REMOVAL OF NITRATE, ARSENIC AND VANADIUM IN BENCH-SCALE BIOLOGICAL FILTERS

by

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Submitted in partial fulfilment of the requirements

for the degree of Master of Applied Science

at

Dalhousie University

Halifax, Nova Scotia

April 2012

DALHOUSIE UNIVERSITY

DEPARTMENT OF CIVIL AND RESOURCE ENGINEERING

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Dated: April 24, 2012

Supervisor: _____

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DALHOUSIE UNIVERSITY

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DEPARTMENT OR SCHOOL: Department of Civil and Resource Engineering

DEGREE: MASc. CONVOCATION: October YEAR: 2012

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Abstract

Nitrate, arsenic and vanadium are all potential groundwater contaminants. Traditional physical/chemical methodologies are often too technical or expensive for rural environments. Biofiltration has been shown to remove a wide range of contaminants depending on the operating parameters. This research examined the possibility of using the denitrifying bacteria, *Paracoccus denitrificans,* to remove nitrate, arsenic and vanadium simultaneously from groundwater with varying iron concentrations. During bench-scale testing nitrate concentrations were reduced by up to 73%, even with the metals present. Without iron, arsenic and vanadium removal was insignificant. Removal increased when iron was added as it was found that arsenic and vanadium could be removed adsorptively by iron hydroxides. With 1 mg/L of iron present, removal rates of 67% and 91% were achieved for arsenic and vanadium, respectively. When the iron was increased to 2 mg/L, the removal rates increased to 85% and 96%, respectively.

List of Abbreviations and Symbols Used

µg	microgram
As	arsenic
AsO_3^{3-}	arsenous acid
AsO_4^{3-}	arsenate acid
C/N	carbon nitrogen ratio
CCL 3	Contaminant Candidate List 3
CFU	colony forming units
Cr	chromium
d	day
EBCT	empty bed contact time
Eh	redox potential
Fe	iron
$Fe(OH)_3$	ferric hydroxide
H	hydrogen
H (H_2)	hydrogen
L	litres
MAC	maximum acceptable concentration
mg	milligrams
min	minute
mL	millilitre
mm	millimeter
N (N_2)	nitrogen
N_2O	nitrous oxide
NO	nitric oxide

NO_2^-	nitrite
NO_3^-	nitrate
NO_3^--N	nitrate expressed as nitrogen
O (O_2)	oxygen
P.	*Paracoccus*
PBS	phosphate buffer saline
pH	potential of hydrogen
rpm	revolutions per minute
sp.	species (singular)
spp.	species (plural)
TOC	total organic carbon
USEPA	United States Environmental Protection Agency

Acknowledgments

I would like to thank Dr. Graham Gagnon for all his help and guidance during this project. I would also like to thank Dr. Lei Liu and Dr. Lisbeth Truelstrup Hansen for reviewing my thesis.

I would also like to thank the rest of the members of Dr. Gagnon research group for their help and advice. Special thanks to Sarah Jane Payne for her mentorship and to Heather Daurie and Shelly Oderkirk for their assistance in the laboratory.

Chapter 1: Introduction

1.1 Project Rationale

Nitrate, arsenic and vanadium are groundwater contaminants which pose health concerns such as various cancers, for the consumers if concentrations surpass a critical level (International Agency for Research on Cancer, 1980; International Agency for Research on Cancer, 2006; Ward *et al.*, 2005). The primary source of nitrate contamination is intensive agricultural practices (Nila Rekha *et al*., 2011). Arsenic and vanadium have both anthropogenic and natural sources. The anthropogenic sources of both are usually related to mining activities (Wang and Mulligan, 2006; Wright and Belitz, 2010). Since groundwater is an important water source, especially for rural Canadians, these contaminants create a public health threat (Agriculture and Agri-Food Canada, 2011).

Biological filtration, or biofiltration, has been shown to be effective at removing various contaminants, depending on the microbial makeup of the biofilm. It is a cost effective, sustainable solution compared to conventional treatment options such as ion exchange and membrane processing (Srivastava and Majumder, 2008).

The mechanism for biological removal of nitrate is well known. Nitrate is converted to nitrogen gas via three intermediate species, nitrite, nitric oxide and nitrous oxide, by denitrifying bacteria in anoxic conditions. This conversion is known as biological denitrification (Karanasios *et al.*, 2010). There are many types of bacteria that are capable of contributing to the denitrification process. *Paracoccus denitrificans* is a common denitrifying bacteria used for drinking water treatment due to its versatility. *P.*

denitrificans also does not produce harmful substances nor is it a causative agent of any human or animal disease (Kim *et al.*, 2004)

The mechanism for metal removal is less known and is highly dependent on the metals and bacteria present. Possible removal pathways include binding to intra or extracellular proteins or adsorbing to metal hydroxides bound to the biofilm (Burger, 2008; Karanasios *et al.*, 2010).

A biofilter that can removal nitrate, arsenic and vanadium simultaneously would be very advantageous for rural communities that are affected by these contaminants but do not have the resources of a larger city. To the author's knowledge the development of a denitrifying biofilter that is capable of removing trace metals has not been reported in the literature.

1.2 Objectives

The main objective of this study is to develop a biological filter that can remove nitrate, arsenic and vanadium from groundwater used for drinking water purposes. The research was split into four stages described herein:

1. Develop and construct a denitrifying biological filter at the bench-scale
2. Assess any toxic effects arsenic and vanadium have on the growth and rate of denitrification of *P. denitrificans*
3. Examine the removal of arsenic and vanadium from synthetic groundwater by denitrifying biological filters inoculated with *P. denitrificans*.
4. Determine whether the addition of iron as a co-precipitate will optimize the removal of arsenic or vanadium during biofiltration.

Chapter 2: Literature Review

2.1 Groundwater

Groundwater is an important drinking water source in Canada. Approximately 25% of Canadians rely on groundwater as their primary water source (Statistics Canada, 2010). Groundwater can be vulnerable to various organic and inorganic contaminants. The source of groundwater contaminants varies. The U.S. Office of Technology Assessment (1984) described six possible sources of groundwater contaminants. The categories and examples of each are summarized in Table 1.

Table 1: Categories and examples of groundwater contaminant sources

Category	Examples
1. Sources which are designed to discharge substances	• Land application of wastewater • Septic tanks
2. Unplanned discharge from sources that are designed to store, treat, and/or dispose of a substance	• Landfills • Graveyards • Underground storage tanks
3. Unplanned discharge from sources designed to retain a substance during transport	• Pipelines
4. Sources discharging a substance as a result of other planned activities	• Irrigation • Fertilizer and pesticide application
5. Activities that induce the discharge of a substance by altering its flow pattern	• Wells • Construction Excavation
6. Naturally occurring sources whose discharge is created and/or amplified by human activity	• Natural leaching from chemical weathering • Salt water intrusion

Possible inorganic groundwater contaminants include cyanide, fluoride, nitrate and sulphate, as well as metals such as arsenic, cadmium, mercury, lead, uranium and vanadium (Ritter, *et al.*, 2002; Wright and Belitz, 2010). Possible organic contaminants

include benzene, benzo[a]pyrene, carbon tetrachloride, dioxins and polychlorinated biphenyls. All of these contaminants pose human health concerns (Ritter, *et al.*, 2002).

Nitrate, arsenic and vanadium were chosen to be examined further. These contaminants were selected because of their negative health affects, their relevance to current research gaps and their importance to Canadians. They will be discussed further in the following sections.

2.1.1 Nitrate in Groundwater

The industrial fixation of nitrogen, known as the Haber-Bosch process, drastically altered agricultural practices; without it, an estimated 40% of the world's population would not be alive (Scharf *et al.*, 2005; Smil, 2001). The process allowed for the manufacturing of fertilizers used to increase crop yield (Nila Rekha *et al*., 2011). Due to its high solubility and mobility in water, nitrate from these fertilizers routinely infiltrates groundwater in areas of intensive agriculture (McLay *et al.,* 2001; Nila Rekha *et al*., 2011). The nitrate concentrations in these regions are often in excess of the Canadian drinking water guideline of 10 mg NO_3-N/L (Health Canada, 2010).

