ASSESSMENT OF BIOREMEDIATION FOR TREATMENT OF ARSENIC IN MINE PIT WATER

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
for the Degree of Master of Science
In the Department of Civil, Geological and Environmental Engineering
University of Saskatchewan
Saskatoon

By
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ABSTRACT

Contamination of arsenic (As) in ground water and surface water is a widespread problem throughout the world. Industrial development and increase of anthropogenic activities such as mining are an issue of concern due to their pollution of the environment. Because of its toxicity to human and environmental health, remediation of As-contaminated water has become a high priority and a number of As treatment technologies have been developed. Common treatment technologies for As treatment are coagulation, oxidation, filtration processes, electrochemical methods, adsorption, phytoremediation, and bioremediation. Common difficulties with conventional treatment techniques may include the potential production of toxic by-products, limited efficiencies, operational difficulties, and high capital and operation/maintenance costs.

Bioremediation may be used to promote the growth of indigenous water and wastewater bacteria, such as sulphate reducing bacteria (SRB), to remove As from these matrices in an effective and environmentally friendly manner. The goal of thesis was to investigate and assess the As bioremediation potential for mine pit water at in situ temperature (8 °C) using molasses as a carbon source. Six sets of experimental batch reactors were prepared including positive controls, negative controls, and molasses amended reactors. A problem with determining As speciation is the need for advanced analytical instruments for the analysis that are not readily available. Thus, suitable sample processing and storage procedures are vital to preserve the species from the time of sampling to analysis. To assess these processing and storage procedures, three methods were used: (1) no acid; (2) ethylene diaminetetra acetic acid (EDTA); and (3) 2% nitric acid (HNO₃). An ion-exchange method was used for the separation of arsenite (As(III)) and arsenate (As(V)) prior to inductively coupled plasma mass spectrometry (ICP-MS), while other metal(loid)s and bacteria were determined using ICP-MS and MiSeq 16S rRNA V4 analyses, respectively.

Results showed that iron reducing bacteria increased the As release rate from waste rock over time leading to higher aqueous concentrations in molasses treatment reactors. For the preservation, it was found that filtered samples stored at 4 °C without acidification provided the best results for maintaining appropriate As speciation. Overall, using molasses as a carbon source led to increased As solution concentrations which was not the anticipated outcome. Thus, bioremediation of As in mine pit water needs further investigation and optimization.
ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Kerry McPhedran, for his tremendous amount of instruction, guidance, and patience throughout this entire process. It would not have been possible to complete this work without his help.

I am also grateful to Dr. Ali Motalebi Damuchali for his encouragement to pursue this opportunity and vision for the direction of this research.

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LIST OF ABBREVIATIONS

AMD: Acid Mining Drainage
ATP: Adenosine Triphosphate
BOD: Biological Oxygen Demand
CEPA: Canadian Environmental Protection Act
CLS: Canadian Light Source
COD: Chemical Oxygen Demand
DMA: Dimethylarsinic Acid
DNA: Deoxyribonucleic Acid
EDTA: Ethylenediamine Tetra acetic Acid
EPA: Environmental Protection Agency
EU: European Union (EU),
EXAFS: Extended X-ray absorption fine structure
FeRB: Iron Reducing Bacteria
HPLC-ICP-MS: High performance liquid chromatography inductively coupled mass spectrometer
HPLC: High Performance Liquid Chromatography Spectrometer
HXMA: Hard X-ray microanalysis
ICP-MS: Inductively Coupled Plasma Mass Spectrometer
MMA: Methylarsonic Acid
NCBI: The National Center for Biotechnology Information
OTUs: The relative abundances of operational taxonomic units
RLU: The Relative Light Unit
SRB: Sulphate Reducing Bacteria
SRC: Saskatchewan Research Council
TLC: Thin-layer Chromatography.
US-EPA: The United States Environmental Protection Agency
USGS: United States Geological Surveys
WHO: World Health Organization
XANES: X-ray absorption near edge structure
XPS: X-ray photoelectron spectroscopy
XRD: X-ray diffraction
Chapter 1: Background and Literature Review

1.1 Introduction

Arsenic (As) is an environmental problem in Canada and around the world due to its high toxicity and ubiquity. It has been estimated that more than 100 million people around the world are chronically exposed to As from drinking water containing high As concentrations (Bang et al. 2005; Parvez et al. 2006). Mine tailings, waste rock, and wastewaters may contain high concentrations of As that necessitate their treatment before release in order to prevent environmental pollution (Wang and Mulligan 2006). For example, As concentrations have been reported in a range of 56 to 9,871 mg/kg for mine tailings in northern Saskatchewan (Moldovan and Hendry 2005) which is the area of current interest for this thesis.

Arsenic is found in four oxidation states (As(-III), As(0), As(III), As(V)) including inorganic and organic compounds that are dependent on the existence of sorbent materials, pH, redox potential (Eh), and microbial metabolic activities. It is impossible to destroy As by any technology methods, it can only change oxidation states (Choong et al. 2007). The level of toxicity and mobility of As depends on its oxidation states with As(III) being ten times more toxic than As(V). Moreover, As(III) species are more soluble than As(V) which makes its removal difficult from various water matrices. In natural waters As normally occurs in the oxidation states As(III) (arsenite) and As(V) (arsenate). Given the As(III) species solubility’s are higher than As(V) making them more difficult to remove from solution, thus, As(III) species typically have to be oxidized to As(V) prior to its removal (Bissen and Frimmel 2003). Canadian Environmental Protection Act (CEPA) lists As in Group 1 of the Priority Substances List which identifies it as being harmful to the environment and human health (Wang and Mulligan 2006). Thus, for environment and human health it is important to determine and implement proper As treatment technologies for various matrices including mining wastes. A large amount of research has focused on the As oxidation states and tried to determine the optimal As treatment technologies, however, there remains a need for further research to optimize treatment and cost efficiencies.

Common As treatment technologies for matrices such as waters, wastewaters and mine effluents are coagulation, oxidation, filtration processes, electrochemical methods, adsorption, phytoremediation and bioremediation (Hu et al. 2012; Kumar and Riyazuddin 2010; Liu et al.
Common difficulties with conventional treatment techniques may include the potential production of toxic by-products, often limited efficiencies, operation difficulties, and high operational and chemical costs. More recently, biological treatment has shown promising potential in As treatment from different water matrices. Biological treatment can be separated into bioremediation and phytoremediation but the most used and common one is bacterial community-based bioremediation. Sulphate reducing bacteria (SRB) have been proposed to be a common and successful bacterium for As remediation (Alam and McPhedran 2019). Compared to other treatment technologies, bioremediation can be inexpensive because it does not rely on advanced technologies, typically does not need pH adjustment or pre-treatment oxidation of As(III) to As(V), needs no chemical inputs, and also generates limited waste sludge (Feldman 1979; Kirk et al. 2010; Muyzer and Stams 2008).

The metabolism of As by various species has an important role in its toxicity. In environmental and biological systems, there can exist nearly two dozen various As species (Chungang and Le 2009). All the As species have different solubility, mobility, toxicity and likely need different techniques for treatment. Thus, knowing the speciation of As is important to develop treatment technologies. Advanced analytical progress has been made in the field of determination of As speciation, however, validation of different analytical procedures is still incomplete. Limits of detection and quantification have been an ongoing problem in As speciation for actual water samples. Another problem is lack of certified reference materials and the inability to use the isotope dilution method for analysis (Komorowicz and Barałkiewicz 2011). Overall, sample preservation is of a great importance in As speciation studies in order to stabilize As species during inevitable time gaps between sampling and analysis. The distribution of As species must be preserved to avoid the changes by processes such as redox reactions, metal oxyhydroxide precipitation, and photochemical oxidation (Bednar et al. 2002).

In spite of the importance of sample preservation, limited research has been conducted to develop an appropriate sample preservation technique for As speciation analysis or to validate existing methods. In addition, a detailed standardized method could not be found in the limited previous reported studies (Bednar et al. 2002). There are agreements and disagreements among reported results on sample preservation for As speciation. There is general agreement among various research in the need for sample filtration (US EPA 1982; USGS 2005) as a step in sample preservation since it removes microbes that can affect As redox species (McCleskey et al. 2004;
Storage of samples at 4 °C as another step of As speciation sample preservation is the other agreement found among previous studies (Daus et al. 2002; Rässler et al. 1998). However, there are inconsistencies among the previous research in the need for acidifying As samples as one of steps of preservation. The inconsistency includes whether samples should be acidified or not and selection of the appropriate acid for sample preservation. For example, a recent study suggested storage at 4 °C without any preservatives (Wolf et al. 2011) while other previous research has suggested acidification as a required step in As speciation sample preservation (Bednar et al. 2002; McCleskey et al. 2004). Adding acid to samples for preservation of As oxidation species has been explained by the theory that acidification (e.g., HCl, EDTA) could reduce oxidation and precipitation of Fe and Mn hydroxides that could precipitate and adsorb As in samples containing As species (Wilkie and Hering 1996; McCleskey et al. 2004). Overall, a detailed standard method of sample preservation for As speciation has not been well-investigated or adequately validated with experimental research.

1.2 Arsenic Background

Arsenic is a naturally occurring metalloid and ubiquitous element that ranks 20th in abundance in the Earth’s crust, 14th in sea water, and 12th in the human body (Mandal and Suzuki 2002). There are more than 300 As-bearing minerals, in addition to other minerals containing As as an impurity, that are naturally occurring worldwide (Hudson-Edward 2013) including sulphides (e.g., arsenopyrite, pyrite, pyrrhotite, marcasite, and chalcopyrite); iron oxides (e.g., haematite, goethite, ferrithydrate, and magnetite); iron oxyhydroxides (hematite, goethite and ferrithydrate); carbonates (e.g., calcite, dolomite, siderite); and other minerals (e.g., apatite, fluorite). Figure 1.1 shows common As species which are generally found in natural water (Komorowicz and Baralkiewicz 2011). The mobility of As species in the environment is influenced by pH, humic substances, clay materials, redox potential, and the presence of absorbents such as oxides and hydroxide of Fe(III), Al(III), Mn (III and IV) (Nriagu et al. 2007). Mine tailings, waste rock, and various industrial or municipal wastewaters may contain high concentrations of As that necessitates their treatment in order to prevent As release to the environment (Wang and Mulligan 2006).
Figure 1.1: Arsenic species generally found in natural water (Komorowicz and Baralkiewicz 2011).
Water pollution by As is one of the most common environmental issues worldwide, resulting in high incidence of arsenicosis in more than 20 countries including Bangladesh, India, Chile, Argentina, and China. The US Environmental Protection Agency (USEPA) has listed As as a primary contaminant for some superfund sites and it is considered as the “king” of poison (Nariagu et al. 2007). The presence of As in groundwater has a major impact on the environment and human health (Jain and Ali 2000). The primary routes of As exposure to humans are ingestion and inhalation with drinking water being the primary concern for human exposure. It has been estimated that more than 100 million people around the world, including parts of Canada, are chronically exposed to As from drinking water containing high As levels (Bang et al. 2005; Parvez et al. 2006). In addition, the World Health Organization (WHO) reported that over 200 million people worldwide have been exposed to drinking water containing As concentrations exceeding its guideline value of 10 µg/L (Herath et al. 2016). Acute effects of As exposure (within 30 min of ingestion) may lead to gastrointestinal discomfort, vomiting, coma, and have even been deadly (Wang and Mulligan 2006). Chronic exposures to As contaminated water cause various diseases such as arsenicosis (i.e., As poisoning); cancer in the skin, bladder, kidney, and lung; diseases of the blood vessels of the legs and feet; diabetes; increased blood pressure and reproductive disorders (Edition 2011; Shankar and Shikha 2014). Given the consequences of these exposures, investigation into the sources of As pollution (both naturally occurring and human-mediated) and development of technologies for the remediation of As from water used for human consumption have become a significant area of research worldwide.

1.3 Sources of Arsenic in the Environment

Arsenic is released to both air and water matrices, then can move into soils and sediments, in the environment from natural and anthropogenic sources. The Earth’s crust is an abundant natural source of As with more than 300 As-minerals present and the most common one being arsenopyrite (Hudson-Edward et al. 2013). It has been estimated that about one-third of the atmospheric flux of As is of natural origin with volcanic action as the primary source and low-temperature volatilization as a secondary source. Natural low-temperature microbial biomethylation and reduction to arsines also releases As into the atmosphere. Arsines released from microbial sources in soils or sediments may undergo oxidation in the air, reconverting the As to non-volatile forms, which settle back to the ground (Wang and Mulligan 2006). Anthropogenic
As is emitted into the atmosphere by high-temperature processes such as fossil fuel combustion plants, metal smelting, wood burning, and waste incineration. As is released into the atmosphere primarily as $\text{As}_2\text{O}_3$ and exists mainly adsorbed on particulate matter. These particles are dispersed by the wind and are returned to the earth by wet or dry deposition (Nariagu et al. 2007).

Naturally occurring aqueous As is found in both inorganic and organic compounds. Inorganic As of geological origin is found in groundwater used as drinking-water in several parts of the world, for example Bangladesh (Olson 2014). Organic As compounds such as arsenobetaine, arsenucholine, tetramethylarsonium salts, arsenosugars and As-containing lipids are mainly found in marine organisms although some of these compounds have also been found in terrestrial species (Jain and Ali 2000). Naturally-occurring dissolved forms of As in water include arsenate, arsenite, thioarsenate, arsenic trisulfide, methylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Figure 1.1). Three major modes of As biotransformation have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of As, and the biosynthesis of organoarsenic compounds (Abernathy et al. 2001). In well-oxygenated water and sediments, nearly all As is present in the thermodynamically more stable arsenate.

