103	0.7	-	5.9	45.9	0.0	8.0	42	1.0
201	-	0.0	45.3	53.2	19.5	327	0.0	-	-	11.2	46.0	0.0	15.2	100	1.0	-	4.9	44.9	0.0	7.4	31	0.8
202	-	0.0	45.1	52.9	19.3	327	0.0	-	-	15.5	46.1	0.0	15.3	91	0.9	-	9.5	46.2	0.0	7.3	12	0.5
203	-	0.0	45.2	53.0	19.5	335	0.0	-	-	0.0	45.4	29.9	20.8	682	0.6	-	0.0	45.5	9.0	-	-	0.3
203	-	-	-	-	-	341	0.0	-	-	0.0	42.2	0.0	17.2	171	0.7	-	0.0	42.2	0.0	12.3	54	0.5
205	-	0.0	40.9	49.1	19.1	328	-	-	-	0.0	41.3	0.0	9.9	110	0.9	-	0.0	41.7	0.0	9.9	53	0.3
206	-	0.0	40.7	48.8	19.0	323	0.0	-	-	4.7	42.2	0.0	15.1	84	0.9	-	12.9	56.2	0.0	11.9	72	0.3
208	-	0.0	35.9	49.8	20.1	329	0.0	-	-	0.0	42.3	0.0	15.3	79	0.9	-	0.2	41.8	0.0	8.9	51	0.3
209	-	0.0	40.2	49.4	18.8	331	0.0	-	-	4.9	41.6	0.0	14.6	81	0.9	-	3.0	42.2	0.0	8.8	15	0.4
211	-	0.0	35.5	50.0	19.6	310	0.0	-	-	-	36.6	0.0	15.7	77	0.7	-	-	36.7	0.0	9.5	38	0.4
212	-	0.0	35.9	49.8	20.0	315	0.0	-	-	-	36.7	0.0	14.8	64	0.7	-	-	37.3	0.0	9.4	29	0.2
214	-	0.0	35.2	49.2	19.5	332	0.0	-	-	-	37.2	0.0	15.1	72	0.8	-	-	36.6	0.0	8.5	20	0.2
216	-	0.0	40.8	49.7	19.2	328	0.0	-	-	-	36.7	0.0	14.8	74	0.8	-	-	36.4	0.0	9.2	31	0.5
217	-	0.0	40.5	51.2	20.6	333	0.0	-	-	-	42.4	0.0	15.8	85	0.8	-	-	41.7	0.0	9.9	51	0.2
218	-	0.0	40.3	50.8	20.5	311	0.0	-	-	-	41.3	0.0	14.6	58	0.8	-	-	41.6	0.0	8.8	26	0.2
219	-	0.0	40.5	50.1	20.0	337	0.0	-	-	-	41.1	0.0	14.4	62	0.8	-	-	41.3	0.0	7.8	25	0.1
220	-	0.0	40.1	49.6	19.9	354	0.0	-	-	31.8	41.8	0.0	15.0	97	0.9	-	-	41.4	0.0	10.4	25	0.2
221	-	0.0	40.0	49.1	19.9	333	0.0	-	-	13.9	41.6	0.0	14.6	80	0.7	-	-	41.6	0.0	7.1	23	0.1

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
222	-	0.0	40.3	48.7	20.2	329	0.0	-	-	11.9	41.2	0.0	14.1	41	0.8	-	-	41.7	0.0	7.3	29	0.2
223	-	0.0	41.7	50.0	20.2	345	0.0	-	-	20.0	41.9	0.0	14.6	65	0.9	-	9.4	42.2	0.0	7.1	35	0.2
224	-	0.0	40.7	48.6	19.7	360	0.0	-	-	-	47.5	0.0	14.2	40	1.0	-	14.7	42.2	0.0	6.8	36	0.0
225	-	0.0	41.0	47.0	20.0	311	0.0	-	-	24.5	41.9	0.0	13.8	17	0.8	-	25.1	42.5	0.0	6.7	11	0.2
227	-	-	-	-	-	319	0.1	-	-	-	-	-	-	23	0.6	-	-	-	-	-	19	0.2
228	-	0.0	40.7	49.5	20.1	308	0.0	-	-	30.4	41.6	0.0	15.9	32	0.8	-	17.7	42.5	0.0	7.0	16	0.0
230	-	0.0	40.5	45.5	20.0	306	-	-	-	30.4	41.3	0.0	14.3	31	-	-	17.7	42.2	0.0	7.8	21	0.2
231	-	-	-	-	-	299	0.1	-	-	-	-	-	-	6	1.4	-	-	-	-	-	0	0.5
233	-	0.0	40.8	49.2	75.4	305	0.0	-	-	88.0	41.0	0.0	66.4	22	1.3	-	82.2	41.1	0.0	6.5	12	0.5
233	-	0.0	40.9	49.2	75.5	300	0.0	-	-	88.0	42.1	0.0	65.9	17	1.3	-	82.0	41.4	0.0	6.3	12	0.6
238	-	0.0	40.5	49.0	21.5	300	0.0	-	-	0.0	41.8	0.0	16.3	72	0.6	-	0.0	41.1	0.0	11.4	27	0.3
238	-	0.0	40.5	49.3	21.5	309	0.1	-	-	35.0	41.9	0.0	14.0	29	0.6	-	26.3	41.9	0.0	6.6	24	0.2
240	-	0.0	40.8	48.1	21.0	323	0.0	-	-	32.4	41.7	0.0	13.6	18	0.6	-	23.4	41.8	0.0	6.4	32	0.2
240	-	0.0	40.2	48.4	21.8	300	0.0	-	-	36.8	41.7	0.0	14.0	45	0.7	-	29.0	42.1	0.0	6.1	16	0.2
243	-	0.0	40.6	48.6	20.0	326	0.1	-	-	-	42.1	0.0	12.2	37	0.9	-	-	42.0	0.0	4.5	13	0.3
243	-	0.0	40.8	47.7	20.1	327	0.3	-	-	-	42.1	0.0	11.8	39	0.8	-	-	42.1	0.0	4.1	11	0.3
244	-	0.0	40.8	48.3	21.5	334	0.3	-	-	-	41.9	0.0	11.6	37	0.9	-	-	42.1	0.0	3.6	27	0.3
246	-	0.0	40.8	48.4	21.2	318	0.0	-	-	-	42.1	0.0	10.7	28	0.8	-	-	42.0	0.0	3.7	16	0.2
246	9.14	0.0	40.6	49.7	21.2	325	0.1	0.9	-	-	42.0	0.0	11.5	32	0.9	-	-	42.3	0.0	2.9	19	0.3
247	9.20	0.0	117.9	49.4	22.3	321	0.1	1.2	-	-	132.6	0.0	14.3	32	0.8	-	-	92.8	0.0	5.9	19	0.3
249	9.26	0.0	91.1	49.2	22.5	322	0.1	1.1	-	-	84.5	0.0	14.7	99	0.7	-	-	42.0	0.0	6.3	45	0.2
250	9.14	0.0	42.0	49.5	23.1	318	0.1	1.6	-	29.0	41.7	0.0	15.5	71	0.8	-	-	40.8	0.0	5.6	26	0.2
251	9.15	0.0	42.2	49.3	23.7	309	0.1	1.3	-	-	42.2	0.0	13.3	80	0.7	-	-	41.9	0.0	5.6	26	0.2
251	9.17	-	-	-	-	-	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
254	9.18	0.0	-	49.9	23.8	321	0.1	0.9	-	23.2	-	0.0	13.6	35	0.8	-	-	42.0	0.0	5.5	17	0.3
254	9.40	0.0	-	48.6	22.6	326	0.1	0.8	-	-	-	0.0	13.6	31	0.9	-	-	-	0.0	5.2	32	0.2