Table 2 summarizes nitrate monitoring results from studies conducted around the world. The most significant finding is the number of wells above the maximum acceptable concentration (MAC) of 10 mg NO_3-N/L; this percentage directly shows the number of people affected by nitrate contamination. The year and wells sampled were used to understand the scope of each study. Maximum nitrate concentrations show the magnitude of nitrate contamination as well as the worst case scenario. Finally, by examining proposed sources, links between land use and nitrate contamination can be observed.

The percentage of nitrate exceedances was also very significant. As reported by Nova Scotia Environment (2009), 19% of wells tested in 2008 in Kings County, Nova Scotia exceeded the Canadian drinking water guideline of 10 mg NO_3-N /L (Table 2). By comparison, a study conducted in the state of Texas (Hudak, 2000) reported concentrations as high as 335 mg NO_3-N /L. Both studies consistently found agriculture to be the predominant source of nitrate. This is concerning as areas of intensive agriculture tend to overlap with regions which rely highly on groundwater for their drinking water. In Canada, 80% of the rural demographic rely on groundwater (Agriculture and Agri-Food Canada, 2011). In Atlantic Canada, similar trends can be found. For example, PEI and New Brunswick rely on groundwater sources for 100% and 50%, of their drinking water, respectively (Statistics Canada, 2003).

Table 2: Summary of nitrate groundwater monitoring results

Region	Wells Sampled	Year	Percentage of wells with concentrations above 10 mg NO_3-N /L	Maximum Concentration (mg NO_3-N/L)	Proposed Source	Reference
Kings county, Nova Scotia, Canada	135	2008	19%	25.5	Unreported	Nova Scotia Environment, 2009
Texas, United States	7793	1990-1998	0-77% depending on the county	335.0	Agriculture	Hudak, 2000
Northern Yucatan Peninsula, Mexico	8	1983-1986	56%	50.4	Human waste, agriculture	Pacheco and Cabrera, 1997
Northern China	69	1993-1994	55%	67.7	Agricultural fertilizers	Zhang *et al.,* 1996
Eskisehir plain, Turkey	51	1986-1988	34.2%	58.0	Agriculture, Municipal and industrial wastewater	Kacaroglu and Gunay, 1997
United States, National Assessment	2130	1992-1995	Up to 15% for 4 of the 33 major aquifers studied	Unreported	Agriculture	Nolan and Stoner, 2000
Ontario, Canada	1212	1991-1992	14%	Unreported	Agriculture	Gross *et al.,* 1998

Region	Wells Sampled	Year	Percentage of wells with concentrations above 10 mg NO_3-N /L	Maximum Concentration (mg NO_3-N/L)	Proposed Source	Reference
Johnstown Castle, Ireland	24	2008	29.2%	13.45	Agriculture, manure	Baily *et al.,* 2011
Bekaa Plain, Lebanon	21	2007-2009	95.2%	>45.2	Agriculture	Darwish *et al.,* 2011
Hashtgerd plain, Iran	26	2007-2008	7.7%	18.0	Agriculture, Municipal and industrial wastewater	Nosrati and Van Den Eeckhaut, 2012
Thessaly plain, Greece	36	2008	Unreported	67.5	Unreported	Stamatis *et al.,* 2011b

Health concerns associated with nitrate are of relevance to public health officials due to a variety of adverse health outcomes. The MAC is set to prevent methemoglobinemia, a disease to which infants are particularly vulnerable. Methemoglobinemia is caused when ingested nitrate is reduced to nitrite in the blood stream. Nitrite is then able to bind with hemoglobin to form methemoglobin. This transformation results in a decrease in the oxygen carrying capacity of the blood (Ward *et al.*, 2005). Additional health risks, such as various cancers and reproductive complications have been shown, however results have been inconsistent (Ward *et al.*, 2005). Cancers which have been found to have a positive association are non-Hodgkin's lymphoma (Ward *et al.*, 1996), colon cancer for certain subgroups relating to vitamin C and meat intake (De Roos *et al.*, 2003), bladder cancer and ovarian cancer (Weyer *et al.*, 2001).

2.1.2 Arsenic and Vanadium in Groundwater

Arsenic and vanadium are both naturally and anthropogenically occurring groundwater pollutants. Arsenic is a global concern, affecting 57 million people in Bangladesh alone with 37 million people exposed to concentrations greater than 50 µg/L (Ahmad, 2001). The current United States Environmental Protection Agency (USEPA) and Health Canada drinking water guidelines have a MAC of 10 µg/L (USEPA, 2011; Health Canada, 2010). Less is known about vanadium and it is currently unregulated in the United States and Canada. However, vanadium is on the USEPA Contaminant Candidate List (CCL) 3, therefore future regulation is anticipated.

Sources of natural arsenic in groundwater are often attributed to sulphide-based minerals, such as pyrite. Minerals containing high levels of arsenic are associated with volcanic activity, marine sedimentary processes, hydrothermal ore deposits and fossil fuels such as

coal (Smedley and Kinniburgh, 2002; Wang and Mulligan, 2006). Anthropogenic activities amplify the amount of arsenic released from these sources. Mineral and fossil fuel mining and processing, including the disposal of mine waste, is a significant source of environmental arsenic (Popovic *et al.*, 2001; Wang and Mulligan, 2006). Vanadium is also widely distributed in the earth's crust. It is able to substitute for iron and is therefore most often seen in mafic rocks. Marine and high organic carbon shales, and mafic igneous rocks such as basalt are common rocks that contain vanadium (Wright and Belitz, 2010). The most significant anthropogenic source of vanadium is from the ferrous metallurgy industry (World Health Organization, 1988; Wright and Belitz, 2010).

A summary of studies that examined arsenic concentrations in groundwater is shown in Table 3. Table 3 utilizes the same categories as Table 2 in Section 2.1.1. All of the studies found cases of exceedances, some with maximum concentrations in excess of 100 times the drinking water guidelines. In North Carolina alone, over 1400 wells were found to have exceedances. This represents a serious public health risk. The sources of arsenic include wastewater discharge, mining waste and coal-based thermal power plants. Cases of natural sources were also found. Nova Scotia Environment (2005) reported that more than 50% of the province has natural geological conditions that are known to contain elevated arsenic. It was reported that these regions frequently have arsenic concentrations above the MAC.

There were fewer studies published that examined vanadium in groundwater. A summary of the studies that were available is shown in Table 4. Again, the same categorizes as Table 2 in Section 2.1.1 were utilized, with the exception of percentage of wells over the MAC. Vanadium does not have a drinking water guideline, therefore a median value was

reported instead. The median value, combined with the maximum concentration shows variations in the concentrations found. The maximum concentration ranged from 19.4 to 2470 µg/L. The sources were generally considered to be natural.

Arsenic exists most often in the +3 and +5 oxidation states in natural water. As(III) is referred to as arsenite and occurs mostly in the acid form AsO_3^{3-}. It is more predominant in anaerobic reducing environments. As(V) is referred to as arsenate and occurs mostly in the acid form AsO_4^{3-}. It is more predominant in aerobic oxidizing environments. Arsenic can also occur in the -3 and 0 oxidation states (Mohan and Pittman Jr., 2007).

Vanadium can exist in +3, +4 or +5 oxidation states in the environment. In water, vanadium (III) and vanadium (IV) rapidly oxidize to vanadium(V) which is the most usual form in the environment (World Health Organization, 2001). Little work has been done on the speciation of vanadium, but it has been suggested that 12 forms can coexist in solution. The 12 species can be cationic, neutral or anionic (Bhatnagar *et al.*, 2008; Naeem *et al.*, 2007).

Arsenic was classified as a human carcinogen in 1980 (International Agency for Research on Cancer, 1980). Chronic consumption of arsenic via drinking water has been shown to increase the risk of contracting certain cancers including bladder cancer (Steinmaus *et al*., 2003), kidney cancer (Hopenhayn-Rich *et al.*, 1998) and lung cancer (Chen *et al.*, 2004; Hopenhayn-Rich *et al.*, 1998). Skin lesions have also been directly linked to arsenic exposure (Ahsan *et al*., 2000). There has been less research on the effects of vanadium, but it has been classified as a possible human carcinogen (International Agency for Research on Cancer, 2006).