Anthropogenic released As into the water environment is considered as the main route for human exposure to As (Garelick et al. 2008). The leaching of As from minerals to water is significantly enhanced by anthropogenic activities such as mining, metal acquisition and processing, wood preservation, use of arsenical insecticides in agriculture, waste disposal and other industrial activities (Garelick et al. 2008). Previously, about 70% of the world As pollution was due to the timber industry use of copper chrome arsenate (CCA). The next largest As sources are agricultural chemicals at 22% (Abernathy et al. 2001). Anthropogenically released aqueous As found in soils can form insoluble complexes with iron, aluminum and magnesium oxide found in the soil surface, and in these forms, As is relatively immobile. In addition, enhanced native As contamination in groundwater has been reported due to several mechanisms including oxidation of As-rich pyrite in an aquifer due to the lowering of the groundwater table (Mandal and Suzuki 2002), reductive dissolution of As-rich iron oxyhydroxides from weathering of metal sulphide minerals (Chowdhury et al. 1999; McArthur et al. 2001), and reductive dissolution of As-bearing ferric oxides from microbial activities (Islam et al. 2004).
Industrial development and increasing anthropogenic activities including mining is an ongoing issue of concern and can seriously pollute water resources. Due to technology advancement mining has become more mechanized and therefore able to process great volumes of waste rock and ore material. In addition, the processing of lower grade ores have also contributed to increasing the amounts of mine wastes which can lead to potentially significant environmental risks unless they are properly managed. For example, some mine waste rocks produced in uranium mining can severely degrade water quality when exposed to air and water by producing sulphuric acid and other contamination (Cullinan and Pigott 2010). The waste often contains heavy metals, including the metalloid As, that can contaminate surface and groundwater through the process of leaching. Pollution of water is also very much dependent on acid mining drainage (AMD). The most common components of AMD are dissolved As, sulphide-bearing minerals, dissolved metals (e.g., As, Cd, Zn, Cu), anions (e.g., sulphates and carbonates), and acidic pH (Razo et al. 2004).

Figure 1.2 shows an example of a giant mine site in Chili (Adopted from Bloomberg.com). About 20 years ago, it was found that about 34 mines around the world are facing As problems with As concentrations in water matrices of these sites and surrounding waterbodies ranging from 0.005 to 72 mg/L (Williams 2001). However, recently these numbers have expanded with over 1,000 orphaned and abandoned mine sites in Canada along having potential As issues. In general, concentration ranges for As in uncontaminated soil are between 1 to 40 mg/kg (Cullinan and Pigott 2010) but much higher concentrations have been found in historical mining areas related with exploitation of base and precious metals. For example, concentrations from 1,000 to 10,000 mg/kg have been found at an abandoned gold mining site in Nova Scotia (Wong, Gauthier, and Nriagu 1999).
Figure 1.2: Escondida copper mining site in Chili (Adopted from Bloomberg.com).
1.4 Geochemistry of Arsenic

For the uranium mining industry, waste rock is defined as any untreated rock that does not contain enough uranium to be economically processed (Boekhout et al. 2015). This residual waste rock can various concentrations of acids, As species, and other toxic metal(loid)s. To prevent acid generation, the waste rock is stored under water in mined out open pits leading to the generation of end-pit lakes that exist well after the decommissioning of the uranium mining operations. However, this conventional storage method of waste rock typically results in the release of acids and metal(loid)s into the storage water and, subsequently, the potential for releases into the surrounding groundwaters (Hudson et al. 1999). Arsenic is a common element found in iron (Fe) or sulphide-bearing mineral deposits, but can also occur in separate As minerals or as solids (FeAsS, Fe(As)2, As2S3, AsS, Cu3AsS4, etc.) in solution (Craw and Bowell 2014).

Since native As and As-bearing sulphides are produced by hydrothermic, mesothermic, or diagenetic processes that generates ore, they are recognized as primary As minerals in the mining-affected environment (Hudson-Edwards and Santini 2013). The weathering and alteration of these minerals by ore processing can also generate secondary As oxides and arsenates. In the mining impacted environments, arsenopyrite and pyrite are the most common As-bearing sulphides (Foster et al. 1998; Hudson-Edward et al. 2005; Meunier et al. 2010). In addition, the less common As bearing sulphides are realgar (AsS) and orpiment (As2S3) (Hudson-Edwards and Santini 2013). Further, enargite (Cu3AsS4) and tennantite (Cu6[Fe3+2(Zn)2]As4S13) occur in Cu-rich deposits (Bruckard et al. 2010; Lattanzi et al. 2008); while gesdorffite (NiAsS) and cobaltite (CoAsS) occur in Ni-rich and Co-rich deposits, respectively (Kwong et al. 2007; Senior et al. 2010). Natural weathering of primary sulphides or metal extraction processes make secondary As-bearing minerals abundant in mine wastes (Fawcett and Jamieson 2011; Walker et al. 2005). Secondary As bearing minerals include Fe arsenates such as scorodite (FeAsO4.2H2O) and kankite (Fe3+AsO4.3.5H2O), Fe sulphoarsenate such as tooeleite (Fe3+6(As3+O3)4(SO4)(OH)4·4H2O), Ca-Fe arsenates such as yukonite (Ca3Fe3+(AsO4)2(OH)3·5H2O), and Ca-Mg such as pharmacolite (Ca(HAsO4))·2H2O (Ashley and Lottermoser 1999; Kossoff et al. 2012; Morin et al. 2003; Walker et al. 2009).

Aqueous As in mine waters could be generated by dissolution of secondary As-bearing minerals, particularly those only stable in extreme environments, such as arsenolite (As2O3) that is stable in alkaline, reduced environments (Jamieson 2014). Crystallization of amorphous As
phases to more crystalline products is another redissolution mechanism which can cause the release of adsorbed arsenates. For example, goethite (FeOOH) and hematite (Fe$_2$O$_3$) have a lower number of sorption sites for arsenate than ferrihydrite ((Fe$^{3+}$)$_2$O$_3$.0.5H$_2$O) so recrystallization of amorphous ferric hydroxide results in release of adsorbed arsenate (Majzlan 2011). Reduction of Fe(III) to Fe(II) also leads to desorption of adsorbed As species and, if it occurs concurrently with the reduction of arsenate to arsenite, it may result in increased solubility as well (O’Day 2006). On the other hand, the presence of sulphide may limit As mobility by forming As sulphides such as pararealgar (As$_4$S$_4$) in highly reducing environments which has been identified in basal sediments from mine pit lakes (Bowell and Parshley 2005).

1.5 Arsenic Remediation

In order to select appropriate As treatment technologies many factors need to be considered including operational complexity, treatment cost, removal performance, operational skill, among others. In addition, as stated previously, the As speciation and compounds must also be considered. This section will include an overview of the common As treatment methods that have been successfully used around the world. These processes are present in an overview schematic in Figure 1.3. Advantages and disadvantages of each of these processes are presented in Table 1.1.
Figure 1.3: A schematic of various arsenic treatment technologies (Adapted from Mohan and Pittman 2007).
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<td>Coagulation</td>
<td>Process simplicity.</td>
<td>High cost of chemicals.</td>
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<td></td>
<td>A wide range of chemicals are available commercially.</td>
<td>Low removal of arsenic.</td>
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<td></td>
<td>Inexpensive capital cost.</td>
<td>High production of sludge volumes.</td>
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<td>Significant reduction on COD and BOD.</td>
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<td>Bacterial inactivation capacity.</td>
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<td></td>
<td>Integrated physicochemical process.</td>
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<tr>
<td>Adsorption</td>
<td>Commercially available and relatively well known.</td>
<td>Needs replacement after four to five regeneration cycles.</td>
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<td></td>
<td>Regeneration may not be required if low cost media is used.</td>
<td>Not standardized; produces toxic solid waste of spent adsorbent.</td>
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<tr>
<td></td>
<td>Media and capacity well-defined; can be independent of pH; specific ion</td>
<td>High cost media; high operation and maintenance cost; As (III) is difficult</td>
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<td></td>
<td>resins exclusive to arsenic removal are available.</td>
<td>to remove; short life of resins.</td>
</tr>
<tr>
<td>Filtration</td>
<td>Well defined and high removal efficiency.</td>
<td>Very high capital and running cost.</td>
</tr>
<tr>
<td></td>
<td>Limited toxic solid waste sludges are produced.</td>
<td>Toxic wastewater produced.</td>
</tr>
<tr>
<td></td>
<td>Capable of removing of other contaminates.</td>
<td>High tech operation and maintenance.</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Simple, rapid and efficient process.</td>
<td>Chemicals required.</td>
</tr>
<tr>
<td></td>
<td>Good elimination of colour and odour.</td>
<td>Pre-treatment is often needed.</td>
</tr>
<tr>
<td></td>
<td>No sludge production and also provides disinfection.</td>
<td>Removes primarily arsenic (V) and accelerates the oxidation process.</td>
</tr>
<tr>
<td></td>
<td>Integrated physicochemical process.</td>
<td></td>
</tr>
<tr>
<td>Biological</td>
<td>The application of microorganisms for the biodegradation of organic</td>
<td>Necessary to create an optimally favorable environment.</td>
</tr>
<tr>
<td></td>
<td>contaminants is simple, economically attractive and well accepted by the</td>
<td>It is often slow due to low biodegradability.</td>
</tr>
<tr>
<td></td>
<td>public.</td>
<td>Poor decolorization ability.</td>
</tr>
</tbody>
</table>
1.5.1 Coagulation

Coagulation is a commonly used method for As treatment. Coagulation is a process where colloid particles are destabilized by neutralizing the forces that keep them apart and allowing them to come together for removal. For example, Gregor (2001) studied the changing species and concentrations of As through an aluminium-based coagulation treatment process for drinking-water treatment plants with raw water coming from a river source. For aluminium-based coagulation with disinfection by chlorination, the form of As most likely to be present in the treated water is soluble As(V) species because final chlorination likely converts any remaining As(III) to As(V). Gregor (2001) found that pre-chlorination before coagulation negatively impacted other water quality parameters such as the formation of disinfection by-products and the release of taste and odour compounds. Ferric salts are also common coagulants used for As removal. For example, Yuan et al. (2003) used ferric sulphate for As removal because it is economic and effective. They reported removal efficiencies of 99% for concentrations of As(V) from 0.1 to 0.5 mg/L. Ferric chloride and ferric sulphate were also used as coagulants for As removal by Han et al. (2002). They concluded that As removal depends on solution pH and ferric dose with an optimized ferric dose up to 10 mg/L at pH 6.2 removing 99% of As from ground water. Additionally, a combined process of coagulation followed by filtration has also been shown to be effective. For example, ferric ion coagulation followed by filtration using 0.22 µm pore size membranes was effective in reducing As concentration (99% removal) in water (Wickramasinghe et al. 2004).

1.5.2 Adsorption

Adsorption is the adhesion of compounds, such as As species, from either a gaseous or liquid solution, to a adsorbent surface. This adhesion is due to van der Waals and electrostatic forces between adsorbent and adsorbate surface structures. Adsorption is suitable even for low As concentrations and can be used in many biological, physical, and chemical systems. Common adsorbents include activated carbon, metal hydrides, and synthetic resins that are used widely for the treatment of industrial wastewaters (Daus et al. 2002). Many other adsorbents have also been considered for As treatment in the literature including zeolites, goethite, clay, kaolinites, chitosan beads, coco-nut husk, coal, fly ash, ferrous iron, alumina, zirconium oxide, red mud, petroleum residues, rice husk, human hair, sawdust, manganese greensand, and orange juice residues (Choong et al. 2007). Activated carbon (Eguez and Cho 1987) and iron-oxide (Huang and Fu 1984)
have been common options as they have been shown to exhibit a high As adsorption capacity. Adsorption completed through filtering water through sand and zero valent iron has also been shown to be a good method to remove As from ground water (Leupin and Hug 2005). In addition, direct filtration with FeCl₃, adsorptive filtration with FeSO₄, and adsorption on granulated ferric hydroxide are three widely used techniques for As treatment (Ruhland and Jekel 2002). For example, the effect of the presence of sulphate and competition with other anions was reported not to influence As(V) sorption by ferrihydrite but resulted in a considerable decrease in As(III) sorption below pH 7, with the largest decrease at the lowest pH (Jackson and Miller 2000).

1.5.3 Filtration

Membrane technology is a promising method use for the removal of As from various water matrices. Membranes are typically synthetic materials with billions of pores or microscopic holes that act as a selective barrier. The structure of the membrane allows some constituents to pass through, while others are excluded or rejected. The movement of molecules across the membranes needs a driving force, such as pressure difference or concentration gradient between the two sides of the membrane. There have been a number of different types of membrane technologies considered for removal of As from water and wastewater including reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and microfiltration (MF) (Uddin et al. 2007). However, use of membranes for treatment of As is not a commonly considered technology worldwide due to high costs of electricity for pumping needed to create the pressure differentials and membrane fouling issues requiring membranes to be cleaned frequently.