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
256	9.38	0.0	-	48.6	22.6	328	0.1	0.8	-	-	-	0.0	13.5	34	-	-	-	-	0.0	5.3	31	-
257	9.42	0.0	-	48.5	22.8	319	0.1	1.0	-	-	-	0.0	13.6	38	0.8	-	-	-	0.0	4.8	33	0.2
257	9.17	-	-	-	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
257	9.12	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
259	9.09	0.0	-	48.3	22.8	318	0.1	0.8	-	28.0	-	0.0	13.6	35	0.7	-	22.1	-	0.0	6.2	7	0.2
260	-	0.0	43.3	48.7	22.9	320	0.1	0.8	7.36	-	44.3	0.0	13.1	50	0.6	-	-	44.3	0.0	4.3	7	0.2
261	9.12	0.0	104.4	47.3	23.2	321	0.0	0.7	7.36	-	89.3	0.0	12.7	48	0.6	-	-	85.8	0.0	4.5	29	0.2
262	9.12	0.0	43.3	48.7	23.0	319	0.0	0.6	7.39	-	44.7	0.0	12.9	34	0.5	-	-	43.9	0.0	3.8	7	0.2
264	9.09	0.0	42.0	49.4	22.5	320	0.0	0.0	7.62	-	42.3	0.0	14.3	82	0.5	-	19.3	43.1	0.0	6.6	72	0.4
264	9.09	0.0	41.3	49.2	22.8	287	0.0	0.0	7.42	-	42.2	0.0	13.8	56	0.5	-	-	42.4	0.0	6.4	23	0.3
265	9.08	0.0	41.3	49.4	22.9	329	0.1	0.0	7.42	-	42.1	0.0	13.4	37	0.4	-	-	42.3	0.0	6.1	16	0.2
265	-	0.0	41.1	48.7	23.6	312	-0.1	0.0	7.36	-	42.4	0.0	13.6	49	0.5	-	-	42.5	0.0	5.7	25	0.2
267	9.09	0.0	41.3	48.3	22.7	306	-0.1	0.0	7.29	-	40.9	0.0	12.7	39	0.6	-	-	112.3	0.0	4.8	19	1.1
267	-	0.0	40.9	48.2	22.5	308	-0.1	0.0	7.33	-	43.9	0.0	13.4	52	0.6	-	-	44.8	1.9	4.9	15	0.2
268	9.06	0.0	42.3	43.4	21.8	326	-0.1	0.0	7.30	-	43.8	0.0	1.3	27	0.8	-	-	44.4	0.0	2.7	14	0.4
269	9.06	0.0	42.4	43.2	24.4	324	0.0	0.0	7.39	-	45.0	-	12.4	33	0.8	-	-	45.3	0.0	4.3	21	0.3
270	8.96	0.0	41.8	43.1	23.0	328	0.0	0.0	7.43	-	45.1	0.0	13.8	54	0.9	-	-	44.7	0.0	4.7	25	0.2
271	8.96	0.0	41.7	43.2	22.9	319	0.0	0.0	-	-	44.7	0.0	13.5	51	1.0	-	-	44.4	0.0	5.0	21	0.0
271	9.01	0.0	41.9	43.0	24.2	322	0.0	0.0	7.29	-	44.7	0.0	13.6	45	0.9	-	-	45.2	0.0	4.4	19	0.2
272	-	0.0	41.7	42.8	22.0	320	0.0	0.0	7.36	-	44.9	0.0	12.9	46	0.9	-	-	42.4	0.0	5.3	21	0.2
274	-	0.0	41.4	49.3	20.5	296	0.0	0.0	-	-	44.0	0.0	9.8	44	-	-	-	45.2	1.2	3.5	18	0.0
275	9.01	0.0	41.4	49.0	20.5	296	0.0	0.0	7.27	-	43.9	0.0	9.6	33	1.0	-	-	43.4	0.0	1.9	14	0.3
276	9.01	0.0	41.3	48.9	20.5	303	0.0	0.0	7.23	-	43.4	0.0	9.6	40	0.8	-	-	43.7	0.0	1.6	18	0.1
277	8.88	0.0	41.1	48.1	20.5	308	0.0	0.0	7.27	-	44.1	0.0	9.1	44	0.9	-	-	44.1	0.6	1.2	14	0.2
279	-	0.0	41.2	48.6	23.3	303	0.0	-	7.28	-	43.8	0.0	12.0	36	0.9	-	-	43.9	0.0	3.3	14	0.2
280	-	0.0	40.9	48.3	23.4	304	0.0	0.1	-	-	44.0	0.0	12.4	40	0.7	-	-	43.9	0.0	2.7	21	0.2

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
281	9.06	0.0	41.5	48.3	23.6	313	0.0	0.0	7.22	-	44.2	0.0	12.0	40	0.8	-	-	43.5	0.0	1.9	18	0.2
282	9.06	0.0	42.3	48.2	23.5	317	0.0	0.0	7.23	-	51.5	0.0	3.0	26	1.0	-	-	43.8	0.0	1.7	20	0.2
285	8.96	0.0	38.7	48.1	21.8	294	0.0	0.0	7.31	18.4	40.9	0.0	11.0	24	0.3	7.29	12.9	41.8	0.0	4.5	12	0.1
285	8.96	0.0	38.6	48.5	21.8	288	-0.1	0.0	7.27	19.6	41.4	0.0	10.6	33	0.6	7.30	16.9	40.7	0.0	1.8	16	0.1
286	9.01	0.0	38.5	48.2	21.8	290	0.0	0.0	7.19	20.4	41.0	0.0	10.2	43	0.7	7.26	20.3	41.6	0.0	2.3	21	0.2
291	9.00	0.0	38.3	47.4	21.8	301	0.0	0.0	7.07	36.2	41.1	0.0	17.8	228	1.7	7.29	19.7	41.4	0.0	9.7	49	0.8
292	9.01	0.0	38.3	45.0	21.4	283	0.0	0.0	7.14	20.7	40.9	0.0	14.1	70	1.2	7.22	16.4	41.3	0.0	4.7	24	0.3
292	9.01	0.0	38.1	47.1	21.8	303	0.0	0.0	7.16	20.8	41.4	0.0	13.0	53	1.1	7.24	19.1	41.3	0.0	4.2	19	0.3
293	8.88	0.0	44.2	43.3	23.5	317	0.0	0.0	7.22	14.0	46.1	0.0	15.5	118	0.8	7.29	10.6	45.9	0.0	7.2	61	0.2
295	-	0.0	43.4	51.2	24.9	323	0.0	0.0	-	17.8	46.0	0.0	13.8	56	0.7	-	15.5	45.6	0.0	5.3	32	0.2
296	-	0.0	42.6	48.0	24.9	332	0.0	-	-	20.3	45.7	0.0	12.7	60	0.7	-	17.5	44.6	0.0	4.3	27	0.1
301	9.07	0.0	24.3	48.0	21.3	304	0.0	0.5	7.12	12.4	24.5	0.0	9.0	39	0.5	7.25	7.5	19.2	0.0	4.3	18	0.1
302	9.07	0.0	24.4	48.1	21.6	296	0.0	0.1	7.11	16.7	26.7	0.0	14.3	107	0.6	7.12	9.5	17.7	0.0	3.1	44	0.2
303	9.03	0.0	24.5	48.2	20.9	306	0.0	0.2	7.12	16.0	26.6	-	13.2	107	0.7	7.25	14.7	26.7	0.0	2.3	23	0.1
303	9.03	0.0	24.2	48.5	21.3	311	0.0	0.5	7.13	17.9	26.7	-	13.8	103	0.8	7.26	14.9	26.3	0.0	3.6	20	0.1
304	8.76	0.0	37.0	49.1	21.5	297	0.0	0.4	7.09	19.0	39.4	-	13.4	84	1.0	7.13	14.1	39.4	0.0	4.0	15	0.1
306	8.72	0.0	36.7	48.4	21.1	298	0.0	0.5	7.09	14.5	38.9	-	14.0	88	1.0	7.13	14.5	39.2	0.0	5.3	23	0.2
306	8.70	0.0	36.7	48.2	21.2	271	0.0	0.7	7.13	14.8	38.7	-	14.5	90	0.4	7.13	15.6	38.7	0.0	6.0	15	0.2
312	-	0.0	40.2	47.5	22.3	288	0.0	-	-	23.2	41.7	0.1	17.3	208	1.2	-	18.9	41.5	0.0	5.1	43	0.3
312	-	0.0	38.1	49.8	22.4	296	0.0	-	-	21.0	41.8	0.0	16.5	158	0.8	-	19.6	41.9	0.0	3.4	39	0.2
315	-	0.0	39.2	48.5	0.0	301	0.0	-	-	24.3	41.8	0.0	0.0	251	1.1	-	23.2	41.4	0.0	0.0	50	0.4
315	9.62	0.0	39.5	49.1	0.0	300	0.0	1.4	7.33	20.1	41.1	0.0	0.0	286	1.1	7.30	20.1	40.9	0.0	0.0	84	0.4
316	9.67	0.0	39.2	48.7	0.0	300	0.0	1.5	7.33	12.3	41.0	0.0	0.0	304	1.3	7.29	15.9	41.3	0.0	0.0	106	0.5
318	9.50	0.0	40.3	48.8	0.0	310	0.0	0.9	7.19	16.4	40.7	0.0	0.0	257	1.4	7.27	17.9	41.3	0.0	0.0	114	0.7
318	9.59	0.0	41.4	48.6	0.0	310	0.1	1.3	7.19	19.0	40.5	0.0	0.0	232	127.6	7.33	14.5	40.1	0.0	0.0	164	0.8
319	9.66	0.0	39.8	49.0	21.4	300	0.1	1.8	7.39	18.9	41.5	0.0	18.4	159	1.3	7.39	18.8	42.0	0.0	3.7	53	0.4