Table 3: Summary of arsenic groundwater monitoring results

Region	Wells Sampled	Year	Percentage above 10 µg/L	Maximum Concentration (µg/L)	Proposed Source	Reference
Delhi Yamuna Flood Plains, India	98	2007	55%	110	Coal-based thermal power plant	Dubey *et al.,* 2012
North Carolina	63856	1998-2010	2.25% with some counties as high as 20%	806	Combination of natural and unknown anthropogenic sources	Sanders *et al.,* 2012
Oropos–Kalamos basin, Attica, Greece	25	2008	Unreported	246.5	Mining waste piles	Stamatis *et al.,* 2011a
Aksaray Province, Turkey	62	2007-2008	50%	201	Natural	Altaş *et al.,* 2011
Pearl River Delta, China	14	2008	42.9%	21	Industrial wastewater discharge	Guanxing *et al.,* 2011
Central–West Chaco, Argentina	86	2007	88%	1073	Natural	Blanes *et al.,* 2011
Osijek, Croatia	30	1996-2007	100%	358	Unreported	Romić *et al.,* 2011

Table 4: Summary of vanadium groundwater monitoring results

Region	Wells Sampled	Year	Median Concentration (µg/L)	Maximum Concentration (µg/L)	Proposed Source	Reference
Oropos–Kalamos basin, Attica, Greece	25	2008	1.4	19.4	Natural	Stamatis *et al.,* 2011a
California, United States	8470	2000-2007	5	140	Natural	Wright and Belitz, 2010
Coronel Dorrego, Argentina	101	2003	510	2470	Unreported	Fiorentino *et al.,* 2007

2.2 Treatment Options

There are a variety of chemical, physical and biological treatment techniques available for nitrate and heavy metals. Nitrate removal techniques include ion exchange, biofiltration, electro-dialysis and membrane processing (Wang *et al.*, 2009). Treatment options for heavy metals include adsorption, biofiltration, ion exchange, membrane processing and chemical coagulation (Wang and Wai, 2004).

2.2.1 Adsorption

Adsorption is a surface phenomenon where contaminants attach to a material surface due to physical, chemical or electrical attraction (Sawyer *et al.*, 2003). Activated carbon is one of the most extensively used adsorbents due to its high surface area to mass ratio (Sawyer *et al.*, 2003).

Adsorption has proven to be effective at removing arsenic. Mohan and Pittman Jr. (2007) described many adsorbents that have been used for arsenic, they include:

- Commercial and synthetic activate carbon
- Agricultural by-products such as rice husks
- Industrial by-products such as chars and coals from pyrolysis reactions, red mud from alumina production, blast furnace slag from steel plants and municipal solid waste incinerators, Fe(III)/Cr(III) hydroxide waste from Cr(IV) production and fly ash from coal combustion
- Manganese greensand and iron oxide coated sand
- Iron oxide/hydroxide
- Zero-valent iron

There is extremely high variability in the performance of each adsorbent depending on factors such as pH, detention time and temperature. A summary of studies conducted using different adsorbents for arsenic removal is shown in Table 5. The table shows the types of water used, the operating parameters and the optimal percent removal. The operating conditions include pH, temperature and contact time, all of which are important adsorptions factors. Iron based adsorbents were predominantly studied. Each adsorbent was very effective at removing arsenic and achieved removals greater than 90%.

Research into adsorbents for vanadium is not as extensive. Naeem *et al.* (2007) found bench-scale filtration columns filled with commercially available iron and titanium dioxide based adsorbents achieved removal efficiencies of 100% with a vanadium concentration of 50 mg/L at a pH of 7.5. Similarly, Bhatnagar *et al.* (2008) found that waste sludge from electroplating processes was able to remove 100% of a 280 mg/L vanadium solution. The sludge was then immobilized as concrete for disposal.

The advantages and disadvantages of adsorption depend highly on the adsorbents used. Adsorbents have a high range of costs, some can be regenerated, some are sensitive to pH and some are sensitive to interference from other contaminants such as nitrate. Picking the correct adsorbent is a very complex decision (Mohan and Pittman Jr., 2007).

Table 5: Summary of arsenic adsorption studies

Adsorbent	Type of Water	pH	Contact Time	Influent Concentration (µg/L)	Temp (°C)	Maximum Removal (%)	Reference
Iron oxide coated sand	Arsenite in distilled water, pH adjusted with NaOH and H_2SO_4	7.5	2 hours	400	27 ± 2	94	Gupta *et al.,* 2005
Iron oxide based sorbent	Arsenate in distilled water	7.5	0.5 minutes	100	Unknown	>90	Zeng *et al.,* 2008
Ferric iron loaded granular activated carbon	Groundwater	7.6-8	1 minute	60	Unknown	>90	Jang *et al.,* 2008
Chitosan coated activated alumina	Arsenic in distilled water, pH adjusted with NaOH and HNO_3	4	12 minutes	200000	25 ± 0.5	100	Boddu *et al.,* 2008
Iron oxide/activated carbon	Arsenic in tap water	Unknown	12 minutes	500	25	99.8	Zhang *et al.,* 2007
Iron coated calcined bauxite	Arsenic in distilled water	7	7.5 minutes	2000	27 ± 2	100	Ayoob *et al.,* 2007

2.2.2 Biofiltration

Biofiltration involves encouraging microbial growth in filters. The microbial growth is able to improve the performance of the filter by including other removal mechanism than just filtration. These mechanisms can include denitrification, nitrification and natural organic matter removal (Droste, 1997).

2.2.2.1 Biological Denitrification

Biological filters are able to remove nitrate via a pathway known as denitrification. This involves the transformation of nitrate to nitrogen gas using facultative autotrophic or heterotrophic bacteria in an anoxic environment (Karanasios *et al.*, 2010). The transformation pathway is seen in Equation 1 (Wang *et al.*, 2009).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO(g) \rightarrow N_2O(g) \rightarrow N_2\,(g) \qquad [1]$$

A summary of various heterotrophic denitrification studies is seen in Table 6. Similarly to Table 5 in Section 2.2.1, Table 6 summarizes the sourcewater type, operating parameters and optimal removals. The primary operating conditions that were considered include carbon source, C/N mass ratio, type of denitrifiers, detention time, pH and temperature. All of these parameters need consideration when designing a biological denitrification system. The carbon sources varied from traditional sources, such as methanol and glucose, to newspaper derived cellulose and corncobs. Each carbon source was very effective as a feed for denitrifying bacteria. The removal rates were generally in excess of 90%. The detention times varied from 13.2 minutes to 48 hours. The C/N mass ratio was important in determining how much carbon source to add. The exact ratio is highly dependent on the type of carbon source used. For complete removal benzoic acid required a ratio greater than 3, whereas glycerol and methanol only required a ratio of 1.

Table 6: Summary of denitrification studies conducted with various carbon sources and reactor types

Source of Denitrifiers	Reactor Type	Carbon Source	Detention Time (hours)	pH	Temp (°C)	Influent Nitrate Conc. (mg N/L)	Optimal C/N Ratio (g C/g N)	Percent Removal at Optimal C/N (%)	Reference
Mixture of activated sludge and anaerobic digester sludge	Batch	Methanol	12	7	30	50	0.9-10.0	93.5-100	Her and Huang, 1995
Mixture of activated sludge and anaerobic digester sludge	Batch	Acetic Acid	12	7	30	50	>1.9	99.7-100	Her and Huang, 1995
Mixture of activated sludge and anaerobic digester sludge	Batch	Glucose	12	7	30	50	>2.0	98.8-99.8	Her and Huang, 1995

Source of Denitrifiers	Reactor Type	Carbon Source	Detention Time (hours)	pH	Temp (°C)	Influent Nitrate Conc. (mg N/L)	Optimal C/N Ratio (g C/g N)	Percent Removal at Optimal C/N (%)	Reference
Mixture of activated sludge and anaerobic digester sludge	Batch	Benzoic Acid	12	7	30	50	3-3.6	92.0-92.1	Her and Huang, 1995
Mixed colony from a denitrification reactor	Batch	Ethanol	48	7.5	Unknown	22.6	1.5	>90	Aslan, 2005
Sludge from a municipal wastewater plant	Membrane bioreactor	Ethanol	19-37	7.04	20-24	30	2.2	>66.7	Buttiglieri *et al.,* 2005
Sludge from a municipal wastewater plant	Membrane bioreactor	Methanol	0.22	7-7.2	Unknown	200	0.41	>97.5	Ergas and Rheinheim-er, 2004