1.5.4 Oxidation

Two oxidation states of As are found in natural water which are As(III) and As(V), the removal of As(III) is typically more difficult than As(V). Therefore, a common strategy is to oxidize As(III) to As(V) prior to its removal. Since the oxidation process in the presence of air or pure oxygen is slow, the oxidation rate can be increased by treatment using ozone, chlorine, hypochlorite, chlorine dioxide, or H₂O₂. The oxidation of As(III) is also possible in the presence of manganese oxide coated sands or by other advanced oxidation processes. For example, As can be removed by coprecipitation with Fe(OH)₃, MnO₂ or during water softening. Oxidation can also be used in combination with other processes. For example, the effectiveness of As removal was tested in the
presence of adsorbents such as FeOOH, activated alumina, ferruginous manganese ore, granular activated carbon, or natural zeolites. It was found that oxidation and activated alumina removed 43 to 51% As from concentrations from 21 to 1,100 mg/L (Jiang 2001). As(III) can be oxidized catalytically in the presence of activated carbon and O₂. Gottschalk et al. (1992) found that 90% of As(III) was oxidized with 5 to 10 g/L activated carbon within 20 to 30 min in a water initially containing 40 mg/L As(III).

1.5.5 Biological

Although biological treatments include both bioremediation and phytoremediation, the more common approach is bioremediation. Bioremediation is an environmentally friendly technology because it uses living organisms (primarily microbes) for the detoxification of toxic contaminants. Unlike organic compounds that can be mineralized to CO₂ and H₂O, heavy metals can only be subjected to processes such as biotransformation, bioaccumulation, biosorption and bioevaporation (Singh and Tripathi 2007). For example, As and other metals (Cu, Zn, Ni, Fe, Al and Mg) were treated in a laboratory scale up flow anaerobic packed bed reactor by using a mixed SRB with more than 77.5% of the initial concentration of As being removed (Jong and Parry 2003). The SRB are an important component of natural and artificial wetlands and contribute significantly to metal immobilization by precipitation (Francisco et al. 2017). They are heterotrophic bacteria which can used a variety of carbon sources for their growth and metabolism. In anaerobic environments, SRB can promote the removal of As by sulphate reduction through the generation of As bearing sulphides (Keimowitz et al. 2007). The As bioremediation by SRB has many advantages, including a limited requirement for use of advanced technologies, limited generation of waste sludges, little to no need for pH adjustment, and low costs (Alam and McPhedran 2019). Stimulating SRB growth in order to increase the sulphate reduction rate, followed by As removal by forming As sulphur precipitates, has been the focus of several studies in acid mine drainage, groundwater, and other matrices (Alam and McPhedran 2019). Although many research studies have reported successful As treatment by SRB bioremediation, some researchers have reported complications in some cases due to stimulating iron reducing bacteria (FeRB) as a result of adding a carbon source (Maguffin and Jing 2018; Sun et al. 2016), making the investigation of As bioremediation potential of SRB necessary in each environment.
In addition to the complications posed by FeRB in SRB bioremediation, other complications may occur regarding to the presence of nutrients in the system. Some researchers suggested that the presence of nitrite and nitrate could inhibit SRB growth or oxidize their generated sulphide back to the elemental sulphur or sulphate (Mohanakrishnan et al. 2008; Okabe et al. 2005). However, it has been shown that SRB activities resumed once nitrate was consumed, reaching similar levels as before the occurrence of inhibition in some of these studies (Mohanakrishnan et al. 2008; Okabe et al. 2005). García de Lomas et al. (2006) suggested that lower sulphite production in the presence of nitrate was not due to lower SRB activity. Instead, they attributed the lower sulphite concentration to the autotrophic denitrification by bacteria that used sulphite as an electron donor in an enriched SRB biomass in the system with long exposure to nitrate. Similarly, Rubio-Rincón et al. (2017) suggested that SRB proliferation occurs in biological nutrient removal systems with sludge retention times longer than 20 days. In conclusion, SRB growth is not likely much affected by the presence of nitrate/nitrite in mine pit lakes with prolonged anaerobic conditions as it can resume after denitrification.

Phosphate ($\text{PO}_4^{3-}$) is an essential nutrient and a component of DNA, RNA, lipid membranes, and the energy carrier ATP that is used to activate sulphate during microbial sulphate reduction (Wu et al. 2019). However, it has been observed that the availability of phosphate does not affect SRB growth (Van den Brand et al. 2015). Instead, SRB and FeRB activities can affect phosphorus cycle in the environment. Positive proportions have been reported between dissolved ferrous ions (Fe(II)) and phosphate ($\text{PO}_4^{3-}$) in both freshwater and marine ecosystem (Mort et al. 2010). Phosphate is usually adsorbed on iron hydroxide minerals (Mort et al. 2010). Dissimilatory iron reduction in minerals by FeRB can release the adsorbed phosphate. Thus, iron cycling, including microbial iron reduction and physicochemical precipitation of Fe(III), controls phosphorus cycling in the pre-industrial non-sulphidic sediments to a certain extent (Olsson et al. 1997). However, sulphur has been involved in iron-phosphorus cycling after the explosive increases of sulphur input due to the developing industry (Hall et al. 2006). The biogeochemical reactions of iron and sulphide influence availability and mobility of P (Azzoni et al. 2005). The sulphate-induced P mobilization is mainly explained by the formation of iron sulphides through sulphate reduction. As a result, soluble ferrous iron becomes fixed as insoluble iron sulphides, and formerly immobile phosphate is released. Moreover, H$_2$S generated by SRB can substitute phosphate from iron
phosphate and thereby generate ferrous sulphide. Iron P is then reduced to soluble ferrous simultaneously with the release of P (Roden and Edmonds 1997).

1.6 Arsenic Sample Preservation and Analysis

Sample preservation is an important step in As speciation research in order to stabilize As species during storage periods between the sampling/processing and analysis stages. Despite the importance of the issue, limited research has been conducted to develop and/or validate appropriate sample preservation techniques for As prior to speciation analysis. For example, a synthetic ground water containing As(III) was found to be stable for 24 h to 72 h, however, all of the As(III) eventually oxidized to As(V) if the sample was not preserved (Samanta and Clifford 2005). Presence of metals such as iron can also influence the sample speciation. For example, Hug et al. (2001) showed that 2 mg/L Fe(II) can readily oxidize 500 mg/L As(III) in about at pH 7.8-8 and in the dark. Preservation using various acids have been shown to maintain As speciation previously. For example, EDTA, HAc or H₂SO₄ were all shown to successfully maintain an As solution speciation for up to 28 d (Samanta and Clifford 2005).

Typically sample filtration and refrigeration are considered to be essential steps for As preservation (Kumar and Riyazuddin 2010). Filtration removes suspended matter and most microorganisms, while subsequent refrigeration helps to limit any further biotic and abiotic reactions. Kumar and Riyazuddin (2010) indicated that storage in the dark is also needed for preservation since this will prevent the reaction of Fe(III) and As(III) which occurs under visible light conditions. For example, an As solution was successful preserved for 77 d at 4 °C for solutions adjusted to ~pH 3 using HCl, HNO₃, or acetic acid and stored in the absence of light (Wu and Pichler 2016). Overall, there is a need to investigate the need and impact of various sample preservation techniques to produce accurate analytical results and, subsequently, implement appropriate treatment technologies informed by the determined As speciation.

Historically, determining the various species concentrations of As has been limited due to the need for low detection limits and high selectivity’s (Andreae 1977). An older method for As speciation is gas chromatography with flame ionization detection (GG-FID) which has been used for decades (Ballin et al. 1994). More recently, higher sensitivity has been achieved using Inductively coupled plasma mass spectrometry (ICP-MS) in combination with high-performance liquid chromatography (HPLC) for As speciation. Currently, the HPLC-ICP-MS is one the most
common methods which is used world-wide for As speciation (Hansen et al. 1992). In addition to separation via HPLC, ion exchange columns can also be used to separate As species prior to ICP-MS detection. A less used and available instrument for As speciation is done by X-ray absorption spectroscopy (XAS) technology which is useful based on its ability to show real-time As(III) and As(V) speciation without preservation. However, the HXMA results are qualitative, not quantitative, thus indicating only the existence of the species and not the actual concentrations.

1.7 Knowledge Gaps

There are two knowledge gaps that this thesis is meant to address:

- Among the various methods for treatment of aqueous As, bioremediation has been attracting increasing attention as it has been shown to be an environmentally-friendly and cost-effective method. More specifically, application of SRB bioremediation has been the focus of many historic and recent studies for groundwaters, acid mine drainage (AMD), wastewaters, and other matrices. The physical, chemical, and biological properties of any environmental matrix affects the potential of bioremediation by SRB. The potential of indigenous SRB bioremediation for mine pit lake As treatment at low, in situ temperatures has not been studied well. Thus, this research investigates the potential for indigenous SRB treatment of As found in uranium mine pit lake water at low in situ temperatures found in northern Saskatchewan.

- The As speciation determines its mobility and toxicity in different aqueous environments. Reduced As species could be oxidized over relatively short periods making the need for sample preservation for determination of As speciation in order to stabilize As species during inevitable time gaps between the sampling and analysis stages. Despite the importance of the issue, limited research has been conducted to develop or validate appropriate sample preservation techniques for aqueous As speciation studies. In addition, a detailed standardized method has not been developed for As preservation. Thus, this research compares different typical preservation techniques suggested by previous research for As speciation sample preservation in order to find the optimum method of aqueous As sample preservation.
1.8 Thesis Overview

Arsenic is a natural element, ubiquitous in the environment, cycling through water, land, air, and living systems. Anthropogenic releases of As are increasing over time and a current issue is the potential release of As from mine waste rock. A mine site located in northern Saskatchewan is anticipated to produce over a million tons of waste rock which contain high concentrations of As. All waste rock is currently, or planned to be, stored in mined out pits under water making the release of this As into surrounding ground water an issue of environmental concern going forward. Given this waste rock in pit scenario and the provided introduction background information, the research questions for this thesis include:

- Can a bioremediation technique be an effective method in As removal from mine pit water at relevant in situ temperatures?
- Can an appropriate preservation method be developed that could preserve As speciation over a long duration (e.g., weeks to months) prior to analysis?
- Is there agreement between As speciation results using ion exchange ICP-MS and HXMA analyses?

Based on these research questions, the main objective of this thesis is to assess the potential for As bioremediation of mine waste rock pit water at 8 °C. Subobjectives will include the determination of appropriate sample preservation for collected As samples and the comparison of ion exchange ICP-MS and HXMA As speciation results.

This research has resulted in one published, peer-reviewed conference proceeding and a second publication currently in preparation:

Chapter 2:

Chapter 3:


1.8.1 Chapter 2

This chapter includes an in-preparation manuscript of the study of molasses-supplemented bioremediation of As-contaminated mine pit water. The novel aspect of this chapter is the study of As bioremediation potential of mine pit water using indigenous bacteria, such as SRB and iron-reducing bacteria (FeRB), at the in-situ mine pit water temperature of 8 °C. Treatment reactors were stored in the Phytotron facility at the University of Saskatchewan and assessed over 6 months experimental duration. My contribution was to aid Dr. Ali Motalebi Damuchali, in the setup, sampling, and analyses of these reactors. Dr. Raquibul Alam assisted us by sharing his experience in developing bioreactors in As bioremediation studies. Dr. Aslan Lee and Dr. Wonjae Chang assisted in the bacterial DNA extraction and analysis including Illumina MiSeq 16S rRNA V4 sequencing. I wrote the initial draft of this chapter including preparation of all tables and figures. In addition, I wrote the first draft of the in-preparation manuscript.

1.8.2 Chapter 3

This chapter includes a published manuscript of the study of As-preservation techniques prior to As speciation analysis. The novel aspect of this chapter is the testing of numerous preservation methods for maintaining As speciation and the determination of the speciation using the HXMA beamline. My contribution was to assist Dr. Raquibul Alam in the setup of these samples and analysis using the HXMA beamline. Dr. Aslan Lee assisted in the HXMA beamline analysis and Dr. Wonjae Chang provided review of the manuscript and funding for Dr. Lee. I wrote the initial draft of this chapter including preparation of all tables and figures.
1.8.3 Chapter 4

Chapter 4 includes a summary of the results found in the previous chapters. This chapter includes the engineering significance of the thesis and provides direction for future work in this research area.

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Chapter 2: Assessment of Indigenous Bacterial Bioremediation of As-Contaminated Mine Pit Water with Molasses Amendment

This chapter’s research addressed the As bioremediation potential for a mine pit lake considering the promotion of indigenous SRB and FeRB using a supplemented carbon source. For the experimental setup, a total of six sets of reactors were prepared, each included one positive, one negative and one molasses treatment reactor. In the treatment reactor, molasses was used as a carbon source to enhance bacterial growth. Each month, one set of the reactors were sacrificed to run various analyses. Bacterial growth was tested by an adenosine triphosphate (ATP) test and metalloid(s) concentrations were measured using ICP-MS. For As speciation, ion-exchange resin extraction was used to separate As(III) and As(V) followed by analysis using ICP-MS. Illumina MiSeq analysis of 16S rRNA V4 amplicons was conducted to determine different bacterial classes and genera. Overall, FeRB were dominant over the six months of experiments that led to the As release into water from the waste rock in the treatment reactor.