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
320	9.65	0.0	39.8	48.4	21.4	300	0.1	1.6	7.19	17.0	41.9	0.0	18.6	97	0.8	7.29	20.8	42.2	0.0	5.2	28	0.3
321	9.66	0.0	39.9	48.9	21.4	304	0.1	0.6	7.33	20.2	41.5	0.0	18.3	93	0.8	7.33	14.8	41.7	0.0	5.0	21	0.2
323	10.02	0.0	40.7	50.3	14.1	286	0.0	0.6	7.56	21.1	43.1	0.0	9.9	177	0.3	7.56	25.0	43.0	0.0	0.5	34	0.1
324	-	0.0	40.1	49.3	14.1	287	0.0	-	-	23.3	42.4	0.0	9.1	173	0.3	-	25.4	42.8	0.0	0.1	35	0.1
325	9.65	0.0	39.6	48.4	14.0	289	0.1	0.5	-	17.9	42.5	0.0	10.1	211	0.2	-	24.0	42.8	0.0	0.7	45	0.1
327	-	0.0	40.4	47.9	14.7	297	0.0	-	7.77	25.1	42.5	0.0	10.6	218	0.3	7.86	25.6	43.0	0.0	0.5	78	0.1
329	9.78	0.0	41.3	47.4	25.4	305	0.0	0.5	7.44	16.6	43.0	0.0	23.4	420	1.3	7.59	12.2	43.0	0.0	10.2	78	0.1
330	9.76	0.0	40.9	55.5	25.4	303	0.0	0.4	7.51	17.0	42.8	0.0	22.8	220	0.5	7.59	12.1	42.8	0.0	9.9	78	0.1
331	-	0.0	40.7	55.2	25.6	302	0.0	0.4	7.36	16.5	42.7	0.0	23.2	182	0.8	7.43	17.7	42.9	0.0	9.0	41	0.1
332	-	0.0	40.0	54.6	25.4	299	0.0	-	-	19.2	42.7	0.0	23.0	182	1.0	-	10.9	43.1	0.0	8.5	52	0.1
333	9.85	0.0	40.2	54.4	24.1	316	0.0	0.5	7.62	11.2	42.8	0.0	21.3	213	0.9	7.59	12.7	42.9	0.0	9.8	71	0.1
334	9.72	0.0	41.8	52.1	24.5	315	0.1	0.8	7.44	18.2	44.4	0.0	20.9	244	0.9	7.54	13.4	43.9	0.0	10.0	75	0.1
335	9.63	0.0	41.7	53.3	23.8	302	0.1	0.6	7.61	16.6	44.2	0.0	21.2	257	0.4	7.61	12.9	43.5	0.0	11.5	80	0.1
336	9.72	0.0	41.8	51.0	25.0	296	0.0	0.4	7.45	10.8	43.1	0.0	20.8	173	0.6	7.54	14.7	43.9	0.0	9.9	52	0.0
337	9.72	0.0	40.1	47.1	22.0	292	0.0	0.6	7.41	28.6	41.8	0.0	19.6	195	0.7	7.55	20.1	41.8	0.0	8.7	53	0.0
338	9.60	0.0	42.3	51.8	25.2	315	0.0	0.2	7.34	18.7	44.1	0.0	23.4	240	1.1	7.34	17.1	44.4	0.0	14.1	110	0.2
339		0.0	41.2	49.4	23.6	303	0.0		-	19.1	44.0	0.0	23.3	241	1.3	-	19.5	44.3	0.0	14.5	64	0.1
340	9.67	0.0	40.3	51.5	24.7	303	0.0	-	7.31	18.8	42.2	0.0	21.9	220	1.0	7.54	19.0	38.5	0.0	7.5	48	0.1
341	9.63	0.0	40.1	51.9	23.9	302	0.0	-	7.43	4.8	42.1	0.0	23.3	280	0.7	7.47	8.1	41.8	0.0	9.8	48	0.1
342	-	0.0	40.2	51.7	24.3	303	0.0	-	7.50	1.5	40.9	0.0	23.7	240	0.6	7.40	0.0	39.6	0.0	10.8	45	0.1
343	-	0.0	40.2	51.7	24.3	303	0.0	-	-	19.2	42.2	0.0	22.9	239	1.1	-	15.5	42.2	0.0	9.8	58	0.1
345	-	0.0	40.2	51.7	24.3	303	0.0	-	7.54	18.8	42.4	0.0	21.7	229	1.2	7.57	20.8	42.6	0.0	11.4	47	0.1
345	-	0.0	39.7	52.6	24.3	297	0.0	-	7.37	19.8	42.4	0.0	22.2	220	0.4	7.62	16.0	42.3	0.0	10.3	44	0.1
346	-	0.0	39.7	52.6	36.8	297	0.0	-	-	14.5	41.8	0.0	34.3	244	0.9	-	8.8	41.8	0.0	20.0	42	0.1
347	-	0.0	40.1	50.2	35.9	298	0.0	-	7.41	-	42.1	0.0	33.5	202	0.9	7.51	-	42.2	0.0	19.0	48	0.0
348	-	0.0	39.7	51.3	35.0	299	0.0	-	7.43	-	43.1	0.0	34.1	186	1.0	7.59	-	42.6	0.0	17.3	43	0.0

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
349	-	0.0	39.6	50.3	36.1	296	0.0	-	7.43	-	41.8	0.0	33.0	176	1.0	7.59	-	41.8	0.0	18.4	47	0.1
350	-	0.0	39.2	49.8	34.8	302	0.0	-	7.65	-	40.2	0.0	33.5	175	1.0	7.68	-	42.1	0.0	15.4	43	0.3
352	-	0.0	40.2	52.3	35.3	300	0.0	-	-	-	38.9	0.0	33.5	172	-	-	-	41.9	0.0	17.7	44	-
352	9.69	0.0	38.8	51.8	25.4	297	0.0	-	7.50	22.1	42.1	0.0	22.1	174	1.0	7.59	24.8	42.4	0.0	11.5	45	0.3
354	9.69	0.0	58.9	51.5	25.6	288	0.1	-	7.51	-	47.1	0.0	21.3	169	12.1	7.59	17.4	49.2	0.0	14.8	15	0.5
355	-	0.0	40.9	52.2	25.2	288	0.1	-	7.01	17.9	42.7	0.0	23.7	171	1.5	7.05	13.3	51.0	0.0	11.6	18	0.9
356	-	0.0	58.9	51.5	25.6	288	0.1	-	7.02	18.5	42.8	0.0	23.5	170	1.6	7.05	13.3	51.3	0.0	12.1	20	1.0
357	8.79	0.0	-	50.8	26.0	288	-	-	7.20	25.0	63.4	0.0	22.6	120	1.4	7.13	15.9	70.7	0.0	10.8	18	0.8
358	-	0.0	58.9	51.5	25.6	288	0.1	-	7.05	16.6	44.8	0.0	21.7	149	1.4	7.13	19.4	49.4	0.0	8.4	15	0.7
359	-	4.0	41.0	52.9	26.6	286	-	0.6	7.09	15.9	43.2	0.0	23.9	158	1.4	7.02	13.6	49.4	0.0	9.2	17	0.8
363	-	-	51.9	33.7	31.0	287	-	0.0	-	-	51.5	0.0	27.9	147	1.4	7.07	-	44.0	0.0	11.4	49	0.5
364	-	0.0	50.3	50.8	29.0	288	0.1	-	7.11	-	56.1	0.0	25.8	178	1.3	7.14	-	48.9	0.0	10.1	22	0.5
365	-	0.0	50.3	50.8	29.0	288	0.1	-	7.07	-	52.9	0.0	26.4	182	1.3	7.06	-	60.6	0.0	10.3	27	0.4
366	-	0.0	50.3	50.8	29.0	288	0.1	-	7.05	-	57.2	0.0	28.0	205	1.5	7.12	-	63.1	0.0	11.5	90	0.5
367	-	0.0	50.3	50.8	29.0	288	0.1	-	7.09	-	67.7	0.0	27.5	164	1.3	7.17	-	66.4	0.0	14.9	59	0.4
368	-	0.0	50.3	50.8	29.0	288	0.1	-	7.09	-	59.0	0.0	28.4	155	1.2	7.12	-	87.0	0.0	10.2	41	0.4
370	8.95	-	51.5	43.6	29.7	302	0.0	0.0	-	-	52.1	0.0	28.7	158	1.5	-	-	57.8	0.0	8.9	46	0.4
371	-	-	51.5	43.6	29.7	302	0.0	-	7.00	-	169.1	0.0	24.6	179	1.8	6.95	-	-	0.0	8.1	58	0.5
372	8.91	-	51.5	43.6	29.7	302	0.0	-	7.05	-	49.2	0.0	25.3	194	1.4	7.03	-	57.3	0.0	4.5	18	0.5
373	8.99	-	51.5	43.6	29.7	302	0.0	-	7.01	-	54.4	0.0	25.3	205	1.4	6.99	-	59.7	0.0	8.1	39	0.7
374	-	0.0	48.1	46.0	29.6	310	-	-	6.99	15.1	56.8	0.8	25.6	212	2.2	6.94	10.9	56.7	0.0	8.3	77	0.6
375	9.04	0.0	48.1	46.0	29.6	310	-	-	6.98	15.9	56.4	0.0	27.3	186	1.9	7.05	11.0	57.8	0.0	8.5	65	0.6
376	-	0.0	48.1	46.0	29.6	310	-	-	6.98	16.3	49.2	0.0	25.0	171	1.6	7.02	10.0	162.5	0.0	7.2	45	0.6
377	-	0.0	48.1	46.0	29.6	310	-	-	7.05	-	69.8	0.0	27.7	195	1.5	7.11	-	69.9	0.0	4.7	56	0.5
378	-	-	47.7	45.3	29.4	315	-	-	7.01	10.3	-	0.0	25.0	189	1.4	7.03	13.9	-	0.0	7.2	63	0.4
379	-	-	47.7	45.3	29.4	315	-	-	7.03	16.2	60.6	0.0	26.5	168	1.4	7.06	12.5	60.6	0.0	6.9	87	0.2