Source of Denitrifiers	Reactor Type	Carbon Source	Detention Time (hours)	pH	Temp (°C)	Influent Nitrate Conc. (mg N/L)	Optimal C/N Ratio (g C/g N)	Percent Removal at Optimal C/N (%)	Reference
Cellulose degrading bacteria isolated from wood infested with termites	Continuous - Column	Newspaper derived cellulose	3	6-8	25 ± 1	22.6	Unknown	100	Volokita *et al.,* 1996
Pseudomonas and *Proteus* spp. isolated from activated sludge	Upflow anaerobic sludge blanket reactor	Glycerol	22	6.9-7.1	20	600	1	98	Grabińska-ńoniewska *et al.,* 1995
Sludge from a municipal wastewater plant	Continuous - Column	Corncobs	5.55	7.5-8.5	28-29	25	Unknown	>85%	Xu *et al.,* 2009
Sludge from a municipal wastewater plant	Continuous - Column	Rice husks	6.7	7.5-8.5	29-30	25	Unknown	>90%	Shao *et al.,* 2009

2.2.2.2 Biological Metal Removal

Biological removal pathways for heavy metals are not as well understood as denitrification. Anoxic bacteria have been shown to remove various heavy metals. Diels *et al.* (2003) studied a sand filter inoculated with *Ralstonia eutropha* CH34, *Pseudomonas mendocina* AS302 and *Arthrobacter* sp. BP7/26. The biofilter was capable of removing 95-100% of zinc and copper, 60-80% of iron and more than 80% of aluminum, silver, chromium, arsenic and selenium.

Denitrifying bacteria have been linked to the oxidation of arsenite to arsenate in sediment and sludges. Arsenate is more susceptible to adsorption to iron and aluminum oxides. Therefore, denitrifying bacteria may be able to aid in decreasing arsenic mobility and toxicity (Sun *et al*., 2008). These processes have not been studied extensively for drinking water treatment applications.

Four possible removal mechanisms for heavy metals have been found. They are:

1. The metal could undergo a redox reaction that results in a less mobile species (Valls and de Lorenzo, 2002)
2. The metal could bind to intra or extra-cellular proteins that have an affinity for the specific metal (Karanasios *et al.*, 2010)
3. The metal could adsorb to exopolymeric substances produced by the biofilm (Panwichian *et al.*, 2011)
4. Finally, the metal could adsorb to metal hydroxides or oxides, such as iron or manganese, bound to the biofilm (Burger, 2008)

2.2.3 Chemical Coagulation

Coagulation involves the addition of a chemical reagent in order to destabilize colloidal particles. The destabilized particles then flocculate to form larger particles which can either settle out or be caught by a filter (Droste, 1997).

Arsenic has been shown to be removed by iron and aluminum based coagulants (McNeill and Edwards, 1997). There are two main mechanisms for arsenic removal during coagulation. Arsenic can either be coprecipitated into a hydroxide or it can adsorb to hydroxide floc (Hu *et al.*, 2012; McNeill and Edwards, 1997). Arsenic flocs formed with both iron and aluminum are very small, and often will not be completely removed using traditional sand filtration (McNeill and Edwards, 1997; Song *et al.*, 2006).

Song *et al*. (2006) used ferric chloride coagulant and coarse calcite to remove over 99% of arsenic from mine drainage water with an arsenic concentration of 5 mg/L. Calcite particles became coated with arsenic floc and were removed via sedimentation or filtration. Hu *et al.* (2012) examined the use of aluminum chloride and polyaluminum chloride coagulants for arsenic removal. During jar tests, a 280 µg/L solution of arsenate was reduced to below 10 µg/L. Micro and ultrafiltration membranes have been used successfully in conjunction with coagulation. Ćurko *et al.* (2011) examined the removal of arsenate from tap water spiked to 100 µg/L using ferric chloride as a coagulant followed by microfiltration in a pilot treatment plant. During the study the effluent was always below the drinking water guidelines, with an average effluent concentration of 3.4 µg/L.

No studies have been reported in the literature concerning the removal of vanadium using coagulation.

2.2.4 Ion Exchange

Ion exchange can be used to remove nitrate and metals. Ion exchange resins consist of large molecular weight organic substances that can exchange one ion for another. Once the resin is spent, it can be regenerated. The regeneration processes creates a brine of the contaminants that were previously removed (Droste, 1997).

Nitrate and arsenic can be removed using a strong base anion exchange resin (Kim and Benjamin, 2004). An *et al.* (2005) found that two different strong base anion exchange resins, as well as a copper loaded polymeric ligand exchanger, were very effective at treating a 75-95 µg/L arsenate solution. Removal rates were in excess of 90% for each resin but the copper loaded polymeric ligand exchanger was able to treat more bed volumes before breakthrough. Similar removals using strong base anion exchange resins were found by Korngold *et al*. (2001). In this study, two resins were examined: Relite-A-490 resin, designed for selective removal of nitrate and Purolite A-505 resin, a type 1, trimethyl ammonium resin. Both obtained removal rates of 99% when the influent arsenate concentration was 600 µg/L. There was significant variation in the number of bed volumes each resin could treat before having an effluent concentration greater than the drinking water guidelines. Relite-A-490 resin was able to treat 3.5 times as many bed volumes as Purolite A-505 resin. This is a promising result as arsenic and nitrate could potentially be removed simultaneously by one resin. This concept was studied further by Kim and Benjamin (2004). Nitrate and arsenate removal by a sulfate-selective, strong base anion exchange resin was studied. The influent concentrations were 40 µg/L of

arsenate and 13.5 mg N/L of nitrate. Complete removal of each species was possible, but the resins preference for sulphate was observable.

Less research has been conducted using ion exchange to remove vanadium from drinking water. Anion exchange has been used to remove vanadium in industrial processes. Hu *et al*. (2009) used a strong base anion exchange resin to remove vanadium from a molybdate solution in order to produce high-quality ammonium molybdate. The molybdate solution examined contained 600 µg/L of vanadium. The strong base anion exchange resin achieved a vanadium removal of 99.5%.

Ion exchange is effective at removing all of the contaminants. A major portion of studies on arsenic focus on arsenate because it is more readily absorbable than arsenite. If arsenite is present, it may have to be oxidized in order to improve the effectiveness of ion exchange. The disadvantages of ion exchange are related to regeneration and costs. During regeneration, a waste brine is generated which requires disposal. Ion exchange resins are also very expensive (Wang *et al.*, 2009).

2.2.5 Membrane Processing

Membranes can be used to remove nitrate, arsenic and vanadium. During membrane treatments, water or constituents in water are driven through a membrane. The driving force can be pressure, concentration gradient or electrical potential. The pore size of a membrane is the most import factor in dictating what can pass through the membrane. Membranes can also be selective toward anions or cations, depending on the membrane surface charge (Droste, 1997).

Nitrate has been removed at full scale using nanofiltration or reverse osmosis membranes. The membranes can be driven using pressure or electrical potential in a process called electro-dialysis (McAdam and Judd, 2006). Electro-dialysis uses electrodes on either side of a membrane to stimulate the migration of charged particles. Anions will migrate to the cathode and cations will migrate to the anode. Electro-dialysis removes nitrate by using a membrane that is selective toward anions between the electrodes, with the cathode on the permeate side. When voltage is applied to the electrodes, nitrate will migrate through the membrane towards the cathode. A concentrated brine will form on the permeate side of the membrane (Droste, 1997).

Table 7 summarizes studies which have utilized nanofiltration or reverse osmosis to remove nitrate, arsenic and vanadium. The summary is similar to Table 5 in Section 2.2.1 and Table 6 in Section 2.2.2.1. The operating parameters for membrane processing include the type of membrane used and the transmembrane pressure applied. Both of these factors have an influence on the removal of each species. Nitrate removal rates had very high variability, ranging from 92% to just 7.5%. The removal rate was highly dependent on the membrane used and the transmembrane pressure. Both nanofiltration and reverse osmosis were found to be effective at removing arsenate, but less effective at removing arsenite. Arsenate removal was generally greater than 90%, while arsenite removal could be as low at 9.8%. There were limited studies done on the removal of vanadium using membranes. High removal rates were shown to be possible though, based on the research of Richards *et al.* (2011).

In summary, membranes are effective at removing nitrate, arsenic and vanadium. They are also relatively simple to operate and can be automated (Wang and Wai, 2004).

Membranes do have disadvantages; they are expensive and they generate a concentrated effluent which must be safely disposed of.