Abstract

In-pit disposal of mine waste rock filled with water is a common technique used by mining companies to limit the movement of waste rock contaminants into receiving environments. However, this method may result in the release of arsenic (As), and other contaminants, from these pits into local groundwaters and soils/sediments. The bioremediation of As via sulphate reducing bacteria (SRB) has been of recent interest given their ability to immobilize As under anaerobic conditions, potential cost-effectiveness, and being environmentally friendly. However, the As bioremediation potential via SRB for mine pit water at low temperatures found in areas such as northern Saskatchewan, Canada is not well understood. Thus, the main objective of the current study was the evaluation of the As bioremediation potential for treatment of mine pit water at an in-situ condition of 8 °C using molasses as a carbon source. Treatments included molasses-amended, positive control (no carbon added), and negative control (sterilized and no carbon added) reactors. Overall, indigenous bacteria increased the release rate of total As (mostly As(V) and marginally as As(III)) from mine waste rock over six months for both positive control (2.5 mg/L) and molasses-amended (3.7 mg/L) treatments. Bacterial community analyses found a variety of
classes and genera including both SRB and iron-reducing bacteria (FeRB). However, the expected bioremediation by SRB was not successful due to the slow rate of SRB growth under the low temperature and pH conditions.

2.1 Introduction

Arsenic (As) is a metalloid which is naturally present in the environment including in water, air, and soil/sediment matrices. Arsenic contamination is an ongoing environmental problem in Canada, and worldwide, particularly due to its presence in drinking water with estimates of more than 100 million people being chronically exposed to As from drinking water containing As concentrations exceeding guidelines (Bang et al. 2005; Parvez et al. 2006). Although As pollutants can originate through both natural and anthropogenic sources, human-mediated releases continue to be a main source of As pollution into the environment. Of current interest, mining operations in Canada create both waste tailings and waste rock that may contain high concentrations of As, and other contaminants, making their treatment necessary in order to prevent environment pollution (Wang and Mulligan 2006).

The Canadian Environmental Protection Act (CEPA) lists As in Group 1 of the Priority Substances List which identifies it as potentially being harmful to both environmental and human health (Wang and Mulligan 2006). Inorganic and organic As species can be found in four oxidation states including As(-III), As(0), As(III), and As(V). The individual species oxidation states impact the As species toxicity and mobility in the environment. For example, As(III) species are ten times more toxic than As(V) species, while also being more soluble, making As(III) more difficult to remove than As(V) from aqueous matrices. The commonly available treatment technologies for As in water matrices include coagulation, oxidation, filtration processes, electrochemical methods, adsorption, phytoremediation, and bioremediation (Hu et al. 2012; Kumar et al. 2004; Liu et al. 2014;; Mohan and Pittman 2007; Nicomel et al. 2015; Ungureanu et al. 2015). Of these, bioremediation has become an area of extensive research for a variety of water and wastewaters due to its limited use of chemicals, potential low costs, perceptions of being a more ‘natural’ process, and promise for effective As treatment.

Recently, As remediation research studies reviewed by Alam and McPhedran (2019) have indicated that biological sulphate reduction technologies show promising potential for removal of As from environmental matrices including mine waters. In aqueous matrices, these technologies
are typically reliant upon the presence of sulphate reducing bacteria (SRB) which are considered to be one of the most common and successful bacterial assemblages for As remediation. For example, Rittle et al. (1995) evaluated the As(III) removal through SRB reduction of sulphate in laboratory microcosms that were operated under strictly anaerobic conditions. They demonstrated that both As(III) and Fe(II) concentrations decreased if sulphide was available in solution with energy-dispersive X-ray (EDS) analysis indicating the precipitation of an iron-arsenic-sulphide solid phase. Alternatively, it has been established that the thermodynamic ladder concept plays an important role in bacterial populations. According to this concept, the FeRB would be expected to dominate in acidic conditions, followed by SRB (Bethke et al. 2011; García-Balboa et al. 2010; Xia et al. 2019). However, this fixed thermodynamic hierarchy was not observed by other researchers including Bethke et al. (2011) who reported a mutualistic relationship between FeRB and SRB populations in long-term experimental bioreactors. Further, FeRB were found to be less abundant than SRBs in other conditions such as in the presence of crystalline Fe(III) (e.g., goethite, hematite) (Ling et al. 2015). Thus, a variety of ecologic and physiologic parameters impact the SRB and FeRB relationships, among other bacteria, highlighting the need to assess individual environmental matrices and conditions to determine their potential for As bioremediation.

The enhancement of SRB growth and metabolism can be accomplished through the supplementation of treatment processes via carbon sources which can lead to the promotion of As remediation. Potential carbon sources specifically aimed towards enhancement of SRB have recently been reviewed by Hussain et al. (2016) with options including sugars, amino acids, alcohols, monocarboxylic acids, dicarboxylic acids, and aromatic compounds. Lactate (or lactose) has been the most common carbon source used for the study of As remediation by SRB in a number of matrices (Alam and McPhedran 2019). However, lactate is considered to be a relatively expensive carbon source that may limit its use on an industrial scale, thereby making the consideration of other carbon sources pragmatic for further evaluation (Nagpal et al. 2000; Bertolino et al. 2014). For example, molasses is low cost, non-toxic, and micronutrient-rich carbon source that has been shown to promote both bacterial growth and metabolism previously (Gerth et al. 2003).

Currently, a common storage method of As-containing mine waste rock in northern Canada is its placement in mined-out pits under water. This storage water becomes contaminated by the waste rock over time, including As species, which can potentially leach into the nearby
groundwater. Thus, development of appropriate treatment technologies for these waters is needed to limit their potentially negative environmental impacts. The main objective of the current study is the evaluation of the As bioremediation potential for treatment of mine pit water at an in-situ condition of 8 °C using molasses as a carbon source. Preliminary experiments in our research group have considered the bioremediation of mine waste rock water using carbon-amended treatments including lactate, molasses, lactate plus sulphate, and molasses plus sulphate. Of these treatments, the molasses treatment was found to be the optimal treatment method making it the preferred carbon source in the current study.

2.2 Materials and Methods

2.2.1 Mine waste rock and water

Mine waste rock and mine pit water sourced from a northern Saskatchewan mining site were provided by Orano Canada Inc. (Saskatoon, Canada). The waste rock was crushed and sieved through a #4 mesh (particles passing 4.76 mm) for use in all of the prepared experimental reactors. The waste rock was crushed and sieved to help create a more homogeneous rock sample that would allow for better reproducibility between experimental replicates given unprocessed rock sizes varied considerably overall. In addition, the crushing resulted in the increase of overall rock surface areas which would be expected to allow for faster mass transfer of bound compounds between rock and water allowing for decreased experimental durations and/or faster equilibrium conditions.

A homogeneous sample of waste rock was acid digested prior to analysis by inductively coupled plasma mass spectrometry (ICP-MS) for determination of the total As concentration of 614 µg/g (Analytical Laboratories and Saskatchewan Research Council (SRC), CA). X-ray diffraction (XRD) analysis and X-ray photoelectron spectroscopy (XPS) analyses were conducted at the Saskatchewan Structural Sciences Centre (SSSC) at the University of Saskatchewan, Canada. The XRD analysis showed that the waste rock mainly consisted of quartz. The XPS analysis was completed on five separate locations of a waste rock sample with major elements found including oxygen (61 to 67%), carbon (6.7% to 16%), potassium (1.2% to 1.7%), sulphur (0.5% to 0.7%), silicon (12% to 14%), aluminum (7.9% to 8.6%), magnesium (0.7% to 1.1%), and iron (0.3% to 0.5%).
2.2.2 Reactor preparation and sampling protocol

An overall schematic of the experimental setup for this project is included in Figure 2.1. Experiments consisted of six reactors (18 reactors in total) for each treatment including molasses, negative controls, and positive controls. Reactors were prepared in 2 L glass vessels each containing 500 g mine waste rock and then filled to a total volume of 1.7 L with the addition of the mine site water. The molasses treatment reactors included the mine waste rock and mine pit water with supplementation of molasses at 1% (v/v) molasses (Fisher Scientific, Canada) as a carbon source to promote bacterial growth. The negative control reactors included sterilized mine waste rock and mine site water (both sterilized via autoclave) amended with sodium azide (500 mg/L; Sigma-Aldrich Canada) to inhibit microbial growth without supplementation of a carbon source. The positive control reactors included mine waste rock and mine pit work without further processing or carbon addition.

The reactors were prepared in six individual anaerobic bag chambers that were continuously flushed with nitrogen gas and the bag chamber air was continuously vacuumed in order to prevent oxidation of As while the reactors were being prepared. Each of the reactors were saturated with nitrogen up to the point that the dissolved oxygen of the water in the reactors was less than 0.1 mg/L, then they were sealed with a layer of parafilm and an airtight polyethylene cap thus creating an anaerobic condition for the duration of the experiments. Each of the individual anaerobic chambers included three reactors: a negative control, a positive control and a molasses treatment reactor. Six sets of anaerobic bag chambers containing 18 reactors in total were prepared and stored in the dark in an 8 °C controlled temperature chamber at the Phytotron facility at the University of Saskatchewan.

Starting after one month, a set of three reactors was sacrificed for sample preparation for completing a series of analyses once a month for a total duration of 6 months. Water samples were collected from each reactor for As speciation (As(III) and As(V)) in months 3 and 6 which was conducted using ion exchange ICP-MS at the environmental laboratory of Saskatchewan Research Council (SRC; Saskatoon, SK). For As speciation samples, 50 mL from each treatment reactor was filtered using a 0.45 µm glass fiber membrane filter (Thermo Scientific, USA). The samples were then acidified with 0.5 mL of concentrated HCl acid and transported in a cooler box to SRC right after collection. At SRC, the samples were stored at 4 °C and analyzed within 72 h. To conduct As speciation, 25 mL of the acidified sample was passed through a glass column (10 cm
X 7 mm) filled with 115 g chloride form resin. At this acidity, the As(III) passes through the resin while As(V) is retained. The As(III) was collected directly from the column, while the As (V) was eluted from the column using 0.12 M HCl. The As(III) and As(V) samples were then analyzed using ICP-MS given a total As in the collected sample since this method cannot be used to separate As(III) and As(V) independently.

For monthly samples, metal(loid)s were quantified via inductively coupled plasma mass spectrometer (ICP-MS) analysis conducted in the Geological Sciences laboratories at the University of Saskatchewan and general chemistry and anion concentrations were determined using Ion Chromatography (IC) in the Environmental Engineering laboratories at the University of Saskatchewan.

Previous research conducted for optimization of carbon sources included replication of reactors (n=3). This study used only single treatment reactors due to space limitations of the controlled temperature chamber. Additionally, eliminating the replication of reactors allowed for the benefit of more sampling intervals over time, with the drawback of no replication. However, many of the previous study replicate samples needed to be combined to save on analytical costs.

2.2.3 Bacterial community analyses

BacTiter-Glo™ Microbial Cell Viability Assays (Promega, USA) were conducted to determine the number of viable bacterial cells in the samples. This method determines the number of viable bacterial cells based on quantifying adenosine tri-phosphate (ATP). In this method, a single reagent is added to bacterial cells in samples and luminescence is measured. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells in samples. To conduct ATP tests, 100 µL of samples collected from each reactor were added to 100 µL BacTiter-Glo™ reagent in opaque-walled 96-well plates in triplicates. After mixing and incubating in an orbital shaker for 5 minutes, the luminescence of content was measured using a GloMax® 96 Microplate Luminometer (Promega, USA) available at the Cancer Cluster Laboratory of Health Sciences at the University of Saskatchewan.

Genomic DNA was extracted using a DNeasy Power Water Kit (Qiagen, USA). The DNA extraction was conducted by passing 500 mL water samples collected from each reactor through a 0.45 µm membrane filter (Thermo Scientific, USA). Illumina MiSeq analysis of 16S rRNA V4 amplicons (with 515F/806R primer) were conducted by RTL Genomics (USA) based on paired-
end sequencing method (Kennedy et al., 2014). To identify available bacteria in each sample, the high-throughput sequencing data obtained from RTL genomics were submitted to the Bio Project archive of the National Center for Biotechnology Information (NCBI, USA). The relative abundances of operational taxonomic units (OTUs) from the treatment samples, sorted with a sequence similarity cut-off of 97%, were calculated.
Figure 2.1: Schematic of the experimental design including molasses treatment, positive controls, and negative controls.
2.3 Results and Discussion

2.3.1 Physio-chemical parameters over time

The pH in the molasses and control treatment reactors was monitored for each month over the six-month experimental duration (Figure 2.2). Overall, the pH decreased between the first and sixth month for all treatments including from pH 4.35 to 3.70 for molasses, pH 4.55 to 4.20 for positive controls, and pH 5.40 to 5.20 for negative controls. Despite this overall decrease, over the first four months the pH values for each treatment remained relatively consistent. Following this stable period, in months 5 and 6 the pH values for all treatments decreased markedly. Interestingly, starting at month 1, the molasses and positive controls clearly had lower initial pH values than the negative controls. The pH values were similar for the first four months for these two treatments, while the pH decrease in the last two months was faster for the molasses treatment vs. positive control treatments.