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
380	-	-	47.2	44.5	29.3	320	0.0	-	7.08	15.9	67.3	0.0	24.8	140	1.3	7.21	11.3	100.4	0.0	7.5	69	0.3
381	-	-	47.2	44.5	29.3	320	0.0	-	7.06	13.4	212.1	0.0	24.9	210	1.4	7.05	9.0	76.3	0.0	7.1	73	0.5
382	8.87	0.0	47.2	44.5	29.3	320	0.0	-	7.17	15.7	50.0	0.0	24.7	149	1.6	7.09	11.6	71.6	0.0	6.6	92	0.4
387	-	0.0	47.2	44.5	29.3	320	0.0	-	7.07	-	51.2	0.0	24.3	153	1.2	7.00	-	59.4	0.0	6.0	37	0.4
388	-	0.0	47.5	44.6	29.5	303	0.0	-	7.11	-	50.6	0.0	26.3	200	1.3	7.03	-	58.5	0.0	9.5	52	0.9
389	-	0.0	47.2	44.5	29.3	320	0.0	-	7.07	-	50.6	0.0	25.7	180	1.4	6.99	-	58.3	0.0	9.2	29	0.4
390	-	0.0	47.4	44.6	26.4	311	0.0	-	7.05	-	-	0.0	26.0	210	1.3	6.96	-	-	0.0	8.5	39	0.4
391	-	0.0	47.4	44.6	26.4	311	0.0	-	7.10	15.9	-	0.0	26.0	192	1.3	6.98	11.5	-	0.0	10.2	31	0.5
392	-	0.0	47.4	44.6	26.4	311	0.0	-	7.09	14.6	-	0.0	26.1	206	1.3	7.01	10.2	55.8	0.0	8.9	41	0.5
395	-	0.0	48.1	46.0	29.6	310	-	-	7.01	-	52.5	0.0	27.3	189	1.4	6.99	-	-	0.0	3.9	33	0.8
399	-	0.0	47.5	44.6	23.5	303	0.0	-	-	-	48.1	0.0	20.6	191	1.7	-	-	55.1	0.0	6.9	57	0.7
402	-	0.0	47.5	44.6	23.5	319	0.0	-	-	-	49.4	0.0	19.8	210	1.4	-	-	59.4	0.0	4.7	43	0.7
405	-	0.0	65.3	44.6	27.8	298	0.0	-	-	7.3	131.5	0.0	22.4	177	1.0	-	11.0	55.1	0.0	5.1	25	0.5
407	-	44.6	48.9	35.0	27.8	298	-	-	-	16.4	44.8	2.1	21.4	183	1.3	-	11.2	55.4	0.0	3.6	24	0.4
408	-	0.0	65.3	44.6	27.8	298	0.0	-	-	15.7	-	0.0	23.8	199	1.3	-	12.0	-	0.0	6.2	25	0.6
409	-	0.0	65.3	44.6	27.8	298	0.0	-	-	18.9	53.3	2.2	24.5	189	1.2	-	14.5	74.5	0.0	6.1	30	0.5
410	-	0.0	65.3	44.6	27.8	298	0.0	-	-	17.1	-	0.0	23.8	190	1.3	-	12.5	-	0.0	6.4	29	0.6
412	-	0.0	65.3	44.6	27.8	298	0.0	-	-	12.4	-	-	24.9	200	1.4	-	9.7	-	0.0	5.6	25	0.5
413	-	0.0	-	32.3	31.1	303	0.0	-	-	13.5	-	-	26.6	190	1.2	-	11.5	49.2	0.0	5.6	19	0.4
414	-	0.0	-	48.5	30.9	303	0.0	-	-	15.8	52.8	1.9	26.4	219	1.2	-	11.7	-	0.0	6.7	34	0.3
415	-	0.0	-	48.5	30.9	303	0.0	-	-	13.1	-	0.3	26.4	207	2.3	-	9.1	-	0.0	6.8	29	0.5
416	-	0.0	-	48.5	30.9	303	0.0	-	-	17.3	47.9	1.2	27.1	227	1.2	-	12.4	53.5	0.0	6.9	32	0.6
417	-	0.0	-	48.9	32.1	308	0.0	-	7.10	13.7	-	1.6	28.5	232	1.4	7.08	9.4	-	0.0	9.7	64	0.7
418	-	0.0	-	48.9	32.1	308	0.0	-	7.19	17.2	-	0.6	28.6	208	1.3	7.15	11.5	-	0.0	8.9	36	0.5
419	-	0.0	-	48.9	32.1	308	0.0	-	-	10.9	-	0.0	28.9	210	1.2	7.16	11.2	-	0.0	8.0	38	0.4
420	-	0.0	-	48.9	32.1	308	0.0	-	7.31	16.8	51.5	0.0	28.6	206	1.2	7.22	13.0	69.3	0.0	8.1	29	0.3

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
421	-	0.0	-	48.9	32.1	308	0.0	-	7.21	19.7	-	0.0	31.1	202	1.1	7.15	17.3	-	0.0	9.5	29	0.4
422	-	0.0	-	48.9	32.1	308	0.0	-	7.20	19.7	-	1.8	30.2	150	0.8	7.12	10.0	-	0.0	8.0	32	0.4
423	9.00	0.0	-	48.9	32.1	308	0.0	-	7.18	13.9	53.3	0.0	17.7	201	0.2	7.13	12.1	69.3	0.0	4.4	29	0.4
424	-	0.0	-	48.9	32.1	308	0.0	-	7.12	11.1	-	0.0	25.3	202	1.4	7.08	13.2	-	0.0	7.4	36	0.5
425	8.97	0.0	-	48.9	32.1	308	0.0	-	7.17	16.4	-	0.0	25.4	182	1.1	7.04	11.8	-	0.0	8.5	33	0.4
426	-	0.0	46.7	46.8	30.8	303	0.0	-	7.15	14.2	-	0.0	25.8	211	1.1	7.00	11.3	-	0.0	6.7	40	0.5
427	9.12	0.0	46.7	46.8	30.8	303	0.0	-	7.11	16.3	48.5	0.0	27.0	176	1.2	7.08	12.1	-	0.0	6.0	29	0.4
428	-	0.0	46.7	49.0	29.8	308	0.0	-	7.28	7.4	-	18.4	26.2	220	1.0	7.26	4.6	-	0.0	7.0	58	0.3
429	9.05	0.0	46.7	69.3	29.8	308	0.0	-	7.24	7.4	-	19.9	26.5	195	1.0	7.11	4.0	-	0.4	6.8	39	0.3
430	-	0.0	-	68.3	30.3	286	0.0	-	7.32	8.8	-	18.3	26.5	196	0.9	7.25	5.0	-	0.0	5.3	53	0.2
431	9.04	0.0	-	68.2	30.0	299	0.0	-	7.22	-	-	21.6	26.6	197	1.1	7.09	-	-	0.0	7.0	52	0.3
433	8.97	0.0	-	69.2	29.8	300	0.0	-	7.37	-	-	19.9	29.4	233	1.1	7.43	-	-	0.0	12.8	58	0.6
434	-	0.0	-	70.0	30.0	299	0.0	-	7.39	-	-	20.6	29.2	220	0.9	7.28	-	-	0.0	10.2	64	0.5
435	-	0.0	-	70.0	30.3	299	0.0	0.1	-	-	-	21.6	28.7	187	0.9	-	-	-	0.0	9.8	69	0.4
436	-	0.0	-	70.0	33.7	308	0.0	-	7.35	-	-	18.5	29.5	195	1.0	7.20	-	-	0.0	12.2	59	0.5
437	-	0.0	45.5	71.2	32.9	303	0.0	-	7.30	10.6	49.2	21.7	27.8	199	0.2	7.24	4.6	66.3	0.0	11.7	102	0.4
438	-	0.0	-	68.6	34.2	298	0.0	-	7.17	6.1	-	22.6	26.2	280	1.4	7.13	3.4	-	0.0	10.7	104	0.6
439	-	0.0	45.5	71.0	32.9	303	0.0	-	7.24	8.8	-	21.5	25.3	239	1.2	7.21	5.2	68.0	0.0	11.1	69	0.4
440	-	0.0	45.5	67.3	31.0	297	0.0	-	7.14	5.8	-	22.5	26.0	285	1.3	7.12	3.2	-	0.0	10.0	115	0.5
441	-	0.0	45.5	70.0	32.9	303	0.0	-	7.21	6.3	-	21.4	30.8	288	1.2	7.09	2.7	-	0.0	10.4	133	0.5
442	-	0.0	50.6	75.7	33.9	297	0.0	-	7.34	8.0	-	21.1	25.7	203	0.6	7.23	4.7	-	0.0	10.5	53	0.4
443	9.16	0.0	-	70.3	32.1	297	0.0	-	7.35	9.1	-	20.0	26.9	202	0.5	7.10	3.9	-	0.0	9.8	66	0.3
444	-	0.0	-	68.3	31.6	297	0.0	0.8	7.25	10.7	-	21.2	26.3	214	0.5	7.19	6.0	-	0.0	9.3	59	0.4
445	8.83	0.0	-	70.3	32.1	297	0.0	0.5	7.08	16.6	-	21.4	26.1	143	1.0	7.02	11.7	-	0.0	9.0	47	0.5
446	-	0.0	-	66.0	30.8	297	0.0	0.5	7.14	18.5	-	22.0	26.3	148	1.1	7.08	11.8	-	0.0	9.2	47	0.6
447	8.90	0.0	-	70.3	32.1	297	0.0	0.4	-	16.2	-	21.1	26.3	140	1.1	7.00	6.4	-	0.0	9.5	48	0.6