Table 7: Summary of membrane processing studies for nitrate, arsenic and vanadium

Membrane Type	Commercial Name	Driver	Type of Water	Influent Concentration	Pressure/Current	Removal (%)	Reference
Nitrate Studies							
Nanofiltration	Dow-FilmTec NF90	Pressure	Groundwater	62.1 mg N/L	1.6 MPa	~92	Santafé -Moros et al., 2005
Nanofiltration	Dow-FilmTec NF270	Pressure	Groundwater	62.1 mg N/L	1.6 MPa	~77	Santafé -Moros et al., 2005
Nanofiltration	Hydranautics ESNA1-LF	Pressure	Groundwater	62.1 mg N/L	1.6 MPa	~34	Santafé -Moros et al., 2005
Nanofiltration	Dow-FilmTec NF70	Pressure	Groundwater	9.9 mg N/L	1.0 MPa	75	Van der Bruggen *et al*., 2001
Nanofiltration	Dow-FilmTec NF45	Pressure	Groundwater	9.9 mg N/L	1.0 MPa	16	Van der Bruggen *et al*., 2001
Nanofiltration	Toray Ind. Inc. UTC-20	Pressure	Groundwater	9.9 mg N/L	1.0 MPa	32	Van der Bruggen *et al*., 2001
Nanofiltration	Toray Ind. Inc. UTC-60	Pressure	Groundwater	9.9 mg N/L	1.0 MPa	11	Van der Bruggen *et al*., 2001
Reverse Osmosis	SS10	Pressure	Amended tap water	22.6 mg N/L	2.76 MPa	76.3	Bohdziewicz et al., 1999
Reverse Osmosis	ST10	Pressure	Amended tap water	22.6 mg N/L	2.76 MPa	66.7	Bohdziewicz et al., 2000
Reverse Osmosis	SR10	Pressure	Amended tap water	22.6 mg N/L	2.76 MPa	65.0	Bohdziewicz et al., 2001

Membrane Type	Commercial Name	Driver	Type of Water	Influent Concentration	Pressure/Current	Removal (%)	Reference
Reverse Osmosis	SF	Pressure	Amended tap water	22.6 mg N/L	2.07 MPa	50.9	Bohdziewicz et al., 2002
Reverse Osmosis	SX	Pressure	Amended tap water	22.6 mg N/L	1.36 MPa	40.4	Bohdziewicz et al., 2003
Nanofiltration	HG19	Pressure	Amended tap water	22.6 mg N/L	0.69 MPa	8.6	Bohdziewicz et al., 2004
Nanofiltration	SX10	Pressure	Amended tap water	22.6 mg N/L	1.36 MPa	32.1	Bohdziewicz et al., 2005
Nanofiltration	SV10	Pressure	Amended tap water	22.6 mg N/L	1.38 MPa	28.0	Bohdziewicz et al., 2006
Nanofiltration	SX01	Pressure	Amended tap water	22.6 mg N/L	1.36 MPa	24.8	Bohdziewicz et al., 2007
Nanofiltration	BQ01	Pressure	Amended tap water	22.6 mg N/L	0.69 MPa	11.5	Bohdziewicz et al., 2008
Nanofiltration	MX07	Pressure	Amended tap water	22.6 mg N/L	0.69 MPa	7.5	Bohdziewicz et al., 2009
Anion exchange	Unknown	Electro-dialysis	Groundwater	47.4 mg N/L	0.12 A	90.0	Sahli et al., 2008

Membrane Type	Commercial Name	Driver	Type of Water	Influent Concentration	Pressure/Current	Removal (%)	Reference
Arsenic Studies							
Nanofiltration	Toray (specifics unknown)	Pressure	Synthetic groundwater	20-90 µg/L	Unknown	>90% for As(V), <9.8% for As(III)	Xia *et al.,* 2007
Nanofiltration	Osmonics Inc NF-300	Pressure	Groundwater	409 µg/L	2.0 MPa	96.0	Saitua *et al.,* 2011
Nanofiltration	TFC NF-300	Pressure	Amended tap water	51 µg/L	5.0 MPa	99.8	Harisha *et al.,* 2010
Reverse Osmosis	Nitto Electric Industrial Co. ES-10	Pressure	Arsenic in distilled water	100 µg/L	1.5 MPa	~95% of As(V), ~75% of As(III)	Kang *et al.,* 2000
Reverse Osmosis	Nitto Electric Industrial Co. NTR-729HF	Pressure	Arsenic in distilled water	100 µg/L	1.5 MPa	~95% of As(V), ~20% of As(III)	Kang *et al.,* 2000
Vanadium Studies							
Reverse Osmosis	BW30	Pressure	Groundwater	22 µg/L	9 MPa	>95%	Richards *et al.,* 2008

2.2.6 Synthesis

For this study, four factors were taken into consideration when deciding on a treatment system. Since the region targeted is rural, the system should be cost effective. It should be diverse enough to handle nitrate and metals. The system should create minimal waste, as access to safe disposal sites is often limited. Finally, the system should by sustainable and utilize green technology.

A summary of the available treatment techniques is seen in Table 8. Adsorption, electro-dialysis and chemical coagulation were eliminated as options because they were not able to remove both contaminants. The remaining options were biofiltration, ion exchange and membrane processing.

Table 8: Summary of the available treatment techniques for each contaminant

Nitrate	Heavy Metals
Biofiltration	~~Adsorption~~
~~Electro-dialysis~~	Biofiltration
Ion exchange	~~Chemical coagulation~~
Membrane processing	Ion exchange
	Membrane processing

Ion exchange and membrane processing are both highly capable but have high capital cost and waste brine disposal issues (Bae *et al.,* 2002; Schoeman and Steyn, 2003; Srivastava and Majumder, 2008). Alternatively, biofiltration is less expensive and is generally considered a more ecologically friendly technology. Capital and operating costs

are particularly concerning considering that the majority of nitrate treatment systems would be designed for rural communities. Biofilters can be relied upon to remove a variety of contaminants depending on the composition of the biofilm (Srivastava and Majumder, 2008). For the reasons stated, biofiltration was chosen as an appropriate treatment technology for this study.

2.3 Biofiltration Design

2.3.1 Loading Parameters

Empty bed contact time and nitrate loading rate were the two key parameters considered when designing the biofiltration setup. Empty bed contact time (EBCT) is the time it takes water to flow through the sand portion of the filters. It is defined by the Equation 2.

$$EBCT = \frac{Volume\ of\ the\ filtration\ media}{Flow\ rate} \quad [2]$$

Nitrate loading is the amount of nitrate added to the filtration column per day. It is defined by Equation 3.

$$Nitrate\ Loading\ Rate = \frac{Nitrate\ concentration}{EBCT} \quad [3]$$

Sison *et al.* (1995) found that nitrate removal rates of 90% could be obtained with an EBCT >60 min or a nitrate loading rate <0.48 g/L•d. The experiment was conducted at the bench-scale and used an upflow granular activated carbon filter. Nitrate was added continuously at 20 mg/L, while the carbon source was only added for 10 minutes every 24 hours.

Experiments conducted with higher nitrate influent concentrations required much longer EBCT. Lee *et al.* (2001) studied denitrification of wastewater. The concentrations considered ranged from 700 to 900 mg N/L. The required EBCT for complete denitrification with a nitrate loading rate of 2.84 g/ L•d was 6.76 hours.

EBCT can vary considerably depending on the target contaminant. In biofilters that target organic matter, an EBCT as low as 4 minutes has been shown to be effect (Hozalski *et al.,* 1995).

2.3.2 Bacteria

There are both heterotrophic and autotrophic denitrifying bacteria. Heterotrophic denitrifying bacteria utilize carbon compounds for electron sources and energy (Karanasios *et al.*, 2010). Possible carbon compounds are ethanol (Aslan, 2005), methanol (Wąsik *et al.*, 2001), acetate (Her and Huang, 1995), benzoic acid (Her and Huang, 1995), glucose (Her and Huang 1995) or glycerol (Kim *et al.*, 2004). The products of heterotrophic denitrification are nitrogen gas, water, carbon dioxide and hydroxide ions (Wang *et al.*, 2009). Alternatively, autotrophic bacteria utilize hydrogen, iron or sulphur compounds (Karanasios *et al.*, 2010). The excess by-products of iron and sulphur autotrophic denitrification are iron oxyhydroxides and sulphate, respectively (Rivett *et al.*, 2008). Hydrogen autotrophic denitrification only produces nitrogen gas and water. The advantages and disadvantages of each type of denitrification are summarized in Table 9.