The molasses and positive control treatments each would be expected to have had native bacteria present in both the mine waste rock and mine pit water that could stimulate bacterial growth in these reactors leading to a decrease in reactor pH (even after only one-month duration). Hoirdahl and Borden (2014) indicated that a decrease (or increase) in pH can potentially be created by stimulating (or inhibiting) microbial growth. The pH decrease could potentially be attributed to the roles of fermentation and syntrophic metabolisms of bacteria which produce acids thereby decreasing pH. For the negative control treatment, the inhibition of these bacterial metabolisms via sterilization of mine waste rock and pit water, and the presence of sodium azide for bacterial inhibition, could explain the relatively stable pH in the negative control at about pH 5.5 over the first four months. For months 5 and 6, it appears the marked increase in bacterial activity (see Section 3.2) in the molasses and positive control reactors could explain the drop in pH in these months. A similar, but smaller increase in bacterial activity for negative controls indicates that, despite the best efforts to impede bacterial growth, the bacterial activities may have started to increase for this treatment after an initial lag phase. This is also evidenced in the bacterial activity results which will be discussed further in Section 3.2. In addition, bacterial community analysis in Section 3.3 will help to elucidate some of the species present in each treatment and their roles in the bioremediation process.
Figure 2.2: pH in treatment and control reactors over a six months duration.
The potential for As treatment via bioremediation or through processes such as adsorption or precipitation are highly dependent on the solution pH. Maguffin and Jin (2018) emphasized the importance of pH changes as being major controlling factors governing the efficacy of As remediation technologies. For example, bioremediation removal rates of dissolved As were found to be higher when the pH of solutions were lower (Maguffin and Jin 2018; Rodriguez-Freire et al. 2014; Sun et al. 2016). In addition to pH, the oxidation of As is also a factor in treatment effectiveness. In the current study anaerobic conditions favoured the As(III) species (see below for speciation discussion) which is more soluble than As(V). Under anaerobic conditions, the optimum pH range for removal of As via adsorption was found to be 5.5 to 6 (Badr and Al-Qahtani 2013). In addition, the As removal from solution is highly dependent on both the initial and final pH of solution that can change throughout the treatment process (Can et al. 2012).

The total As aqueous concentrations for each experimental treatment for each month of the six month experimental duration are shown in Figure 2.3. For the molasses treatment, the As concentration increased slowly from months 1 to 5 starting at 0.58 mg/L the first month, followed by a marked increase to 3.70 mg/L in month 6. The positive control reactors had a similar trend to the molasses reactors, despite lower concentrations, with a month 1 value of 0.39 mg/L and month 6 value of 2.45 mg/L. There was an unexplained spike in the positive control treatment for month 2 which returned to about month 1 levels in the following month 3 sample reactor. The negative control reactors had the lowest overall As concentrations in the range of 0.1 to 0.5 mg/L, except for the first month where the concentration was 3.4 mg/L.

The increased aqueous As concentrations in the molasses and positive control treatments in the sixth month coincide with the decreased pH discussed previously (Figure 2.2) and the increased bacterial activity to be discussed in the following section (Section 2.3.2). Based on the literature, common As treatment related bacteria include SRB as recently reviewed by Alam and McPhedran (2019) in addition to iron reducing bacteria (FeRB) that have been shown to release mineral-bound As into solution thereby competing with SRB for the carbon source during treatment processes (Islam et al., 2004; Wang et al., 2019). According to bacterial community analysis (Section 2.3.2), the FeRB were the dominant group in both the molasses and positive control reactors. The FeRB would be expected to promote the dissolution of As, thus, once the bacterial activity increased, the FeRB metabolism increased the As concentrations. Discussion of specific bacteria community analysis is presented in Section 2.3.2.
Figure 2.3: Total aqueous As in treatment and control reactors over a six months duration.
The second highest aqueous As concentration of any individual treatment reactor was found in month 1 for the negative control treatment at 3.4 mg/L (Figure 2.3). The inhibition in bacterial growth appeared to be successful in this reactor based on bacterial activity (see Section 2.3.2) and the pH was higher relative to other treatments having high As concentrations associated with lower pH values. Thus, it is speculated that the autoclaving may have led to higher availability of As to be released from the mine waste rock in this treatment. In addition, the sterilization may have also caused the release of bacterial-bound As into solution. This As could have been quickly precipitated out by the second month, leading to reducing As concentrations for the following month prior to an increase in month 6. However, this is speculative and further research for this peak in As for the negative control would be needed for validation.

The aqueous As(III) and As(V) are presented in Figure 2.4 for all the treatments for the third and sixth months. The As speciation was only conducted on these two sampling periods due to high analysis costs for As speciation. Overall, the As(III) dominated all reactors which was expected given the anaerobic condition and relatively low pH of all the treatment reactors. The molasses treatment had the highest As(III) concentrations for both sampling periods at 0.89 mg/L for month 3 and increasing markedly to 3.47 mg/L for month 6. Similar to molasses, the positive control treatment had increasing As(III) concentrations from month 3 to month 6. In contrast, the negative control treatment had only a marginal increase in As(III) over time. For month 3, the As(V) concentrations were negligible for all treatments, while increasing to detectable concentrations in month 6 of 0.21 mg/L, 0.08 mg/L, and 0.03 mg/L for molasses, positive control, and negative control treatments, respectively (Figure 2.4).

In general, the As(III) concentrations closely followed the total As concentrations (Figure 2.3) as would be expected (despite being measured on different instruments). The presence of As(V) in all treatment reactors at month 6 can be the result of the increased total As in this sampling period, as well as the possibility of bacterial metabolism creating As(V) from the available As(III). The speciation of As is important to determine since its toxicity is dependant on both the speciation as well as the solution pH. For example, Fulladosa et al. (2004) showed that pH impacted the toxicity of both arsenate and arsenite to a luminescent bacterium (Vibrio fischeri).
Figure 2.4: Aqueous As(III) and As(V) concentrations in treatment and control reactors at the 3rd and 6th month sampling event.
Figure 2.5 shows the concentration of four metals over the six months for the molasses treatment reactors only including iron (Fe), cadmium (Cd), aluminum (Al), and selenium (Se). The positive and negative control treatment concentrations of these metals have been omitted from these figures for clarity; however, trends will be discussed herein (data not shown). For Fe, the molasses treatment concentrations remained stable for months 1 to 5 followed by a spike in month 6 reaching about 350 mg/L (Figure 2.5(e)). In contrast, both the positive and negative controls had stable Fe concentrations over the entire six months duration with concentrations under 50 mg/L. For Cd, all three treatments had similar concentrations ranging from 1 to 5 µg/L over the six months with no discernible trends over time except for a decrease in month 6 similar to the Al and Se reductions for the molasses treatment only (Figure 2.5(b)). For Al and Se, the positive control treatment closely followed the molasses treatment trend with a decrease in months 5 and 6 for Al, and month 6 for Se, from the previous months (Figure 2.5(c) and Figure 2.5(d)). In contrast, the negative control treatments had almost negligible Al concentrations for the duration of the study. Additionally, the negative control Se was under 100 µg/L for all months.

Of most interest currently is the increase in the Fe concentration for the molasses treatment in month 6 (Figure 2.5(a)). The peak in Fe coincides with the peak in As (3.7 mg/L), the lowest pH value (pH 3.7), and the increase in bacterial activity (Figure 2.6; Section 2.3.2). The biological treatment of As can be reliant upon the presence of Fe. For example, White et al. (2013) indicated that As can be precipitated or adsorbed concurrently with the oxidation of As and/or Fe. In addition, As removal has been shown to increase in the presence of Fe via formation of FeAsS, and other compounds (Sahinkaya et al., 2015; Liu et al. 2018). The formation of FeS followed by adsorption to create FeAsS is especially favourable under anaerobic conditions found in the current study. Thus, although both the Fe and As aqueous concentrations were elevated at month 6, the expectation would be that Fe/As compounds would form and precipitate out in the following months. Given the presence of Fe, the bacterial activity at month 6 would be expected to be related to FeRB. This is, in fact, the case currently as discussed in Section 2.3.2. Unfortunately, it was expected that the bioremediation process would be faster and only six months was considered in the current study. Extension of the bioremediation process to longer duration is currently being considered; however, inclusion of adsorption in conjunction with bioremediation is also being studied to help improve the time duration for the treatment process.
Figure 2.5: Aqueous concentrations of (a) Fe; (b) Cd; (c) Al; (d) Se; and (e) SO$_4$ in the negative control, positive control, and molasses treatment reactors over the six months duration. Note Fe values are in mg/L and the Cd, Al, Se, and SO$_4$ are in µg/L.
Although not a focus of the current study, the marked reduction of Cd, Al, and Se in the sixth month for the molasses treatment reactors was interesting. It appears that these metals are impacted by the increases of As and Fe in solution leading to their potential dissolution during this month. The positive control also had similar decreases for Al and Se, with Cd perhaps following molasses treatment declines if the reactors were processed after 6 months. For example, the Se aqueous concentration variation in the reactors could be explained by microbial Se oxyanion reduction to \( \text{Se}^0 \) precipitates via *Paenibacillus* in the positive control and treatment reactors (Lusa et al., 2015). In addition, the concentrations of Cd are very low in all reactors potentially due to the presence of *Pseudomonas* which has been shown to precipitate out Cd by forming cadmium sulphide (Wang et al., 1997). The markedly lower concentrations of Al and Se in the negative control reactors may be attributed to these metals not being released from waste rock and/or the higher pH values in these reactors over time vs. molasses and positive control treatments.

Aqueous sulphate concentrations were also investigated in all reactors for last three months of reactors. The dissolved sulphate concentration decreased in all reactors from the fourth month to sixth months (Figure 2.5e). In the molasses treatment, the dissolved sulphate concentration decreased from 960 to 681 µg/L from the fourth month to sixth month with similar decreases shown in both the negative and positive control treatments. It was observed that sulphate reduction rate was the lowest in the negative control treatment likely due to the inhibiting of SRB. Sulphate reduction rate was slightly higher in the molasses treatment compared to the positive control treatment in the fourth and five months likely due to the growth of *Pantoea* which belongs to non-traditional SRB (de Matos et al. 2013). However, sulphate reduction became higher in the positive control treatment that could be attributed to the growth of the *Desulfosporosinus* genera which belongs to the traditional SRB groups. The bacterial community analysis is discussed in detail in the following sections.

### 2.3.2 Bacterial activities

Figure 2.6 shows the bacterial activities via the Relative Light Unit (RLU) in all three reactor treatments over 6 months of the experiment. It should be noted that the RLU values recorded by the luminometer are directly proportional to ATP with the higher RLU values representing ATP activities, and, subsequently, a higher number of viable bacterial cells in the samples. However, it should be noted that these values do not provide any information regarding bacterial community
compositions. For the molasses treatments, the initial first month RLU value was 19,112 with a significant drop in the second month followed by increases until the sixth month where the RLU value was 46,939. Both the positive and negative controls followed similar trends as the molasses treatment, albeit with lower values, ending at six months with 6,506 and 2,422 RLU values, respectively.

The initially higher RLUs can be explained via the release of bacteria from waste rock and the initial bacteria in the mine pit water available in the molasses and positive control treatments. For the negative controls, bacteria that survived the sterilization of the waste rock and mine pit water may have recovered enough after one month to have marginal activities at that sampling point. For the molasses and positive control treatments, the increase in RLU for the sixth month coincides with the concurrent decrease in pH (Figure 2.2) increase in As (Figure 2.3) for both of these treatments at this time. Overall, the general trends of increasing RLUs over time also compare well with the As concentrations and pH values. The only exception is the first month where the higher RLU values do not appear to correlate well with any of the physico-chemical parameters.

Knowledge of the bacterial activities, and specific bacterial communities below, is important in the understanding of As fate in bioremediation. Arsenic can readily enter bacterial cells through existing cell-wall transporters due to the analogy of As species to other molecules (Rosen and Liu 2009). Bacterial processes may also impact As speciation during treatment processes. For example, many heterotopic bacteria are capable of oxidizing As(III) to detoxify their environment, while other bacteria use As(III) as an electron donor in aerobic oxidation, anaerobic nitrate and selenite-dependent respiration or phototrophy (Fisher and Hollibaugh 2008). In addition, some bacteria can transform As(V) into As(III) in the process of detoxification (Cavalca et al. 2013). Arsenic-rich environments, such as acid mine drainage, are rich in specific bacteria that can obtain energy from the redox transformation of As(V) as it enters the cell via phosphate transporters where it can then interfere with oxidative phosphorylation by replacing phosphate (Rosen 2002).
Figure 2. 6: Bacterial activities as represented via RLU\textit{s} in treatment and control reactors over a six months duration. Note that error bars represent standard deviation (n=3) of replicate samples from individual reactors.
2.3.3 Bacterial classes

Figure 2.7 shows the relative abundances (%) of various bacterial classes in treatment and control reactors at the 1st, 3rd, and 6th sampling months as determined by Illumina MiSeq analysis. It should be noted that these values only indicate relative abundances, hence, the total bacterial concentrations are not considered. In addition, the negative control treatment bacterial concentrations were generally too low (see Figure 2.6) to provide enough DNA sample for relevant sequencing with the sixth month sample sequencing being unsuccessful due to this issue. In contrast, both the molasses and positive control treatments had adequate DNA for sequencing purposes. In general, bacterial classes were variable both within treatments and over time for both molasses and positive control treatments.

For the molasses treatments there were 20 bacterial classes found over the six months. There were only two common classes found in high relative abundances (>0.5%), namely the Gammaproteobacteria and Betaproteobacteria (Figure 2.7). For the first month, the Gammaproteobacteria were almost the only class identified with 99% abundance and only Betaproteobacteria being relevant in this month at 0.5% abundance. In month 3, the dominant bacterial class shifted to the Betaproteobacteria (54.5%) followed by Actinobacteria (9.8%), Gammaproteobacteria (9.6%), Planctomycetia (4.8%), and Alphaproteobacteria (4.7%), respectively. By month 6, the Bacilli class became dominant with 55% abundance followed closely by the Clostridia at 35.3%.