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
449	9.20	0.0	-	68.9	31.3	210	0.0	0.1	7.00	25.6	53.2	26.9	28.5	164	1.9	6.85	19.7	63.0	2.0	21.1	84	3.0
450	-	0.0	-	68.3	36.3	205	0.0	0.2	7.11	13.2	67.8	23.0	27.8	162	1.5	6.98	6.9	-	0.0	13.5	75	1.8
451	9.18	0.0	-	72.0	30.3	210	0.0	0.5	7.03	19.8	43.8	22.9	27.8	162	1.4	-	20.4	-	2.7	15.4	43	1.1
452	-	0.0	50.3	69.3	30.8	210	0.0	-	-	12.9	59.3	21.3	27.0	137	1.3	6.98	8.5	-	0.0	11.1	51	1.2
453	9.11	0.0	91.5	66.8	36.3	205	0.0	0.1	7.20	15.3	48.4	21.0	27.3	143	1.1	7.09	11.9	52.1	0.0	10.2	46	0.5
454	9.02	0.0	74.6	68.3	33.3	208	0.0	0.3	7.02	15.1	50.5	21.0	27.4	148	1.1	7.02	11.9	57.8	0.0	10.6	44	0.5
455	-	0.0	49.4	71.0	31.3	213	0.0	0.3	-	15.5	20.9	23.2	27.6	135	1.4	-	14.4	-	0.0	10.5	31	0.8
456	-	0.0	50.4	73.0	30.6	217	0.0	-	-	12.7	-	21.3	27.6	143	1.0	-	8.4	-	0.0	14.5	42	0.4
457	-	0.0	48.3	69.3	31.2	209	0.0	-	7.10	11.3	-	19.4	27.2	130	1.3	7.00	7.5	-	0.0	13.6	37	0.8
458	-	0.0	49.4	68.9	32.6	213	0.0	0.1	7.11	19.0	46.4	21.0	27.4	114	1.2	7.09	14.2	-	0.0	12.8	28	0.6
459	-	-	49.4	70.3	30.9	213	0.0	0.1	7.04	24.4	-	18.0	26.4	108	1.2	6.98	15.5	-	0.0	13.3	26	0.7
460	-	0.0	49.4	72.0	31.3	213	0.0	0.2	7.02	34.5	-	19.3	22.3	119	1.2	7.00	14.6	54.7	3.1	12.7	36	0.8
461	-	0.0	44.0	70.1	30.0	213	0.0	0.1	7.03	30.0	65.0	18.7	24.8	120	1.1	7.00	16.6	-	0.0	14.2	27	0.8
462	-	0.0	63.3	67.2	31.7	213	0.0	0.1	7.03	23.2	54.2	10.9	27.9	108	1.1	7.02	15.4	-	0.0	13.5	28	0.7
463	-	0.0	44.7	71.2	32.1	214	-	0.2	7.04	27.8	66.3	19.2	26.4	101	1.1	7.00	23.8	-	0.5	20.1	26	0.7
464	-	0.0	76.5	70.1	31.9	213	0.0	0.1	-	-	53.4	6.7	26.5	101	1.1	-	12.7	53.7	0.0	14.1	31	0.9
465	-	0.0	45.4	69.3	31.8	213	0.0	0.1	7.02	17.6	47.0	0.0	26.3	112	1.1	6.95	14.6	53.6	0.0	14.3	30	1.0
466	-	0.0	47.2	68.2	33.2	213	0.0	0.3	6.99	21.9	62.4	0.0	32.3	106	1.2	6.94	17.9	-	0.0	15.9	33	0.9
467	-	0.0	42.6	54.0	30.8	213	0.0	0.0	6.99	21.7	62.2	0.0	30.7	132	1.3	7.01	15.9	-	0.0	16.4	44	1.2
468	-	0.0	48.2	53.8	31.7	213	0.0	0.2	7.11	21.9	-	0.0	30.6	151	1.3	7.02	15.7	-	0.0	16.0	48	1.1
469	-	0.0	45.4	53.9	32.2	213	0.0	0.3	7.15	24.1	52.8	0.0	32.1	132	1.2	7.07	14.7	-	0.0	17.1	32	0.8
470	8.83	0.0	45.4	56.7	32.2	213	0.0	0.8	7.00	16.6	-	0.0	32.2	150	1.2	7.05	12.3	-	0.0	18.6	51	0.7
471	-	0.0	45.4	56.7	32.2	213	0.0	0.3	7.10	16.4	46.1	0.0	27.3	112	1.2	7.00	13.0	60.7	0.0	15.5	30	0.9
472	-	0.0	53.2	46.2	32.1	209	0.0	0.3	7.12	16.6	43.8	0.0	27.7	112	1.1	7.00	13.5	52.3	0.0	15.2	35	1.0
473	-	0.0	57.0	43.8	31.6	221	0.0	0.2	7.12	14.2	56.1	0.0	26.7	107	1.1	7.15	13.5	44.3	0.0	16.7	34	1.2
474	8.96	0.0	53.2	46.2	31.3	209	0.0	0.6	7.18	14.4	45.4	0.0	27.9	139	1.1	7.04	11.3	46.0	0.0	14.9	48	0.9

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
476	8.98	0.0	52.0	47.1	31.0	214	0.0	0.2	7.24	14.7	45.8	0.0	20.3	153	1.2	7.07	11.5	55.1	0.0	9.0	52	1.0
477	-	0.0	53.2	46.2	33.2	209	0.0	0.2	7.14	15.0	46.4	0.0	20.4	155	1.1	7.02	10.1	-	0.0	10.9	73	1.2
478	-	0.0	49.4	47.6	31.0	196	0.0	0.2	7.18	14.2	57.6	0.0	20.2	155	1.1	7.04	11.5	48.3	0.0	10.3	57	1.0
481	-	0.0	44.4	46.7	23.2	210	0.0	0.2	7.12	15.2	42.0	0.0	19.4	152	1.2	7.02	12.5	44.2	0.0	7.5	39	0.9
482	8.90	0.0	54.1	48.7	23.5	210	0.0	0.2	7.29	11.8	-	0.0	18.8	152	1.2	7.14	3.8	43.2	0.0	8.9	43	0.9
483	-	0.0	63.8	50.8	23.8	210	0.0	0.3	7.20	14.2	51.3	0.0	18.9	154	1.2	7.08	11.5	-	0.0	8.2	49	0.9
484	-	-	43.2	50.4	24.7	211	0.0	0.2	7.22	10.5	-	0.0	18.5	154	1.2	7.11	11.3	44.5	0.0	8.9	65	1.0
485	-	0.0	43.2	49.3	24.3	211	0.0	0.2	7.17	15.4	43.1	0.0	19.1	157	1.2	7.11	11.9	-	0.0	8.8	55	1.0
486	-	-	43.2	50.4	24.7	209	0.0	0.1	7.19	13.1	48.2	0.0	18.4	151	1.2	7.10	10.5	53.4	0.0	7.7	46	1.1
487	-	0.0	43.2	51.0	24.9	211	0.0	0.2	7.10	11.4	-	0.0	18.2	147	1.1	6.99	6.4	-	0.0	6.4	47	0.9
488	-	0.0	43.2	51.0	24.9	211	0.0	0.7	6.98	13.4	43.2	0.0	18.4	139	1.2	6.95	10.1	49.2	0.0	7.3	36	0.8
490	-	-	42.2	50.1	23.3	196	0.0	-	-	11.1	-	0.0	17.5	124	1.1	-	12.2	-	0.0	5.3	34	0.7
491	-	-	40.0	48.5	22.7	196	-	-	-	14.5	-	0.0	17.6	123	1.2	-	6.3	-	0.0	5.7	38	0.7
492	-	-	40.0	48.5	22.7	196	0.0	-	-	8.7	-	0.0	18.7	124	1.0	-	9.5	-	0.0	4.8	34	0.5
493	-	0.0	37.9	47.0	22.1	196	0.0	-	-	15.3	-	0.0	18.5	120	1.0	-	11.8	-	0.0	5.8	33	0.5
494	-	0.0	40.0	46.4	22.9	197	0.0	-	-	13.2	-	0.0	18.6	133	1.0	-	8.7	-	0.0	5.5	42	0.4
495	-	0.0	-	46.2	23.0	195	0.0	-	-	12.8	-	0.0	18.4	121	1.8	-	10.9	-	0.0	6.6	35	0.6
496	-	0.0	37.0	46.2	22.9	198	0.0	-	-	13.6	-	0.0	18.7	122	0.9	-	10.8	-	0.0	6.4	32	0.6
497	-	0.0	37.0	46.2	22.9	198	0.0	-	-	10.5	-	0.0	18.4	146	1.2	-	7.1	-	0.0	6.2	39	0.9
498	-	0.0	37.0	46.2	22.9	201	0.0	-	-	8.3	-	0.0	18.5	172	1.2	-	9.5	-	0.0	5.7	44	0.6
499	-	0.0	37.0	46.2	22.8	198	0.0	-	-	13.2	70.7	0.0	17.8	170	1.2	-	10.3	-	0.0	4.1	49	0.6
504	-	0.0	37.0	46.2	22.8	198	0.0	-	-	15.1	40.0	0.0	19.2	168	0.8	-	12.4	49.5	0.0	8.6	68	0.5
505	-	0.0	35.2	46.3	22.4	229	0.0	-	-	14.5	36.8	0.0	17.3	154	0.8	-	12.8	53.0	0.0	7.9	60	0.4
506	-	0.0	35.2	46.7	23.1	229	0.0	-	-	14.6	-	0.0	17.7	164	0.9	-	11.9	-	0.0	7.8	73	0.5
507	-	0.0	-	44.0	22.7	236	0.0	-	-	14.2	-	0.0	17.6	153	1.0	-	11.9	-	0.0	8.0	63	0.6
509	-	-	-	43.8	22.8	238	0.0	-	-	14.4	-	0.0	18.4	154	0.9	-	11.3	-	0.0	8.1	77	0.6