Table 9: Summary of the advantages and disadvantages of each type of denitrification

Type of Denitrification	Advantages	Disadvantages	Reference
Heterotrophic	High rate, very reliable	External carbon sources can be expensive	Park and Yoo, 2009
Iron autotrophic	Does not require a carbon source	Ammonia can be formed abiotically	Devlin *et al.*, 2000; Park and Yoo, 2009
Sulphur autotrophic	Does not require a carbon source	Slower, produces sulphate	Park and Yoo, 2009
Hydrogen autotrophic	Does not require a carbon source, lower sludge yields	Longer startup period, requires an explosive substance	Park and Yoo, 2009

There are various bacteria genera and even some archaea and fungi that can perform denitrification (Philippot and Hallin, 2005). The taxonomy of denitrifying bacteria is very diverse. Bacteria that have been isolated in denitrifying systems include *Pseudomonas*, *Alcaligenes*, *Hyphomicrobium*, *Paracoccus*, *Hyphomicrobium*, *Bacillus*, *Methylobacterium, Rhodobacter* and *Blastobacter* spp. (Neef *et al.,* 1996).

A species that has been widely studied is *Paracoccus denitrificans*. It has been isolated from both water and wastewater denitrification systems. Neef *et al.* (1996) found that denitrifying sand filters in a wastewater treatment plant had a relative abundance of *Paracoccus* spp. of 40-50% while receiving supplementary methanol. Szekeres *et al.* (2002) confirmed the presence of *P. denitrificans* in hydrogen gas autotrophic denitrifying sand filters used for drinking water treatment.

P. denitrificans are biofilm forming bacteria. The clusters they form are shown in Figure 1. They are a facultative bacterium, capable of anoxic and aerobic denitrification (Kim *et*

al., 2004; Till *et al.,* 1998; Stouthamer *et al.,* 1997). Under aerobic conditions, the product of denitrification tends to shift towards nitrous oxide gas rather than nitrogen gas. Davies *et al.* (1995) found that aerobic conditions decreased nitrogen removal by just 6% compared to anoxic conditions. Under aerobic conditions *P. denitrificans* were still able to remove 92% of a 27.8 mg NO_3-N/L solution.

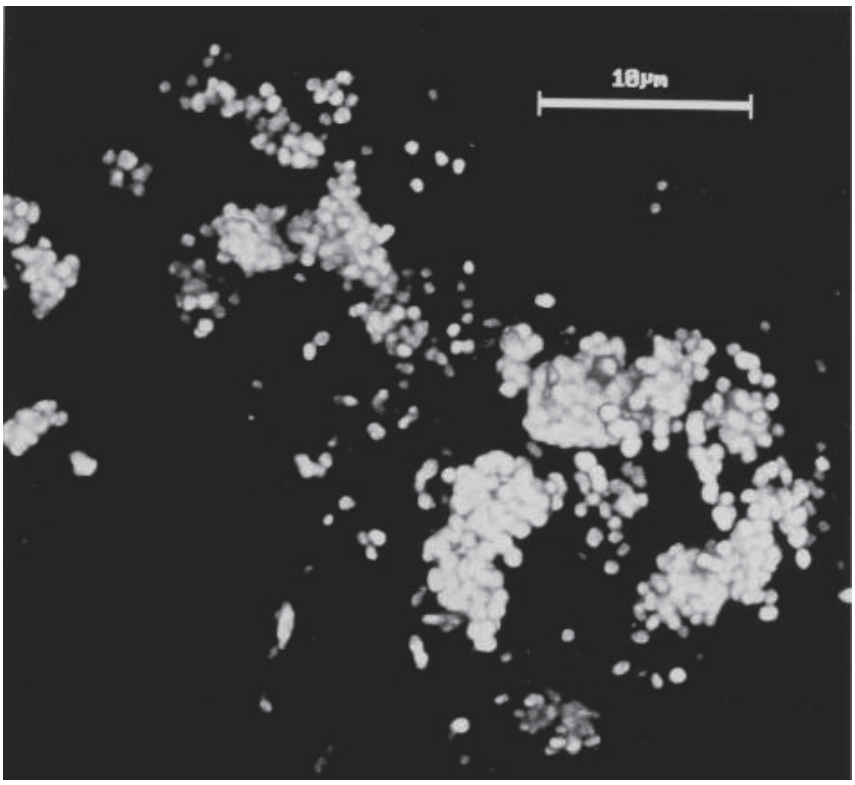

Figure 1: Clusters of *Paracoccus* sp. from detached biofilms (Neef *et al.*, 1996)

An important factor considered was whether the bacteria used are harmful to humans. *P. denitrificans* does not produce harmful substances nor is it a causative agent of any human or animal disease (Kim *et al.*, 2004). Due to its identification in full scale plants,

its performance under varying treatment conditions and its lack of health risks, *P. denitrificans* is an ideal candidate for drinking water treatment.

Heterotrophic denitrification with *P. denitrificans* was selected for the biofilters. This scheme was picked because of its high rate and reliability. Due to its availability and ease of use, glycerol was used as the external carbon source.

Chapter 3: Materials and Methods

3.1 Analytical

Water samples were analyzed for various parameters. Nitrate was analyzed using ion chromatography (Metrohm 761 Compact IC with 788 Filtration Sample Processor). Total organic carbon (TOC) and total nitrogen was analyzed using a total organic carbon analyzer with a total nitrogen measurement unit (Shimadzu TOC-VCSH TOC Analyzer with TNM-1 TN Measurement Unit). Arsenic, vanadium and iron were analyzed using inductively coupled plasma mass spectrometry (Thermo Fisher XSeries 2 ICPMS). Dissolved oxygen and pH measurements were done using a multi-parameter meter and associated probes (Fisher Scientific Accumet Excel XL50).

The detection limit for each parameter is summarized in Table 10.

Table 10: Summary of the detection limits for the analytical parameters measured

Parameter	Detection Limit
Nitrate	0.7 µg/L
Total Nitrogen	0.2 mg/L
Total Organic Carbon	0.6 mg/L
Arsenic	0.4 µg/L
Vanadium	0.4 µg/L
Iron	7 µg/L

3.2 Stock Preparation

Stock solutions of nitrate and iron were prepared by dissolving salts in milli-Q water. The stock nitrate solution prepared using sodium nitrate (Sigma-Aldrich) had a concentration of 10000 mg NO_3/L. The stock iron solution prepared using ferric chloride (Fisher Scientific) and had a concentration of 10000 mg Fe/L. A stock glycerol solution was made by diluting pure glycerol (Fisher Scientific) with milli-Q water. Dilution was needed because of glycerol's high viscosity. The final stock solution was 70% glycerol. None of the stock solutions were autoclaved as sterility was not a concern.

Commercial prepared stock solutions of arsenic and vanadium (Spex CertiPrep) were used. The arsenic concentration used was 10000 mg/L stored in 5% nitric acid. The vanadium concentration used was 1000 mg/L stored in 2% nitric acid.

3.3 Microbiological

P. denitrificans were obtained from American Type Culture Collection (ATCC #13543) and cultured in nutrient broth (BD Difco). The presence of *P. denitrificans* was confirmed by spread plating on nutrient agar (BD Difco) and R2A agar (BD Difco). Numeration of colonies during the toxicity assessment was done by spread plating on R2A agar. All the spread plates were incubated at 26°C. Plates with R2A were incubated for 7 days and plates with nutrient agar were incubated for 3 days.

3.4 Statistical Analysis

An analysis of variance was used to determine whether effluent concentrations under various conditions were statistically different. The analysis was conducted using the statistics software Minitab, version 16 (Minitab Inc.).

3.5 Filtration Setup

A general schematic of the setup is shown in Figure 2. A peristaltic pump (Cole-Parmer Masterflex) and tubing (Cole-Parmer PharMed, size L/S 14) were used to pump synthetic groundwater from the storage bottles to the filtration columns (Kimble Chase Flex Column, 2.5 cm ID by 20 cm) at a flow rate of ~0.75 mL/min. The storage bottles were 4 litre amber jugs capped with rubber stoppers wrapped in Teflon tape. The stoppers had a hole in them to allow for the tubing. The stoppers were used to decrease oxygen from entering the headspace. Because of the hole for the tubing, a perfect seal could not be made.