For the positive control treatment there were 21 bacterial classes found in the three sampling periods. Interestingly, the positive controls also had the same two common classes found in high relative abundances (>0.5%) as the molasses treatments, namely the Gammaproteobacteria and Betaproteobacteria (Figure 2.7). In the month 1, the dominant class was Betaproteobacteria at 37.5% followed by Actinobacteria (18.2%), Gammaproteobacteria (5.6%), Sphingobaceteria (4.9%), and Bacilli (4.6%). For the third month, the Betaproteobacteria (54.3%) became the dominant class with high abundances of Alphaproteobacteria (14.0%), Gammaproteobacteria (10.5%), and Actinobacteria (5.2%). In the last sample month, the Betaproteobacteria continued to be the dominant class increasing to 77.0% abundance. The Clostridia (13%) and Deltaproteobacteria (6.8%) were the second and third most dominant classes, followed by the Gammaproteobacteria at 1.7%. For the negative control treatments only four bacterial classes were found in months 1 and 3, with no bacteria being determined in the sixth month sampling due to
Figure 2.7: Relative abundance (%) of various bacterial classes in treatment and control reactors at 1\textsuperscript{st}, 3\textsuperscript{rd}, and 6\textsuperscript{th} months. Note that the negative control samples had limited DNA available for extraction due to low bacterial numbers, see text for further information.
limited DNA being extracted. In month 1, the main class found was Fusobacterilia (64.0%) followed by Alphaproteobacteria (12.0%) and Gammaproteobacteria (12.0%). In month 3, only Epsilonproteobacteria were detected accounting for 100% abundance.

2.3.4 Bacterial genera

Following the bacterial class discussion in Section 2.3.3, this section will present the dominant bacterial genera followed by discussion of their potential role in As bioremediation and mobilization in Section 2.3.5. Figure 2.8 shows the relative abundances (%) of various bacterial genera in treatment and control reactors at months 1, 3, and 6. All genera with abundances greater than 5% of the total abundance for each individual sample are shown in Table 2.1. Overall, 179 bacterial genera were identified in all treatment reactors with only 19 genera being present at greater than 5% of total abundance, thus, being discussed further herein. As for the previous section, focus will be on molasses and positive control treatments given the low bacterial abundances determined for the negative control reactors.

The Pseudomonas genera dominated the month 1 abundance for the molasses treatment reactor at 99.1% (Figure 2.8 and Table 2.1). For the month 3, the Pseudomonas declined drastically leading to the dominance of Methylophilus (17.9%), Rhodoferax (16.7%), and Legionella (8.4%). Similar to the changes in month 3, the month 6 dominant genera became Sporolactobacillus (50.8%) and Paenibacillus (11.8%). For the positive control in month 1, there were many unclassified genera at 40.7% of the total abundance. For classified bacteria, no genera exceeded 10% of the total abundance with the top three genera including Methylophilus, Legionella, and Geobacillus at 7.1%, 6.7%, and 6.2%, respectively. In contrast, month 3 samples had three genera exceeding 10% including Rhodoferax, Methylophilus, and Legionella at 22.2%, 14.7%, and 13.4%, respectively. While months 1 and 3 had marked overlaps in abundant genera, there was a noticeable shift in month 6 which was dominated by Polaromonas at 63.0% of the total abundance. Lastly, for the negative controls in months 1 and 3 the Arcobacter and Sneathia where dominant at 100% and 72.7%, respectively. As discussed previously, no data were available for month 6 for the negative controls.

It should be noted that not all classes and genera would be expected to contribute to As bioremediation or mobilization. In general, As and Fe reducing genera would be expected to play an important role in increasing As release rate from mine waste rock, while SRB could potentially assist either As precipitation or immobilization As through biosorption. The following section will discuss the bacterial roles in As bioremediation and mobilization.
Figure 2.8: Relative abundance (%) of various bacterial genera in treatment and control reactors at 1st, 3rd, and 6th months. Note that the negative control samples had limited DNA available for extraction due to low bacterial numbers, see text for further information.
Table 2.1: Relative abundance (%) of bacterial genera in treatment and control reactors at 1st, 3rd, and 6th months with abundance greater than 5%.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Molasses 1st month</th>
<th>Molasses 3rd month</th>
<th>Molasses 6th month</th>
<th>Positive control 1st month</th>
<th>Positive control 3rd month</th>
<th>Positive control 6th month</th>
<th>Negative Control 1st month</th>
<th>Negative Control 3rd month</th>
<th>Negative Control 6th month</th>
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2.3.5 Bacterial role in As bioremediation and mobilization

This section will present an overview of potential bacterial groups that may have influenced As bioremediation and mobilization in the treatment reactors over the experimental duration. These bacteria may include the following processes: sulphur reduction via sulphur-reducing bacteria (SRB); iron reduction via iron-reducing bacteria (FeRB); bacterial-mediated reduction of As(V) to As(III); bacterial bioadsorption or bioaccumulation; and bacterial-mediated As methylation. Each of these groups will be discussed including their context within the current experiment treatments that contain each of these groups.

The SRB may impact As bioremediation by reducing sulphate to sulphide that could then complex with Fe and As and precipitate out of solution. Common SRB that have been discussed in the literature include members of the families Desulfovibrionaceae and Desulfbacteraceae from Deltaproteobacteria (Minz et al. 1999; Fourçans et al. 2008). Common genera recognized as SRB include Desulfovibrio, Desulfotomaculum, Desulfosarcina, Desulfococcus sp. Desulfbacca acetoxidans, Desulfobulbus, Desulfomicrobium, Desulfbacter, Desulfosarcina, Desulfopila, Desulfonema, and Desulfobacterium (Alam and McPhedran 2019). Interestingly, none of these genera were observed in molasses treatment reactors, however, Desulfosporosinus (13.2%) was found in the positive control reactor in the sixth month sample. Instead, other genera with the capability of sulphate reduction were observed in the molasses treatment reactors. For example, Pantoea is considered to be a non-traditional SRB (de Matos et al. 2013) that was found with an abundance of 64% in the molasses treatment reactor at month 3. One of the most dominant bacteria in the month 6 molasses reactor was Clostridium (27.9%) with some Clostridium species being well-known SRB (Sallam and Steinbüchel 2009). For the positive control treatments, Corynebacterium was observed in the months 1 and 3 reactors at low abundances of 0.11% and 0.79%, respectively. This genus is also known to be capable of reducing sulphate at a low rate (Krömer et al. 2008).

The FeRB may increase the As release from the mine waste rock by reducing the Fe in As-Fe bearing compounds thereby releasing both As and Fe into the solution. In addition, some FeRB genera can uptake Fe via siderophores, thus leading to higher mobilization of Fe from the mine waste rock in conjunction with As. The molasses treatment reactors had the highest FeRB population among the reactors. In the month 1 reactor, the most dominant genera was Pseudomonas (99.07%) that are capable of uptaking Fe via siderophores which could have resulted
in the increased As release from the waste rock in this reactor early in the experimental duration (Cornelis and Dingemans 2013). At month 3, the abundant genera *Rhodoferax* (16.7%) is commonly known for the reduction of Fe(III) (Finneran, Johnsen, and Lovley 2003). Lastly, at month 6 the most dominant bacteria was *Sporolactobacillus* (50.80%) that has been categorized as FeRB genera (Bignall 1994). The species *Pantoea*, also found at low abundance (0.6%) in this reactor, could also contribute to As mobilization as a species belonging to this genus called *Pantoea agglomerans* has been identified as being capable of reducing Fe(III) (Francis et al. 2002; Liu et al., 2015; Lovely et al., 2004;). Other FeRB found in various treatments, and in various abundances, included *Bacillus* (Das et al. 2016; Nealson 1994; Saffarini 1994) and *Arcobacter* (Roalkvam et al. 2015).

A third group of potentially important bacterial genera that were found in various treatments include those able to promote As mobilization from the mine waste rock via reduction of As(V) to As(III) followed by the dissolution of the more soluble As(III) into solution. For the molasses treatment in month 1, *Pseudomonas* were found with 99% abundance. Interestingly, despite this high abundance the *Pseudomonas* population decreased over time to only 0.1% at month 3, and not being found in month 6. It has been reported that *Pseudomonas* could reduce As(V) in minerals (Freikowski, Winter, and Gallert 2010; Joshi et al. 2008; Srivastava and Mukhopadhyay 2013), thus, could have reduced bound As(V) to As(III) in the molasses reactors. For the month 3 molasses reactors, a low abundance of *Kocuria* (0.1%) was the only genera found that have been identified as As(V) reducers previously (Bachate et al. 2009; Li et al. 2017). However, Li et al. (2017) showed that despite the *Kocuria* reducing As(V) to As(III), this reduction did not result in the reductive dissolution of As. Therefore, this genus may not have contributed to the mobilization of As from waste rock into solution through As(V) reduction in this reactor. Lastly, for the month 6 reactor a dominant genus was *Paenibacillus* at 11.8% abundance. The *Paenibacillus* have been shown previously to mobilize mineral-bound As (Corsini et al. 2010; Das et al. 2016), thus, could have potentially led to the mobilization As from the current study mine waste rock via dissimilatory As(V) reduction.

A fourth group of potentially important observed genera were bacteria that are capable of removing As from solution via bioadsorption and/or bioaccumulation. The genera *Zoogloea* were found in very low abundance in the month 3 molasses treatment reactor at 0.02%. The *Zoogloea* have been shown to be able to oxidize As(III) to As(V), thus facilitating As removal from aqueous
solution (Weeger et al. 1999). The *Lactobacillus* were more abundant than the *Zoogloea*, but still low in abundance in the month 3 and 6 molasses reactors at 1.7% and 0.7%, respectively. The *Lactobacillus* have been reported to be capable of removing As (Monachese et al. 2012) in addition to Cd and Pb (Daisley et al. 2019) from water via biosorption. The *Corynebacterium* were found in both the month 3 (0.8%) molasses treatment and the month 1 (0.1%) positive control treatment reactors. The *Corynebacterium* is a genus that has been shown to remove As from water through bioaccumulation (Villadangos et al. 2014). Lastly, the *Pseudomonas* that has been discussed previously as having potential to be an As(V) reducer, has been also found to bioaccumulate As (Mohapatra et al. 2017).

The fifth and final group of potentially important observed genera were bacteria that are capable of As methylation followed by As removal from aqueous solution through volatilization of methylated As. The *Pseudomonas* have already been discussed herein as having genera capable of both As(V) reducing and Fe uptake activities, however, have also been shown to be capable of As methylation which can ultimately lead to the volatilization of As (Zhang et al. 2015). In addition, a species of *Sphingomonas* that was discussed previously as having the potential to reduce As(V), called *Sphingomonas desiccabilis* was previously found capable to be capable of methylation and volatilization of soluble As (Liao et al. 2011).

### 2.4 Conclusions

In this study, the As bioremediation potential of mine pit waste rock and water was investigated over six months with the supplementation of molasses as a potential carbon source for indigenous bacteria. Initially, SRB were considered to be favoured in these experiments, however, FeRB were found that could also potentially impact the As bioremediation. Overall, FeRB were dominant over the six months period which led to increasing the release rate of As (as As(V) and marginally as As(III)) and Fe from mine waste rock after six months for both positive control and molasses-amended treatments. SRB growth did not become dominant during the experiments likely due to the low activities rate caused by the low temperature. In conclusion, bioremediation by SRB was not successful at the low in situ temperature of 8 °C used in the current study after six months experimental duration. This lack of bioremediation is attributed to the outcompeting of SRB by FeRB over the six months. It is expected that SRB may become dominant and help As precipitation at a later point, however, the low in-situ temperature makes the process
The results of this study suggest that As bioremediation through stimulating SRB may not be an appropriate method of As treatment in iron-rich mine pit lakes with acidic pH, so further research is needed to investigate the potential of other As treatment methods.

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Chapter 3: Arsenic Bioremediation: Importance of Sample Preparation for Environmental Arsenic Species Identification

This chapter’s research includes the assessment of preservation methods for preserving aqueous As speciation in produced samples. All the samples were filtered by using 0.45μm filters and maintained in 4 °C temperature in a negative pressure dark room. Then, two sets of samples were acidified by EDTA and HNO₃ and one set left without acidification. It was found that the filtered samples stored at 4 °C without acidification provided the best As speciation preservation as compared to both acidified samples. In this study, this methodology preserved appropriate amounts of As(III) species in aqueous samples as compared with acidified samples that oxidized As(III) species. This result can help As speciation research in sample preservation that is a current need due to inevitable time gaps between sample collections and analysis. It may also simplify the collection of arsenic samples, especially in the field, given the elimination of the need for acids to be used. However, the impact of the elimination of acidification may need to be determined for accurate analyses of other metal(loid) in preserved samples.

Abstract:

Industrial development and increasing anthropogenic activities such as mining continue to be of concern as they lead to pollution of waters. Mine wastes contain heavy metals, such as arsenic (As), that can contaminate surface waters and groundwater. Arsenic is found in four oxidation states in species including inorganic and organic compounds that are dependent on the existence of sorbent materials, pH, redox potential, and microbial metabolic activity. The As(III)/As(V) states dominate in water given their species solubility’s, with As(III) being 10 times more toxic than As(V). Thus, knowing the specific As species in wastes is important for understanding both their toxicity potential and for development of specific treatment technologies. The problem with determining As speciation is the need for advanced analytical instruments for the analysis that are not readily available. Thus, suitable sample processing and storage procedures are vital to preserve the species from the time of sampling to analysis. Currently, three methods for As preservation
were considered including: (1) no acid; (2) ethylene diaminete tetra acetic acid (EDTA); and (3) 2% nitric acid (HNO₃). The overall objective of our research program is to investigate and mitigate As from mine waste rock that will be stored in water in Saskatchewan, Canada, with focus on bioremediation of As at relevant in situ temperatures. Thus, the samples preserved were taken from experimental reactors created as part of this extended research. Overall, the no acid treatment resulted in the most consistent speciation preservation for experimental samples. Interestingly, both standard method acid treatments resulted in unexpected oxidation of As, thus, indicating the need for validation of standard methods that may not be appropriate for all metal/metalloids.