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
511	-	-	-	43.8	22.8	238	0.0	-	-	19.1	37.7	3.5	20.2	176	1.1	-	13.4	43.4	0.0	8.3	75	1.0
512	-	0.0	-	43.6	23.0	239	0.0	-	-	16.3	-	0.0	19.7	156	1.0	-	13.6	-	0.7	8.4	73	1.0
515	-	0.0	34.8	43.2	22.9	225	0.0	-	-	16.1	64.1	0.0	19.5	120	0.6	-	12.4	78.9	0.0	7.4	70	0.3
516	-	0.0	34.8	45.9	23.9	206	0.0	-	-	15.6	37.6	1.7	19.0	145	0.8	-	17.4	45.0	0.0	8.9	62	0.6
518	-	0.0	34.8	44.6	23.4	221	0.0	-	-	16.2	39.1	0.0	23.3	163	0.1	-	10.9	45.0	0.0	9.0	65	0.4
519	-	0.0	-	46.0	24.3	215	0.0	-	-	16.1	39.3	0.0	23.0	158	0.1	-	12.5	46.8	0.0	5.2	31	0.3
520	9.55	0.0	35.3	46.5	23.9	204	0.0	0.2	7.18	17.0	46.4	2.4	21.9	247	0.1	7.09	14.1	50.7	0.0	7.6	45	0.5
521	-	0.0	35.3	46.5	23.9	203	0.0	0.3	7.06	15.3	39.1	0.0	22.1	218	0.2	6.97	12.2	54.0	0.0	7.5	35	0.5
522	9.57	0.0	35.3	46.5	23.9	203	0.0	0.3	6.87	10.3	40.0	0.0	21.4	227	1.4	6.92	3.5	51.2	0.0	7.5	40	0.7
523	-	0.0	37.0	45.7	23.7	217	0.0	0.1	-	16.0	36.3	0.0	21.7	40	1.2	-	12.5	48.4	0.0	9.0	25	-
524	9.52	0.0	46.8	44.0	22.6	210	0.0	0.3	7.22	18.5	37.9	0.0	21.6	76	1.1	7.07	15.1	47.8	0.0	9.8	51	-
525	-	0.0	-	44.0	22.5	211	0.0	0.5	6.81	87.2	45.4	0.0	21.6	29	0.9	6.89	126.6	92.2	0.0	6.6	36	-
526	9.62	0.0	46.8	44.0	22.6	210	0.0	0.1	7.26	21.4	38.4	0.0	22.5	106	1.0	7.13	14.4	43.3	0.0	10.6	65	-
527	-	0.0	34.3	42.3	21.7	203	-	0.2	7.30	16.6	43.3	0.0	20.6	88	-	7.16	14.9	44.6	0.0	6.5	39	-
528	9.58	0.0	46.8	44.0	22.6	210	0.0	0.2	7.26	17.2	36.1	0.0	21.1	95	-	7.20	13.2	46.4	0.0	6.8	36	-
529	-	0.0	36.4	43.5	22.3	202	-	0.1	7.25	19.3	38.2	0.0	20.9	88	-	7.19	15.3	44.7	0.0	6.4	39	-
531	-	0.0	34.6	43.0	23.0	201	-	0.3	7.30	18.1	36.1	0.0	20.8	77	-	7.16	15.8	42.1	0.0	7.5	38	-
532	-	0.0	34.4	43.1	23.2	202	-	1.3	7.26	16.6	36.0	0.0	20.3	69	-	7.02	12.9	56.1	0.0	4.8	19	-
533	-	0.0	34.6	43.0	23.0	201	-	1.3	-	17.2	36.7	0.0	22.1	71	-	6.90	13.5	50.2	0.0	6.2	16	-
534	8.76	0.0	32.9	42.3	23.4	200	-	0.2	6.89	17.5	36.9	0.0	21.0	84	-	6.83	13.4	50.9	0.0	7.2	14	-
535	-	0.0	34.0	44.0	23.3	211	-	0.2	6.70	17.6	37.2	0.0	22.9	60	-	6.84	14.2	43.5	0.0	6.7	17	-
536	9.01	0.0	34.0	43.8	23.4	211	-	0.4	6.70	17.2	36.7	0.0	22.1	71	-	6.90	13.5	50.2	0.0	6.2	16	-
537	8.92	0.0	34.1	43.5	23.5	211	-	0.3	6.92	16.2	37.6	0.0	20.6	73	-	6.92	12.1	43.6	0.0	5.7	19	-
538	-	0.0	34.0	43.8	23.4	211	-	-	-	17.5	35.8	0.0	20.5	82	-	6.89	13.9	42.7	0.0	7.6	20	-
539	8.86	0.0	34.0	43.8	23.4	211	-	0.5	6.85	19.2	35.7	0.0	21.0	75	-	6.89	15.6	47.2	0.0	10.2	20	-
540	8.95	0.0	33.0	43.5	22.4	200	-	0.6	7.00	17.0	34.5	0.0	20.3	96	-	7.14	13.5	40.3	0.0	7.7	24	-

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
541	-	0.0	32.5	43.5	22.6	203	-	0.5	6.83	17.7	36.6	0.0	20.7	76	-	6.80	14.5	41.3	0.0	6.8	19	-
542	-	0.0	32.8	43.2	22.8	210	-	0.6	6.80	17.3	34.7	0.0	20.1	90	-	6.69	13.7	41.2	0.0	7.5	19	-
543	8.92	0.0	31.7	43.8	22.6	200	-	0.7	6.92	18.6	35.6	0.0	19.9	83	-	6.90	14.4	43.1	0.0	7.1	20	-
544	-	0.0	32.5	43.5	22.6	203	-	0.3	6.89	18.2	35.9	0.0	20.2	78	-	-	15.6	40.6	0.0	8.0	23	-
546	-	0.0	32.5	43.5	22.6	203	-	-	6.89	18.2	35.9	0.0	20.2	93	-	-	15.6	40.6	0.0	10.2	49	-
547	-	0.0	32.7	43.5	22.1	204	-	-	-	8.6	35.4	0.0	21.7	107	-	7.02	10.0	41.1	0.0	11.5	52	-
548	-	0.0	32.7	43.5	22.1	204	-	-	-	17.3	36.5	0.0	21.5	120	-	-	14.6	42.5	0.0	9.7	39	-
549	-	0.0	32.7	43.5	22.1	204	-	-	-	8.2	36.7	0.0	22.0	95	1.2	-	9.3	43.0	0.0	11.0	47	-
550	9.45	0.0	33.7	48.4	23.7	202	0.0	0.1	7.54	-	34.3	0.0	23.0	113	1.1	7.02	5.4	39.6	0.0	13.4	49	-
551	-	0.0	33.5	48.4	23.5	201	0.0	0.2	7.21	16.4	39.0	0.0	20.0	76	1.2	7.19	13.2	46.4	0.0	8.5	45	0.5
553	9.52	0.0	33.7	48.4	23.7	202	0.0	0.4	7.10	19.6	36.5	0.0	20.7	90	1.3	7.03	15.5	44.4	0.0	8.9	40	0.5
554	-	0.0	33.9	48.3	24.0	202	0.0	0.2	7.26	15.1	36.6	0.0	19.9	82	1.3	7.11	11.9	43.3	0.0	8.4	42	0.5
555	9.58	0.0	33.7	48.4	23.7	202	0.0	0.1	7.19	15.1	37.3	0.0	20.0	64	1.4	7.15	12.7	45.2	0.0	7.0	32	0.5
557	9.51	0.0	33.7	50.5	23.2	202	0.0	0.2	7.11	15.2	37.2	0.0	19.9	-	-	7.17	12.7	45.0	0.0	7.1	-	-
558	8.83	0.0	32.9	47.1	23.6	216	0.0	0.1	6.78	-	15.3	0.0	20.8	-	-	6.78	12.8	43.9	0.0	7.5	-	-
559	8.88	0.0	33.6	46.5	24.0	215	0.0	0.3	6.85	-	15.3	0.0	18.7	53	2.0	6.92	14.5	70.0	0.0	7.6	19	0.9
560	8.84	0.0	32.5	46.3	23.8	216	0.0	0.4	6.78	-	16.9	0.0	19.6	52	-	6.82	13.8	42.1	0.0	7.0	14	0.8
561	-	0.0	32.7	46.4	23.7	216	0.0	-	6.82	-	18.8	0.0	19.7	46	1.8	6.77	14.3	42.0	0.0	6.7	13	0.9
562	8.86	0.0	31.7	45.7	23.6	216	0.0	0.5	6.87	-	17.9	0.0	19.6	49	1.8	6.91	14.5	43.0	0.0	5.3	14	0.9
563	-	0.0	32.7	46.4	23.7	216	0.0	0.5	6.72	-	17.7	0.0	18.9	38	1.7	6.74	14.9	43.3	0.0	7.0	14	1.0
565	8.84	0.0	34.0	45.3	22.4	213	0.0	0.9	6.86	18.3	36.7	0.0	17.8	38	1.6	6.83	15.0	46.0	0.0	4.8	15	0.9
567	8.77	0.0	33.2	43.5	22.4	216	0.0	0.6	6.89	16.0	35.5	0.0	17.2	33	1.6	6.78	14.4	44.5	0.0	4.8	12	0.7
568	-	0.0	33.6	44.4	22.4	214	0.0	0.4	6.84	4.0	35.3	0.0	9.3	29	1.1	6.83	11.8	44.6	0.0	4.2	11	0.6
570	9.22	0.0	33.1	51.7	24.0	213	0.0	0.2	7.07	18.2	37.2	0.0	18.4	34	1.5	7.11	15.4	52.5	0.0	5.2	11	0.5
572	8.79	0.0	32.9	45.2	21.4	210	0.0	0.3	6.87	14.0	35.3	2.3	15.2	30	1.5	6.87	14.7	42.5	0.0	3.4	11	0.6
574	8.89	0.0	32.9	45.8	20.5	210	0.0	0.7	6.85	26.9	38.1	0.0	18.5	40	1.5	6.85	13.6	42.5	0.0	4.3	12	0.4