The filtration columns contained 20 grams of borosilicate beads, 60 grams of filter sand and 20 g of gravel. The borosilicate beads had a diameter of 2 mm and were used to decrease the fluid pressure on the top of the filter sand layer. The beads will also distribute the schmutzdecke throughout its length, lowering the headloss compared to a filter which has the schmutzdecke caked on the top of the sand layer. The filter sand is where the biofilm mainly formed. The gravel was used to retain the filter media.

Due to the small diameter of the tubing, it was occasionally replaced if it became clogged. The filters were backwashed as needed when the filters were clogged. The filters were backwashed by injecting dechlorinated tap water into the sample port tubing using a 50 mL syringe.

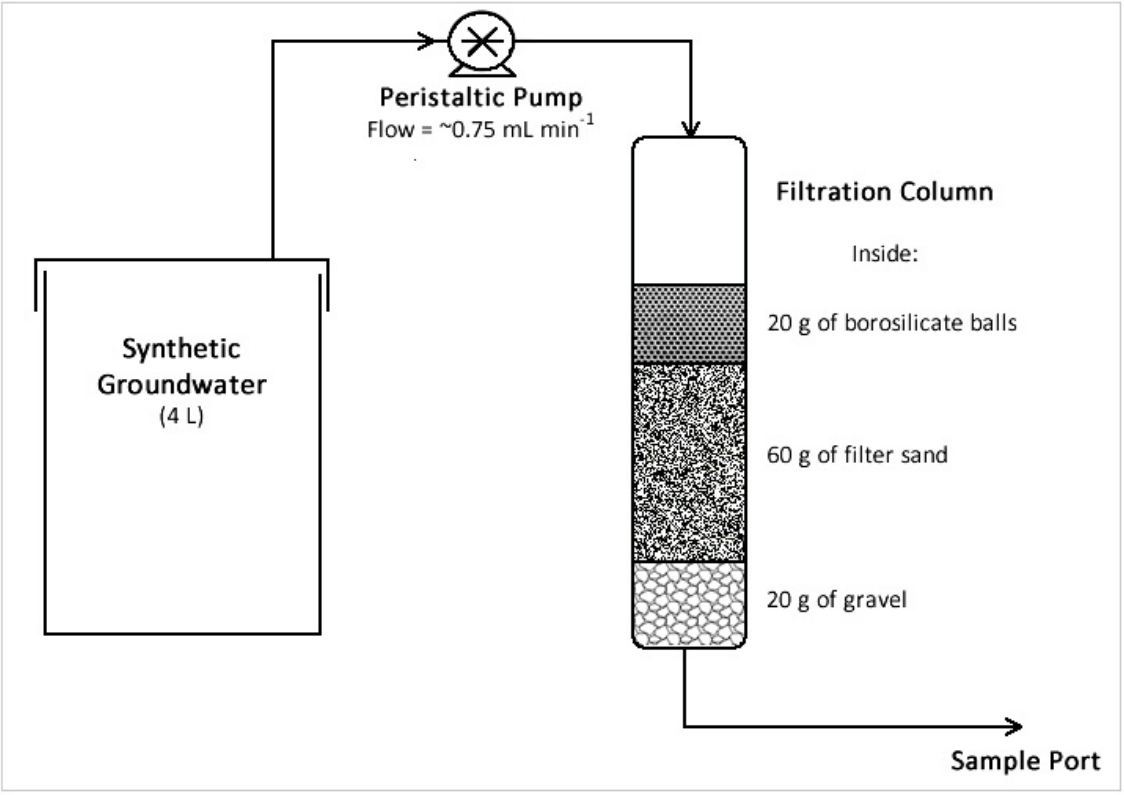

Figure 2: Process diagram for the bench-scale setup

3.6 Synthetic Groundwater Preparation

Synthetic groundwater was created by modifying tap water from Halifax, Nova Scotia. The tap water was passed through an activated carbon filter to remove chlorine, spiked with the appropriate chemicals and then de-gassed with nitrogen gas for at least 30 minutes in order to decrease the concentration of dissolved oxygen (<1 mg/L). The synthetic groundwater was not sterilized and had an approximate pH of 6.0±0.5.

3.7 Experimental Approach

3.7.1 Toxicity

To test for toxicity, *P. denitrificans* was first triple washed with phosphate buffer saline (PBS) solution. Washing was done by centrifuging (Thermo IEC Centra CL2) at 1000 rpm, replacing the supernatant with new PBS, and vortexing (VWR Digital Mini Vortexer) at 3000 rpm. A set of serial dilutions was then made in order to obtain an appropriate plate count. 100 μL of each dilution was spread plated onto R2A agar (Difco) containing each metal. The agar contained arsenic or vanadium at concentrations of 10^0, 10^1 and 10^2 times that of the purposed synthetic groundwater concentration. The purposed concentration for arsenic and vanadium was 50 and 25 μg/L, respectively. The metals were added after the agar was autoclaved. The plates were incubated for 7 days at 26°C then the number of colonies counted.

Previous research on metal resistant bacteria formed the basis for this method (Kumar *et al.,* 2009; Madhaiyan *et al.,* 2007)

3.7.2 Bench-scale Denitrification

Bench-scale biofiltration experiments were conducted continuously for 50 days at an operating temperature of 21°C. The experiments utilized the bench-scale filtration setup seen in Figure 2.

To make the filtration columns biologically active they were inoculated with a pure strain of *P. denitrificans*. A pure strain was used because it has been found to not form any harmful substances and is not a causative agent for any diseases (Kim *et al.*, 2004). *P. denitrificans* were cultured in nutrient broth (BD Difco) then triple washed using PBS

according to the method described in Section 3.7.1. The washed cells in a PBS solution were pumped into the filtration columns for 24 hours at ~0.75 mL/minute. The bacteria concentration in the PBS solution was approximately $5.5x10^4$ colony forming units/mL. The pumps were then turned off for 24 hours to encourage biofilm attachment and growth. At the end of 24 hours, the total number of cells pumped into the filters was approximately $5.94x10^7$ CFU. Following the resting period, synthetic groundwater was pumped into the filtration columns. The inoculation process only occurred once.

The filters were backwashed as necessary. The synthetic groundwater was not sterile so other bacteria could be present in the biofilm. However, due to the initial inoculation, it was assumed that the biofilm was mainly populated *P. denitrificans*.

A series of factorial experiments were designed to consider the effect of arsenic, vanadium, nitrate and glycerol. Experiments with synthetic groundwater that contained both arsenic and vanadium did not occur; therefore two, three parameter factorial experiments were used. There were four overlapping scenarios, so the resultant factor experiment consisted of 12 unique scenarios. The concentrations of each parameter are seen in Table 11.

Table 11: Concentrations for the denitrification bench-scale experiment

Parameter	Concentration (mg/L)	
	High Level	Low Level
Arsenic	0.050	0
Vanadium	0.025	0
Nitrate-N	15	5
Glycerol – Nitrate High	35	0
Glycerol – Nitrate Low	11.7	0

The concentration of arsenic was based on the previous drinking water guideline. Vanadium was based on the 90th percentile of detections as reported by the USEPA (2009) in the CCL 3 fact sheets. Nitrate-N was based on the 90th percentile of detection as reporting by Nova Scotia Environment (2009) for 135 wells monitored in 2008. The low nitrate concentration was chosen to ensure that denitrification could still occur, even though it was not necessary to meet the guidelines. Glycerol was added according to Equation 4 to make sure complete denitrification could occur. The amount of glycerol necessary for complete denitrification is 0.53 mg/mg of nitrate.

$$14NO_3^- + 5C_3H_8O_3 \rightarrow 15CO_2 + 7N_2 + 13H_2O + 14OH^- \quad [4]$$

where:

Molecular weight of NO_3^- = 62.0 g/mol

Molecular weight of $C_3H_8O_3$ = 92.1 g/mol

Samples from the output tubing were taken three times per week and analyzed for nitrate, total nitrogen, total organic carbon, arsenic and vanadium.

Based on the purposed filtration setup and nitrate concentration, the EBCT was 60.8 minutes and the nitrate loading rate was 0.25 g/L•d for the high level. These parameters are similar to those of Sison *et al.* (1995) when a nitrate removal rate of 90% was achieved.