### 3.1 Introduction

Water is a vital natural source and fundamental for human and environmental health and sustainability. Industrial development and increasing anthropogenic activities such as mining continue to be of concern as they can result in the pollution of water resources. Mine wastes often contain heavy metals, such as arsenic (As), that can contaminate surface waters and groundwater. Thus, investigating the vulnerability of water resources due to mine activities is essential to ensure healthy aquatic ecosystems. The occurrence of As in ground water worldwide has had numerous documented negative impacts on both environment and human health (Jain and Ali 2000). The primary routes of As exposure are ingestion and inhalation with ingestion through drinking water being the primary concern for human exposure, especially in developing countries lacking sophisticated drinking water treatment technologies. The World Health Organization (WHO) reported that over 200 million people worldwide are exposed to drinking water containing As concentrations exceeding its guideline value of 10 µg/L (WHO 2017). Arsenic is a naturally occurring metalloid and ubiquitous element that ranks 20th in abundance in the Earth’s crust, 14th in the sea water, and 12th in human body (Mandal and Suzuki 2002). The United States Environmental Protection Agency (USEPA) has listed As as a primary contaminant for some superfund sites and it is considered as the “king” of poison (Nriagu et al. 2007; Sundaram et al. 2008).

Popular treatment technologies that are being used or considered for the remediation of As include coagulation, oxidation, various membrane processes, electrochemical methods, adsorption, and biological options such as phytoremediation and bioremediation (Kumar et al. 2018).
Arsenic is found in four oxidation states (As(-III), As(0), As(III) and As(V)) in a variety of species including inorganic and organic compounds that are dependent on the existence of sorbent materials, pH, redox potential (Eh), and microbial metabolic activity (Yong and Mulligan 2004). The As(III) and As(V) species are dominant in water given their higher solubility’s and As(III) being shown to be potentially 10 times more toxic than As(V) (Jain and Ali 2000). Being more soluble than As(V), As(III) generally needs to be oxidized to As(V) in order to be efficiently removed from various waters (Issen and Frimmel 2003). The determination of the redox state of dissolved As in water samples is important to understand and estimate its toxicity, migration, and geochemical transformation in the environment. Therefore, proper sample preservation technique is essential to stabilize the As states prior to analysis in order to obtain truly representative data in As speciation studies. Knowledge of the oxidation states is also needed for the implementation of appropriate treatment technologies whose efficiencies are typically dependent on the As speciation.

Sample preservation is of a great importance for As, and other metalloids and heavy metals, speciation research in order to stabilize As species during inevitable time gaps (at least days but sometimes weeks to months) between the sampling and analysis stages. The distribution of As species must be preserved to avoid changes in speciation by processes such as redox reactions, metal oxyhydroxide precipitation, and photochemical oxidation (Bednar et al. 2002). Despite the importance of this issue, limited research has been conducted to develop and/or validate appropriate sample preservation techniques for As prior to speciation analysis. In addition, a detailed standardized method could not be found in the limited previous reported studies (Feldman 1979; Bednar et al. 2002). Further, there are agreements and disagreements among reported results on sample preservation for As speciation. For example, there is agreement among various research studies that using filtration (Feldman, 1979; US EPA 1982; McCleskey et al., 2004; USGS 2005) is a necessary step prior to sample preservation since it removes microorganisms that can affect As redox species (Wilkie and Hering 1996; McCleskey et al. 2004). As well, the maintenance of samples at 4 °C as a needed step for As speciation sample preservation is commonly agreed upon among previous studies (Quevauviller et al. 1995; Rassler et al. 1998; Daus et al. 2002). However, there are inconsistencies among the previous research studies on the need for acidifying As samples as a necessary step for As preservation. The inconsistency includes whether samples
should be acidified or not, in addition to the selection of an appropriate acid for use in the preservation of samples. For example, a more recent study suggested storage without any acidification or addition of other preservatives as necessary to preserve speciation of As (Wolf et al. 2011). In contrast, other researchers have suggested that acidification is needed as one of the steps in As speciation sample preservation (Bednar et al. 2002; McCleskey et al. 2004). The need for the addition of acid to samples for preservation of As oxidation species has been explained by the theory that acidification could reduce oxidation and precipitation of Fe and Mn hydroxides that could potentially precipitate and adsorb As in samples containing arsenic species (Wilkie and Hering, 1996; McCleskey et al. 2004). Clearly, there is a need to investigate the need and impact of various sample preservation techniques to produce accurate analytical results and, subsequently, implement appropriate treatment technologies informed by the determined As speciation.

Given the gap in understanding of sample preservation needs, the developing of a detailed standard method of sample preservation for As speciation has not been well-investigated. Hence, the objective of this paper is to develop an appropriate sample preservation method for maintaining of As speciation in samples through comparison of three different recommended methods based on our literature review including: 1) acidifying using nitric acid (HNO₃); 2) acidifying using ethylenediaminetetraacetic acid (EDTA); and 3) without acidification. All samples were filtered prior to preservation for each method and stored at 4 °C prior to analysis.

The As-containing samples considered currently were taken from ongoing experiments on As bioremediation being conducted by our research team (currently Chapter 2 of this thesis). These experiments have included the addition of a carbon source, molasses, to experimental reactors with a goal of releasing As from mine waste rock and then precipitating the As via microbial activity. The use of real-world samples in the preservation investigation is essential as using standard As solutions would not provide for the potential impacts of matrix effects on the preservation technique. However, we will also include standard arsenic As(III) and As(V) solutions, and mixtures, in future research work as standards for analytical comparisons. Standard solutions of As(III) and As(V) (as well as mixtures) were analyzed without preservation using the hard X-ray microanalysis (HXMA) beamline at the Canadian Light Source (CLS) synchrotron prior to commencing the current project. The X-ray absorption near edge structure (XANES), HXMA beamline methodology is used as a comparison to the preserved samples in the current study given its ability to show real-time As(III) and As(V) speciation without preservation. It should be noted...
here that the HXMA results are qualitative, not quantitative, thus indicating only the existence of
the species and not the actual concentrations. In addition, the use of the CLS is prohibitively
expensive for consideration as an As speciation methodology for general samples.

3.2 Materials and Methods

3.2.1 Overview of Arsenic Bioremediation Experiments

For clarity of understanding of the samples used in preservation experiments and results, the
bioremediation experimental setup is described briefly herein (following Chapter 2 of this thesis).
The As bioremediation experimental set up included molasses-amended, positive control, and
negative control treatment reactors (Figure 3.1). The positive control reactors included waste rock
and mine water without supplementation with a carbon source. The negative control reactors
included sterile (achieved by autoclaving) waste rock and water amended with sodium azide to
inhibit microbial growth.

The molasses treatment reactors included molasses added as a carbon source to help promote
microbial growth and metabolism, mine water, and mine waste rock. The waste rock and water
were collected from a uranium mining site in northern Saskatchewan, Canada. The reactors were
prepared in 2 L glass vessels containing 500 g of mine waste rock and filled to a total volume of
1.7 L with mine water. The reactors were prepared in several anaerobic bag chambers that were
continuously flushed with nitrogen and air was continuously vacuumed to prevent oxidation of As
in air (Figure 3.2). Each of the reactors were saturated with nitrogen up to the point that the
dissolved oxygen of the water in the reactors was less than 0.1 mg/L, thus creating an anaerobic
condition for the duration of the experiments. Each anaerobic chamber included three reactors: a
positive control, a negative control and a molasses amended reactor. Six sets of anaerobic bag
chambers containing 18 reactors in total were prepared and stored in the dark in an 8 °C controlled
temperature chamber at the Phytotron facility at the University of Saskatchewan. Starting after one
month, a set of three reactors was sacrificed for sample preparation for completing a suite of
analyses once a month for a total duration of 6 months.
Figure 3.1: Schematic of experimental setup to assess bioremediation potential for arsenic removal from waste rock water. Notes indicate experimental details with analysis including arsenic (As) speciation, metals, physicochemical and microbial properties.
Figure 3.2: An example experimental anaerobic bag chamber shown in the controlled temperature chamber in the Phytotron facility of the University of Saskatchewan.
Water samples were collected from each reactor for As speciation As(III) and As(V) that was conducted using an ion exchange ICP-MS speciation technique at the environmental laboratory of Saskatchewan Research Council (SRC). Prior to the As speciation by ion exchange, 50 mL from each sample were filtered using 0.45 µm glass fiber membrane filters. The samples were then acidified with 0.5 mL of concentrated HCl acid before being stored at 4 °C before sending to the SRC. The final set of sampled reactors (after 6 months of storage) were used for the experiments at the Canadian Light Source Synchrotron HXMA beamline (CLS).

3.2.2 Overview of Sample Processing and Preservation

The experimental schematic used to investigate the different sample preservation methods of the bioremediation experiment samples is depicted in Figure 3.3. Sample preservation for As speciation was investigated using three methods including: (1) acidifying with 1% EDTA; (2) acidifying with 2% HNO₃; (3) and without acidification. Both the EDTA and HNO₃ were analytical grade acids. Each of these methods was used for each of the treatments including molasses, positive control, and negative control using the 3 month sample of the bioremediation experiments. Samples were prepared directly in the prepared anaerobic bag chambers under anaerobic condition in which the chamber was flushed with nitrogen and inside air was vacuumed continuously to maintain the anaerobic conditions. All samples were filtered using a 0.45 µL filter and placed into Nalgene plastic bottles. Acidification with EDTA and HNO₃ were conducted in compliance with the procedures recommended by United States Geological Surveys (USGS) and US EPA Method 600, respectively (US EPA 1982; USGS 2005). The prepared samples were maintained in an anaerobic chamber filled with nitrogen at 4 °C and a dark negative pressure room until removal for analyses.
Figure 3.3: Schematic of the sample preservation techniques including no acid, 1% EDTA, and 2% HNO3. Positive and negative controls follow Figure 1 notes.
**Table 3.1:** Sample preservation details and timeline.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Preservation process</th>
<th>Date of analysis (fresh sample)</th>
<th>Date of analysis (preserved samples)</th>
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<td></td>
<td>28 Jan/2019</td>
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<tr>
<td></td>
<td>1% EDTA</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>No acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2.3 Sample Preservation Details and Timeline

Table 3.1 summarizes the sample preparation details and timeline. To investigate the different proposed preservation methods, sampling from the reactors were conducted first on November 2018. In total, 12 samples were prepared from three different reactor treatments (molasses, positive and negative controls) of which three samples were analyzed immediately to determine As species concentrations of the fresh samples. The remaining nine samples (three for each treatment) were preserved for the later analyses for comparison to the fresh sample speciation. Each of the samples collected from the reactors were preserved via the three different preservation methods as discussed in section 3.2.2. Analysis of the preserved samples were conducted in SRC lab on January 2019 after maintaining the samples for 106 days (approximately 3 months).

2.4 Brief Overview of CLS Arsenic Speciation Experiments

For the preparation of standard arsenite (As(III)) solutions (using As$_2$O$_3$), sodium hydroxide was added to the solution to increase the pH as As(III) does not readily dissolve in water at neutral pH. The solution was then neutralized using sulphuric acid and subsequent dilutions were used to prepare various standard solution concentrations ranging from 1 to 1000 ppm. No pre-treatment of the water was needed for preparing the stock solution of arsenate (As(V)) and similar dilutions were used to created concentrations ranging from 1 to 1000 ppm. Standard As(III) and As(V) solutions (various ratios) were prepared and pipetted into a CLS beamline sample holder and covered with Kapton tape prior to placement in the HXMA beamline. All the beamline experiments As conducted at room temperature.

For arsenic speciation, a technique was developed to determine arsenic speciation in water samples. It was previously reported that X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) were able to measure thioarsenates and thioarsenites in solution (Suess et al. 2009). Thus, a similar experimental design was used for the current As speciation experiments. It was found that the EXAFS experiments required the HXMA beamline (BL) to use a Si(111) monochromator crystal and an Rh mirror. During the experiments, the BL monochromator energy was first calibrated by using a gold metal standard foil kindly provided by XAFS Materials Inc. The data collection mode was in the transmission mode for concentrated samples and the fluorescence mode for the low concentration samples. The gold
standard foil was placed between the ion chamber detectors I1 and I2 throughout the experiment. A stepwise energy calibration was made for each XAFS scan. The As K edge data acquisition configuration was front edge (-200 to -40 eV), 10 eV / step, 1-2 sec / point; edge area (-30 to 50 eV), 0.25 to 0.3 eV / step, 1-2 Seconds/point; EXAFS area (50 eV to 13 k), 0.05 k / step, 1-2 to 5-10 seconds / point.