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
578	8.89	0.0	33.4	46.2	21.1	216	0.0	0.7	8.35	-	31.2	19.2	21.3	870	-	8.22	-	45.3	4.0	23.2	1429	0.1
579	-	0.0	33.4	46.2	21.1	216	0.0	0.2	8.53	-	47.2	0.0	24.2	738	-	7.82	0.0	45.5	4.3	29.6	1660	-
582	9.18	0.0	35.5	43.8	19.5	210	0.0	0.2	7.13	12.3	44.8	0.0	12.7	102	-	7.11	8.6	42.5	0.0	7.0	399	-
585	8.80	0.0	32.8	42.1	18.9	187	0.0	0.1	6.77	12.4	35.7	0.0	16.4	29	-	6.76	9.6	43.1	0.0	4.3	45	-
587	-	0.0	37.6	51.0	25.2	202	0.0	0.1	6.85	21.0	40.0	0.0	18.3	38	1.1	6.73	16.8	46.3	12.8	4.5	24	1.5
588	-	0.0	37.6	51.0	25.2	202	0.0	0.1	6.74	19.8	40.8	0.0	17.3	24	1.2	6.71	17.9	48.6	0.0	5.6	25	1.5
589	-	0.0	37.6	51.0	25.2	202	0.0	0.1	6.84	20.6	40.5	0.0	18.9	33	1.1	6.74	20.3	48.1	0.0	6.1	20	1.1
590	-	0.0	37.6	51.0	25.2	202	0.0	0.4	6.91	20.0	40.2	0.0	17.1	34	1.2	6.73	17.8	48.2	0.0	5.7	23	0.9
591	-	0.0	37.6	51.0	25.2	202	0.0	0.5	6.87	21.0	40.9	0.0	18.5	30	1.0	6.77	19.2	48.3	0.0	5.6	18	0.8
593	8.60	0.0	37.2	50.9	25.7	205	0.0	1.0	6.80	18.1	39.3	0.0	18.6	31	1.1	6.75	16.1	51.7	0.0	6.7	11	0.8
594	-	0.0	35.9	50.9	23.2	210	0.0	0.4	7.07	17.4	42.6	0.0	15.5	31	0.8	6.95	14.3	47.2	0.0	3.4	11	0.4
595	-	0.0	35.9	50.9	23.2	210	0.0	0.4	6.85	16.8	37.5	0.0	14.5	23	0.9	6.67	14.4	45.6	0.0	4.1	7	0.6
596	-	0.0	35.9	50.9	23.2	210	0.0	0.6	6.96	18.0	37.8	0.0	14.8	28	1.1	6.94	15.2	54.0	0.0	3.9	9	0.6
597	-	0.0	35.9	50.9	23.2	210	0.0	0.3	6.96	19.4	39.2	0.0	14.7	19	0.7	6.89	16.4	54.3	0.0	3.3	8	0.3
598	8.92	0.0	34.6	50.9	20.8	216	0.0	0.2	6.96	19.6	37.8	0.0	15.3	24	0.8	7.01	16.6	50.8	0.0	1.0	10	0.3
599	-	0.0	24.1	47.0	23.9	218	0.0	0.6	6.86	17.0	39.9	0.0	16.1	36	1.0	6.95	13.3	52.1	0.0	3.7	11	0.5
600	-	0.0	24.1	47.0	23.9	218	0.0	0.7	6.94	12.1	39.3	0.0	15.5	25	0.8	6.96	15.3	54.0	0.0	3.2	9	0.4
601	-	0.0	24.1	47.0	23.9	218	0.0	0.9	6.90	18.9	43.6	0.0	13.4	13	0.7	6.87	16.7	53.2	0.0	3.5	7	0.4
602	-	0.0	24.1	47.0	23.9	218	0.0	1.5	7.03	19.6	26.4	0.0	16.1	32	0.9	7.20	16.0	48.8	0.0	3.2	10	0.6
603	-	0.0	24.1	47.0	23.9	218	0.0	-	7.02	20.6	37.9	0.0	17.7	36	0.9	7.10	16.7	46.0	0.0	3.4	8	0.6
605	8.84	0.0	32.2	48.1	23.9	216	0.0	0.4	7.09	21.4	35.2	0.0	14.2	18	0.6	6.96	19.1	42.6	0.0	BDL	10	0.6
606	8.85	0.0	32.1	46.0	24.5	221	0.0	0.5	7.13	16.8	35.6	0.0	14.3	19	0.6	7.10	19.2	44.0	0.0	3.5	9	0.6
607	-	0.0	32.2	47.1	24.2	218	0.0	0.5	7.17	17.1	35.9	0.0	16.9	18	0.5	7.28	14.8	44.9	0.0	14.4	420	1.8
608	-	0.0	32.2	47.1	24.2	218	0.0	0.6	6.95	19.8	38.2	0.0	17.8	18	2.0	7.08	14.7	44.8	0.0	13.1	148	3.7
609	-	0.0	32.2	47.1	24.2	218	0.0	0.7	7.29	17.6	35.6	0.0	15.5	14	0.6	7.01	15.6	43.0	0.0	13.2	171	3.8
610	-	0.0	32.2	47.1	24.2	218	0.0	0.9	7.16	0.0	34.0	0.0	18.1	24	0.6	7.10	16.0	52.3	0.0	12.6	63	4.2

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
611	8.99	0.0	34.4	52.0	24.4	215	0.0	1.0	7.20	17.2	36.3	0.0	16.6	14	0.7	7.10	16.7	42.9	0.0	13.3	20	2.4
612	-	0.0	34.4	52.0	24.4	215	0.0	1.2	7.13	16.5	36.8	0.0	11.3	13	0.7	7.11	15.8	45.6	0.1	10.9	19	2.1
613	-	0.0	34.4	52.0	24.4	215	0.0	1.3	7.09	15.5	37.1	0.0	14.5	12	0.6	7.05	20.4	46.9	0.0	11.5	17	2.0
614	-	0.0	34.4	52.0	24.4	215	0.0	1.6	7.33	11.0	36.3	0.0	16.2	18	0.6	7.10	17.6	47.3	0.0	11.3	47	2.1
615	-	0.0	34.4	52.0	24.4	215	0.0	2.4	7.20	16.3	36.8	0.0	14.4	11	0.6	7.02	17.4	45.4	0.0	8.3	22	2.0
616	-	0.0	34.4	52.0	24.4	215	0.0	2.3	-	18.8	37.0	0.0	13.7	12	0.8	-	17.5	45.5	0.0	9.5	19	2.3
617	-	0.0	33.1	49.7	24.3	214	0.0	1.4	7.19	31.5	37.8	0.0	15.3	14	0.8	7.08	17.2	45.8	0.0	7.3	15	1.9
618	-	0.0	33.1	49.7	24.3	214	0.0	1.4	7.01	17.6	37.1	0.0	13.9	13	0.8	7.22	17.4	47.3	0.0	9.7	14	2.1
619	8.66	0.0	34.4	50.7	24.6	215	0.0	1.3	6.95	17.9	36.6	0.0	14.1	12	0.8	7.13	16.8	45.0	0.0	8.9	12	2.0
620	-	0.0	33.1	49.7	24.3	214	0.0	1.3	7.21	0.0	34.7	0.0	26.0	192	0.6	7.02	1.9	43.0	0.0	12.1	160	2.3
621	-	0.0	33.1	49.7	24.3	214	0.0	1.4	7.02	17.7	36.6	0.0	13.6	10	0.0	6.84	15.4	41.0	0.0	11.9	117	3.0
622	-	0.0	33.1	49.7	24.3	214	0.0	1.6	7.18	18.1	36.7	0.0	12.2	13	0.6	6.85	15.9	45.9	0.0	7.7	38	2.4
623	-	0.0	31.7	48.6	23.9	213	0.0	-	6.97	17.8	36.9	0.0	11.4	9	0.5	6.70	17.1	46.5	0.0	7.6	11	1.9
624	-	0.0	31.6	48.2	23.0	211	0.0	1.1	6.90	18.6	33.9	0.0	12.2	19	0.7	6.88	20.8	43.6	0.0	6.7	9	1.5
625	-	0.0	31.6	48.2	23.0	211	0.0	1.0	6.97	20.9	33.7	0.0	11.9	14	0.7	6.99	20.1	42.8	0.0	8.0	9	1.8
626	-	0.0	31.6	48.2	23.0	211	0.0	0.9	6.99	20.4	33.5	0.0	12.7	15	0.8	6.93	18.3	41.9	0.0	7.0	10	1.9
627	-	0.0	31.6	48.2	23.0	211	0.0	0.9	7.03	15.3	33.5	0.0	14.0	31	0.7	7.21	14.4	35.5	0.0	9.2	14	0.7
628	-	0.0	31.6	48.2	23.0	211	0.0	1.2	7.30	12.2	32.3	0.0	12.5	20	0.5	7.09	11.5	42.8	0.0	7.6	13	0.9
629	-	0.0	30.3	48.2	22.0	216	0.0	-	7.09	12.7	32.5	0.0	12.4	23	0.5	7.03	10.3	41.5	0.0	6.3	13	0.9
630	8.72	0.0	30.3	48.2	22.0	216	0.0	-	7.27	11.2	32.9	0.0	12.9	27	0.4	7.14	9.0	62.9	0.0	7.9	18	1.2
631	-	0.0	30.3	48.2	22.0	216	0.0	-	7.16	18.9	32.9	0.0	12.2	13	0.6	7.05	18.9	40.8	0.0	5.3	9	1.0
632	8.85	0.0	30.3	48.2	22.0	216	0.0	-	7.05	18.7	32.7	0.0	12.1	14	0.6	6.82	17.6	38.6	0.0	5.0	7	0.8
633	-	0.0	30.3	48.2	22.0	216	0.0	0.6	7.04	21.6	33.2	0.0	12.2	16	0.6	6.90	20.4	39.4	0.0	8.0	7	0.9
634	-	0.0	30.3	48.2	22.0	216	0.0	0.7	7.30	16.8	34.2	0.0	13.2	30	0.5	7.08	9.1	40.2	0.0	11.5	21	0.8
635	-	0.0	30.3	48.2	22.0	216	0.0	0.7	7.10	19.8	34.4	0.0	12.1	17	0.7	7.03	18.5	42.4	0.0	7.7	20	1.5
636	-	0.0	32.9	48.8	23.2	212	0.0	0.7	6.96	20.0	35.9	0.0	12.2	24	0.8	6.96	18.6	44.0	0.0	8.0	14	1.9