3 Results and Discussion

3.1 HXMA Beam Analysis

For the As speciation in the HXMA beamline of CLS, standard solutions of each of the individual As species were used to determine the detection limit of the beamline. The detection limit research is out of scope for the current study and will be presented elsewhere. As seen in Figure 3.4, six variations of the different As species solution mixtures were tested with As(III)/As(V) concentrations including 125/375 ppm, 250/250 ppm, 375/125 ppm, 500/500 ppm, 50/50 ppm and 750/250 ppm, respectively. For the first sample tested in the CLS, 125 ppm As(III) solution and 375 ppm As(V) solution were mixed and pipetted into the sample holder, covered with Kapton tape. The result indicated a low concentration for As(III) and high concentration for As(V) were found in the analysis for this sample. For the second sample, 250 ppm solution for both As(III) and As(V) were equally mixed. For this sample, two peaks were expected: one for As(III) and for As(V). However, only As(V) peaks were observed in the analysis as shown in Figure 4. In all other samples where higher concentration of As(III) solutions were mixed with lower concentration of As(V) solution, peaks for As(V) were observed in HXMA beam analysis as depicted in Figure 3.4. These results suggested that the use of room temperature experiments and the creation of aerobic conditions were leading to the rapid oxidation of As(III) to As(V). This was an interesting result showing that samples collected for analysis that are anaerobic (likely containing As(III) as the dominate species) could easily be oxidized and incorrectly determined to be As(V). Therefore, this result indicates the need for sample preservation as an essential issue in As speciation studies. Further HXMA beamline studies were later conducted using flash-freezing of samples as a sample preservation technique specific to the CLS beamline. However, this technique is not feasible for standard analytical instruments and for use by those collecting samples for analysis under typical conditions in the field or laboratory environments.
Figure 3.4: Standards of various arsenic species mixtures measured using the HXMA beamline of the CLS at room temperature.
3.2 Evaluation of Preservation Methods

Initial As(III) and As(V) concentrations (Day 0) were measured for samples prepared from a molasses, a positive control and a negative control reactor in November 2018. However, these samples were incorrectly processed by the SRC laboratories due to improper amounts of resins used for the ion exchange speciation method. This resulted in inaccurate concentrations, however, the results could be compared on a qualitative rather than quantitative method (results are not presented herein due to their inaccuracy). Based on this, it was apparent that the As(III) concentrations were significantly higher than As(V) concentrations as would be expected given the anaerobic conditions of the processed samples. Using this information, the expectation was that the preserved samples should also have As(III) dominated concentrations if the preservation technique was successful.

Preserved samples were analyzed for As speciation after 106 days in January 2019 as illustrated in Figure 3.5. In general, without taking preservation into consideration the overall As concentrations were highest in the molasses treatment and similar for the positive and negative controls. This result was unexpected for the bioremediation experiments as it was expected that the carbon amended treatment would release As from waste rock and then bacteria would metabolize and precipitate out the As (see Chapter 2 for further details). However, this process was not occurring, or was rate limited, leading to the bacteria aiding the release of As from waste rock and increasing the water concentration.
Figure 3.5: Comparison of three preservation treatments for different samples speciation analysis.
As shown in Figure 4.5, the preservation via acidification with HNO$_3$ oxidized all three of the treatments with the decrease of As(III) and subsequent increase in As(V). This oxidization was highest for the molasses sample, considerable for negative control sample, and low for positive control samples. Oxidization by acidification was also observed for the preservation via EDTA with a similar magnitude to HNO$_3$ for the molasses treatment samples where As concentration was initially higher than two control treatment samples. However, unlike HNO$_3$, the oxidization impact of EDTA showed little impact on the preservation of the positive and the negative control samples in which initial As concentrations were considerably lower than molasses sample. In contrast to the acid treatments, the no acid preservation treatment showed an excellent performance for sample preservation of As speciation for both of the molasses and positive control treatment samples. This better performance of preservation lacking the use of an acid for As samples was suggested previously in a study conducted by Wolf et al. (2011). However, a precipitation issue for the no acid preservation was observed in the negative control treatment sample that was not found in the acidified samples. This phenomenon of adsorption of As to Mn and Fe hydroxides in the absence of an acid, followed by their co-precipitation has also been reported by previous studies (Wilkie and Hering 1996; McCleskey et al., 2004). It is unknown why this occurred in only the negative control reactors, thus further work is needed to determine if this result was only an outlier for the current research. Overall, we suggest that the elimination of acidification would be the best approach for sample preservation as it simplifies the sample processing and provided the most consistent performance for the preservation of speciation.

4 Conclusions

Industrial activities like mining lead to the creation of wastewaters that need to be treated prior to release into the receiving environments. The selection and implementation of appropriate treatment technologies relies on the accurate characterization of contaminants found in these waters for their efficient treatment. Currently, our research group is investigating the treatment of As-contaminated mine waters using bioremediation (see Chapter 2 of this thesis). This research success is highly dependent on the knowledge of As speciation in each treatment. In general, samples used for analysis of As need to be properly preserved prior to their analysis to have accurate speciation results. It was found that filtered samples stored at 4 °C without acidification provided the best results for maintaining appropriate As speciation. For the current study, this
methodology maintained the appropriate As(III) species composition as compared with acidification which created As(V) not found in the non-preserved samples (or would be expected under anaerobic treatment conditions). This result will simplify the collection of As samples, especially in the field, given the elimination of the need for acids to be used. However, the impact of the elimination of acidification may need to be determined for accurate analyses of other metals and metalloids in preserved samples.

References


Chapter 4: Discussion and Recommendations for Future Work

4.1 General Discussion:

Arsenic toxicity in water is an environmental issue that has affected millions of people around the world. In Canada and northern Saskatchewan, the current method of storing As-bearing mine waste rock is to place it under water in mined-out pits. This storage method could result in As release from this waste rock into the mine pit water that could then result in the contamination of both adjacent soils and local groundwaters. There are several treatment technologies available for the treatment of aqueous As in various water matrices, however, the majority of these technologies cannot readily be used for the treatment of mine pit water. In addition, this mine pit water can be an ongoing issue, especially after a mine’s decommissioning, as many of the treatment technologies need both electrical power and equipment maintenance via a local technician over time. Thus, bioremediation of this pit water may be a favourable technology as there would be no need for ongoing mine pit treatment, while also be an environmentally-friendly alternative. In this thesis, the bioremediation potential of indigenous sulphate-reducing bacteria (SRB) and iron-reducing bacteria (FeRB), among other bacteria classes and genera, in the treatment of As in the mine pit water at the in situ temperature of 8 °C was investigated. In bioremediation by SRB, indigenous microbial growth is stimulated by a carbon source to promote sulphate reduction. Increased sulphate reduction in the water could provide available sulphide in the water to complex with the released As from the waste rock and precipitate it from water. The previous research in our research group compared different carbon sources frequently used in the relevant literature, including molasses and lactate with and without the addition of sulphate. According to the result of that research, molasses was selected as the optimal carbon source considering the cost and observed SRB growth rate. In this thesis, the performance of molasses in stimulating SRB and As precipitation was investigated over six months duration.

The As speciation is an important concept in As treatment studies. As speciation determines the level of its toxicity and mobility and the optimum method in its treatment. The As speciation in water samples could change over time due to available oxidizers in water sample matrices. There is usually a time gap between sample collection and analysis caused by distance from the sampling locations and laboratories and or As speciation instruments in the lab usually are not ready to
operate on an ongoing basis. This inevitable time gap makes As sample preservation necessary in
As research studies. However, there has not been an agreed upon standard method for As
speciation sample preservation. In this thesis, the most common methods of As sample
preservation were investigated in order to find the appropriate method.

In Chapter 2, the performance of molasses in stimulating indigenous SRB for the As treatment
was investigated at in situ temperature of 8 °C over 6 months. Eighteen experimental reactors in
six sets of three were developed in anaerobic bags and maintained in dark and at 8 °C in a
controlled chamber in the Phytotron facility at the University of Saskatchewan. Each set of three
reactors included a molasses, a positive and a negative control treatment reactor. All the reactors
included mine waste rock (500 g) and mine site water (1.7 L). To inhibit microbial growth in the
negative control reactor, mine site water and waste rock were sterilized by autoclaving and adding
500 mg/L sodium azide. To promote microbial growth in the molasses treatment reactor, 30 mL
molasses was added as the carbon source. Each month (six months in total) a set of the three
reactors was sacrificed for sampling of the reactor water for a variety of physicochemical analyses.
In months 3 and 6, bacterial community analyses were also completed. Results indicated that
adding molasses led to the stimulation of FeRB that led to the increased rate of As and Fe release
from the mine waste rock. It was speculated that these FeRB were able to reduce Fe in As-Fe
bearing bonds in the mine waste rock thereby releasing As and Fe into solution. However, the
expected As precipitation did not occur in the system due to the low rate of sulphate reduction
accomplished by the SRB which may have been limited in abundances due to both the low
temperature and pH of the molasses and positive control reactors. Thus, results of this study
suggest that bioremediation by SRB could be very slow and require longer than six months to
accomplish appreciable As precipitation. Overall, the bioremediation study recommends the
investigation of other treatment technologies, such as adsorption for As, for faster and more
efficient treatment of As in mine pit water.

In Chapter 3, different preservation methods were presented for the preservation of As
speciation in aqueous As samples. All of the As contaminated samples were filtered through a
0.45 μm filter as this is considered a necessary step to remove bacteria from samples. Treatments
considered for sample preservation included addition of 1% EDTA, 2% HNO₃ and no
acidification. All prepared samples were maintained in an anaerobic bag filled with dry nitrogen
and stored at 4 °C in order to maintain the As speciation prior to analysis using ion-exchange ICP-
Overall, the preservation technique without acidification provided the best results in maintaining As(III) and As(V) in the water samples over three months. This result could simplify the collection of As samples, especially in the field, given the elimination of the need for acids to be used. However, the impact of the elimination of acidification may need to be determined for accurate analyses of other metals and metalloids in preserved samples.

4.2 Engineering Significance

This thesis presents an overview of the application of bioremediation using molasses as a carbon source for the treatment of As in mine pit water. Bioremediation by SRB is commonly assumed as an effective technique for As treatment in sulphate-rich environments such as mining-affected waters. However, its performance in the As treatment of mine pit water has not been well-investigated. Mine pit waters in Saskatchewan are a challenging matrix for As treatment as they are at a low in situ temperature of 8 °C, while also being at a low pH under 5. In general, there has been limited or no previous research that has investigated the ability of SRB to treat As at low temperatures. In addition, some recent studies indicated complications in the SRB bioremediation method for As treatment caused by the specific biogeochemical conditions of the treatment matrices. Thus, it is necessary to investigate the bioremediation potential of SRB as a common environmentally-friendly and cost-effective As treatment option. This study investigated As treatment by SRB bioremediation and revealed that As precipitation did not occur after six months due to the low SRB population caused by the low in-situ temperature. The presence of FeRB as the dominant bacterial population led to the release of iron and As from the mine waste rock into the water. Clearly more research is needed to determine the optimum bioremediation method for As-contaminated waste rock stored in mine pit water.

The other important aspect of this thesis was to investigate different preservation techniques for As speciation samples. The As speciation is important since it impacts the mobility, toxicity, and treatment options for As treatment. The As speciation could change shortly after collection in environmental samples due to the presence of different oxidizers. There are usually inevitable time gaps between sampling collection and analysis time making sample preservation necessary. However, no standard method has been developed for As speciation sample preservation. This thesis investigated different preservation techniques for aqueous As speciation samples and concluded that filtering samples through 0.45 µm, followed by storage at 4 °C in air tight Nalgene
bottles placed in an anaerobic bag filled with nitrogen without acidification could preserve samples over three months.

4.3 Future Work

This study suggests further research in the following areas:

- According to the literature, the FeRB and SRB populations depends on the ecologic and physiologic parameters of the system. As discussed in Chapter 2, some studies suggested FeRB growth occurs first followed by SRB growth based on the thermodynamic ladder concept, while the others reported their concurrent growth. In this study, the FeRB growth dominated over the first six months in the experimental reactors at the low in situ temperature of 8 °C. It is expected that SRB growth would become significant after a longer storage duration as the low temperature of system slowed down the treatment processes. Thus, this study suggests the need to investigate bioremediation potential for periods longer than 6 months so that the effects of adding molasses on SRB and FeRB growth can be used to determine the potential for As release/precipitate cycles in the mine pit water.

- As discussed in Chapter 2, the As bioremediation could have been negatively impacted by the addition of molasses that stimulated FeRB growth leading to an increasing As release rate from the waste rock. Based on the literature, it is expected that SRB growth would commence after FeRB growth, however, this study shows that this process takes a long time due to the slow rate of FeRB and SRB growth and metabolism caused by low in situ temperature. Thus, mitigation of the released As may be considered by use of a second treatment process such as the addition of an adsorbent to capture this As from the water.

- Chapter 3 evaluated different preservation techniques for As speciation samples. Results suggested that filtration using a 0.45 μm filter and maintaining the samples at 4 °C without acidification provides appropriate preservation for aqueous As speciation samples. However, the research did not evaluate preservation techniques using standard As solutions and the effect of filtration on preservation given all samples were filtered prior to analysis. Thus, this study suggests further research on the preservation techniques using standard solutions and to investigate the potential effect of filtration on preservation techniques.
Unfortunately, most samples need to be filtered prior to analysis by laboratories to prevent negative impacts on their instrumentation.

- Mine pit waters in Saskatchewan are a challenging matrix for As treatment as they are found at a low in situ temperature of 8 °C, while also being at a low pH under 5. In general, no research has investigated the potential for SRB bioremediation for As treatment of mine pit water at low in situ temperature. Similar to this study, some recent studies indicated complications in the SRB bioremediation method for As treatment caused by the specific experimental biogeochemical conditions. Hence, this study suggests further investigation of the biogeochemical reactions in the mine pit water. This can be accomplished through biogeochemical modelling that could provide an insight into the dominant biogeochemical interactions between mine waste rock and mine pit lake governing As treatment and transport in the mining sites.