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
638	-	0.0	32.9	48.8	23.2	212	0.0	-	7.04	20.8	36.2	0.0	11.8	20	0.7	6.93	20.4	44.9	0.0	7.6	16	2.2
639	-	0.0	32.9	48.8	23.2	212	0.0	-	7.06	20.3	36.3	0.0	12.4	32	0.8	6.86	19.2	44.7	0.0	7.5	18	2.2
640	-	0.0	32.9	48.8	23.2	212	0.0	-	7.09	21.8	35.6	0.0	10.8	28	0.8	6.92	9.0	43.9	0.0	6.9	18	2.1
640	-	0.0	29.6	47.4	22.7	213	0.0	-	6.96	22.2	33.9	0.0	10.9	29	0.7	6.99	15.5	44.2	0.0	5.6	19	1.9
640	-	-	-	-	-	-	-	-	7.05	11.0	24.7	0.0	8.6	51	0.9	7.00	11.5	34.6	0.0	3.6	21	1.4
640	-	-	-	-	-	-	-	-	7.03	17.2	31.1	0.0	11.1	40	1.0	6.95	10.8	34.0	0.0	4.1	22	1.4
640	-	-	-	-	-	-	-	-	-	-	33.9	0.0	12.8	28	1.5	-	-	39.5	0.0	5.5	23	2.0
640	-	-	-	-	-	-	-	-	7.05	18.9	33.3	0.0	11.7	20	0.0	6.93	18.2	41.7	0.0	5.5	12	0.0
641	-	0.0	32.1	47.9	22.4	205	0.0	-	7.02	19.5	32.8	0.0	11.6	25	0.8	6.92	18.4	41.8	0.0	6.7	16	2.0
642	-	0.0	32.1	47.9	22.4	205	0.0	-	7.06	18.7	33.0	0.0	12.4	34	0.9	6.92	13.3	28.8	0.0	4.4	13	1.9
643	-	0.0	32.1	47.9	22.4	205	0.0	0.3	6.79	18.4	33.9	0.0	12.2	26	0.8	6.81	7.5	45.6	0.0	6.0	14	1.8
644	-	0.0	32.1	47.9	22.4	205	0.0	-	6.96	20.2	32.9	0.0	12.1	27	1.0	6.89	14.6	41.6	0.0	6.0	12	1.8
645	-	0.0	32.1	47.9	22.4	205	0.0	-	6.97	13.6	32.6	0.0	11.5	29	0.9	6.94	4.9	37.3	0.0	4.7	13	1.7
646	-	0.0	32.1	47.9	22.4	205	0.0	-	7.04	11.8	45.9	0.0	14.1	37	7.9	7.11	2.2	35.1	0.0	5.1	26	1.5
647	-	0.0	32.1	47.9	22.4	205	0.0	0.4	6.93	14.0	35.9	0.0	12.6	34	0.9	7.05	12.5	43.2	0.0	8.9	14	0.0
648	-	0.0	32.1	47.9	22.4	205	0.0	1.1	6.86	23.9	34.9	0.0	11.9	28	1.0	7.10	0.0	42.1	0.0	6.5	19	2.2
648	-	0.0	32.1	47.9	22.4	205	0.0	1.4	6.92	15.5	37.5	0.0	11.9	29	0.8	6.80	14.1	51.4	0.0	6.0	17	1.7
649	-	0.0	32.1	47.9	22.4	205	0.0	-	7.14	14.1	38.5	0.0	11.8	31	0.8	7.04	8.9	43.6	0.0	5.5	18	1.5
650	-	0.0	32.1	47.9	22.4	205	0.0	-	7.02	14.0	36.9	0.0	11.7	27	0.8	6.97	12.5	44.9	0.0	5.2	14	1.5
651	9.41	0.0	76.0	49.8	23.2	216	0.0	0.7	7.20	9.6	36.8	0.0	12.2	47	0.5	-	12.1	42.1	0.0	5.0	23	1.0
652	-	0.0	76.0	49.8	23.2	216	0.0	-	7.25	16.2	35.2	0.0	12.2	38	0.6	7.11	11.8	43.0	0.0	4.9	23	1.0
653	-	0.0	76.0	49.8	23.2	216	0.0	-	7.19	13.7	35.2	0.0	13.2	64	0.7	7.22	10.7	41.8	0.0	5.8	41	1.3
654	-	0.0	76.0	49.8	23.2	216	0.0	-	7.13	16.1	34.9	0.0	12.2	34	0.5	7.19	14.3	44.0	0.0	5.3	28	1.1
655	-	0.0	76.0	49.8	23.2	216	0.0	0.4	7.08	14.8	36.8	0.0	11.5	23	0.4	7.22	14.1	42.1	0.0	4.4	21	1.0
656	-	0.0	76.0	49.8	23.2	216	0.0	-	7.11	17.4	36.0	0.0	11.6	21	0.4	7.20	16.1	42.8	0.0	4.7	16	0.9
657	-	0.0	32.7	48.5	22.9	206	0.0	-	6.16	49.9	12.3	0.0	6.5	194	1.2	7.14	15.3	40.5	0.0	3.0	12	0.7

658	9.44	0.0	32.7	48.5	22.9	206	0.0	-	6.93	25.3	83.4	0.0	14.2	56	34.8	7.10	17.4	43.2	0.0	7.4	22	1.4
659	-	0.0	32.7	48.5	22.9	206	0.0	-	7.16	17.6	39.2	0.0	14.1	39	1.3	7.23	16.4	43.3	0.0	7.3	33	1.9

Time days	Influent Tank								Effluent From Reactor A (EA)							Effluent From Reactor B (EB)						
	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	DO mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L	pH	Ac^- as C mg/L	Cl^- mg/L	NO_3^- mg/L	SO_4^{2-} mg/L	As_T µg/L	Fe_T mg/L
660	-	0.0	32.7	48.5	22.9	206	0.0	-	7.09	18.5	37.9	0.0	13.7	33	0.7	7.18	17.1	43.8	0.0	6.6	34	1.7
661	-	0.0	32.7	48.5	22.9	206	0.0	2.2	7.13	17.2	34.4	0.0	13.9	41	0.6	7.25	17.1	42.6	0.0	7.1	28	1.3
662	-	0.0	32.7	48.5	22.9	206	0.0	1.8	7.10	19.0	35.0	0.0	13.7	26	0.7	7.30	16.5	42.9	0.0	6.1	24	1.3
663	-	0.0	32.7	48.5	22.9	206	0.0	1.9	7.15	17.9	35.7	0.0	14.0	29	0.6	7.21	15.7	42.6	0.0	5.8	29	1.4
664	-	0.0	32.1	48.4	15.4	229	0.0	1.7	7.23	17.2	35.5	2.0	8.8	30	0.7	7.36	15.4	42.8	0.0	3.1	35	1.7
665	-	0.0	32.1	48.4	15.4	229	0.0	1.5	7.19	17.9	36.1	0.0	8.8	36	0.7	7.34	16.3	42.8	0.0	2.4	38	1.6
666	-	0.0	32.1	48.4	15.4	229	0.0	1.4	7.24	22.7	35.6	0.0	5.9	25	0.6	7.35	17.9	43.1	0.0	0.0	32	1.6
667	-	0.0	32.1	48.4	15.4	229	0.0	3.1	7.25	16.4	35.2	0.0	8.7	36	0.6	7.39	15.0	43.5	0.0	0.0	49	1.3
668	9.23	0.0	32.1	48.4	15.4	229	0.0	1.9	7.25	17.9	35.6	0.0	8.8	32	0.6	7.36	17.8	43.3	0.0	2.5	34	1.9
669	8.96	0.0	32.1	49.2	15.7	236	0.0	2.2	7.02	16.3	34.8	0.0	8.2	-	-	7.23	15.1	42.6	0.0	2.4	20	2.2
670	-	0.0	32.1	49.2	15.7	227	-	2.1	7.00	18.3	35.1	0.0	7.9	15	0.9	7.19	15.1	42.9	0.0	2.3	17	2.2