3.7.3 Bench-scale Enhanced Metal Removal

Enhanced metal removal bench-scale experiments were conducted for 68 days. The experiments utilized the bench-scale filtration setup seen in Figure 2.

The experimental design for the bench-scale denitrification experiments was used with one alteration. Iron was substituted for glycerol in the factorial design described in Section 3.7.2. The high concentration for iron started at 1 mg/L, then increased to 2 mg/L at 46 days, while the low concentration remained at 0 mg/L. Table 12 summarizes the concentrations used. Glycerol was added to all the filtration columns at a concentration of 35 and 11.7 mg/L for the high and low nitrate condition, respectively. This was done to allow for complete denitrification. To account for the glycerol addition to all the filters, the system was run for 1 month with synthetic groundwater in order to reach a steady state before the start of the enhanced metal removal experiments.

Table 12: Concentrations for the enhanced metal removal bench-scale experiment

Parameters	Concentration (mg/L)	
	High Level	Low Level
Arsenic	0.050	0
Vanadium	0.025	0
Nitrate-N	15	5
Iron	1-2	0

3.7.4 Metal Removal with Inactive Filters

The effect of iron on arsenic and vanadium was tested on filtration columns that were not biologically active. The same system and source water was used as described in Section

3.7.3, but the filters were not inoculated with *P. denitrificans*. The iron concentration used was 1 mg/L. The system was run for 15 days. A natural biofilm may have grown in the filters, but due to the short duration any impacts were assumed to be insignificant.

Chapter 4: Results

4.1 Toxicity Assessment Plate Counts

Bacterial counts on spread plates containing arsenic or vanadium are shown in Table 13. Comparisons between arsenic plate counts and vanadium plate counts cannot be made as they came from separate PBS test tubes, and thus have different initial concentrations of bacteria.

For arsenic, the highest plate count occurred on agar that did not contain arsenic and the lowest plate count occurred on agar containing 5 mg/L arsenic, however the difference between the two was small. Statistical tests were done on the results to check for any significant differences. An analysis of variances revealed no significant difference among the different metal concentrations ($p=0.428$). For vanadium, the highest plate count occurred on agar that did not contain vanadium and the lowest plate count occurred on agar containing 2.5 mg/L vanadium, however again the difference between the two was not significant. An analysis of variances revealed no significant difference among the different metal concentrations ($p=0.440$). In both cases, it was inferred that arsenic and vanadium have no toxicological effects on *P. denitrificans* at the studied concentration range.

Table 13: Bacterial counts for spread plates containing arsenic or vanadium

Metal Concentration	Plate Count (CFU/mL)
Arsenic	
0 mg/L	8.2E4 ± 3.4E3
0.05 mg/L	9.2E4 ± 2.6E4
0.5 mg/L	8.1E4 ± 1.4E3
5 mg/L	6.6E4 ± 8.5E3
Vanadium	
0 mg/L	1.6E5 ± 6.0E4
0.025 mg/L	9.4E4 ± 4.9E3
0.25 mg/L	1.3E5 ± 5.9E4
2.5 mg/L	9.2E4 ± 2.1E3

4.2 Bench-scale Denitrification

4.2.1 Denitrification Rates

The effluent nitrate concentrations for filters which contained glycerol are shown in Figure 3. The dashed line represents the influent concentration of nitrate. The mean nitrate concentration after day 9 for the filters without arsenic and vanadium, with arsenic and with vanadium was 7.2, 7.9 and 6.2 mg N/L, respectively. The filter with arsenic usually had the highest effluent concentration, while the filter with vanadium usually had the lowest effluent concentration. Overall the differences between filters were not significant. An analysis of variance indicated no significant difference in denitrification rates among filters with arsenic, with vanadium and without arsenic or vanadium ($p=0.319$).

On day 16, 23, 39 and 46 there were effluent nitrate concentrations which exceeded the MAC. These days correspond with days when storage bottles were replenished. Just before the bottles are filled, there was considerably less synthetic groundwater in the bottles and more air. The water will become less anoxic as oxygen re-diffuses into the water. An increase in DO would result in a decrease in the rate of denitrification and a peak in the effluent concentration. This cycle of oxygen re-diffusing into the water also explains the general trend of peaks and valleys of each line. There were a few samples which had concentrations higher than the influent concentration. This could be due to imperfect mixing or human error with the analytical measurements.

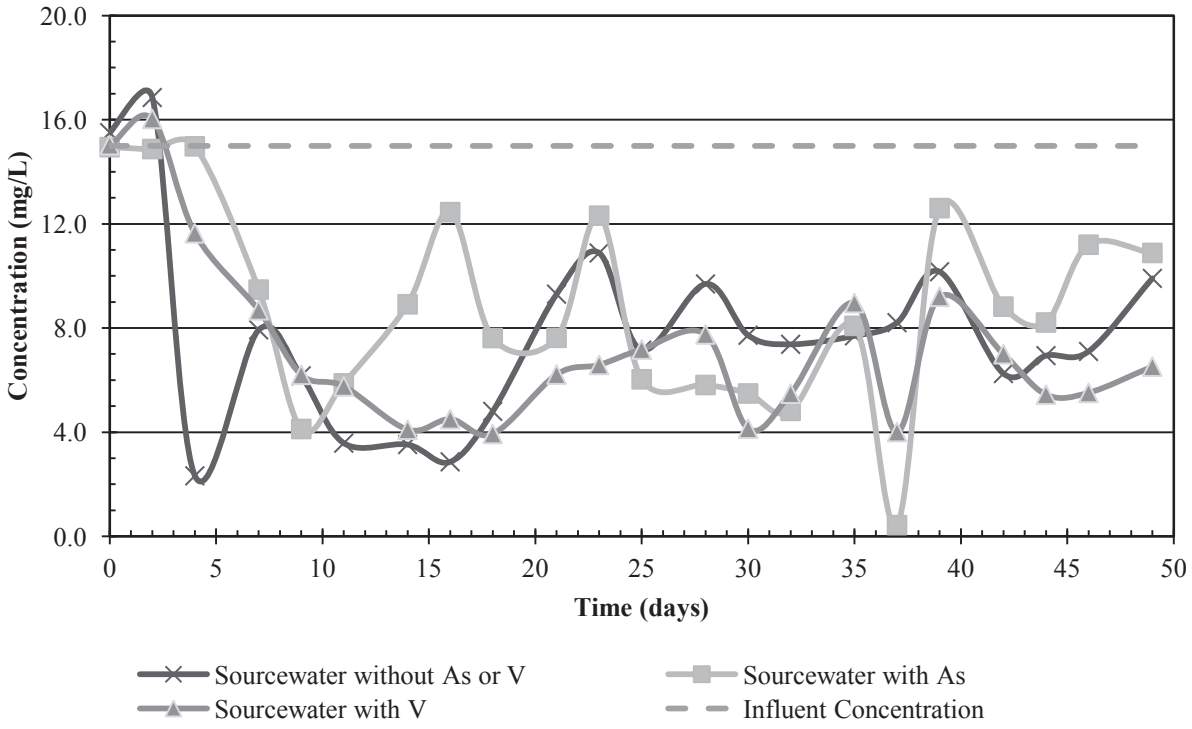

Figure 3: Effluent nitrate concentrations from biofilters inoculated with P. denitrificans and continuously fed with glycerol

The effluent nitrate concentrations for filters that did not have glycerol are shown in Figure 4. The dashed lined represents the influent concentration of nitrate. There was little removal of nitrate in filters that did not receive glycerol. The mean nitrate concentrations without arsenic and vanadium, with arsenic and with vanadium were 14.4, 14.0 and 13.9 mg NO_3-N/L, respectively. An analysis of variance indicated no significant difference in denitrification rates among filters with arsenic, with vanadium and without arsenic or vanadium ($p=0.429$). Thus, the lack of nitrate removal was not affected by the presence of metals. Because it was previously established that the metals did not cause adverse outcomes to bacteria, it is likely that the filter operations presented in Figure 4 were not operating biologically or chemically to remove the nitrate. This was perhaps due to the lack of sufficient carbon sources to allow for the heterotrophic life style of the denitrifying microbial community.

Similar to Figure 3, there were samples with concentration above the influent concentration. Again, this could be due to imperfect mixing or human error with the analytical measurements.

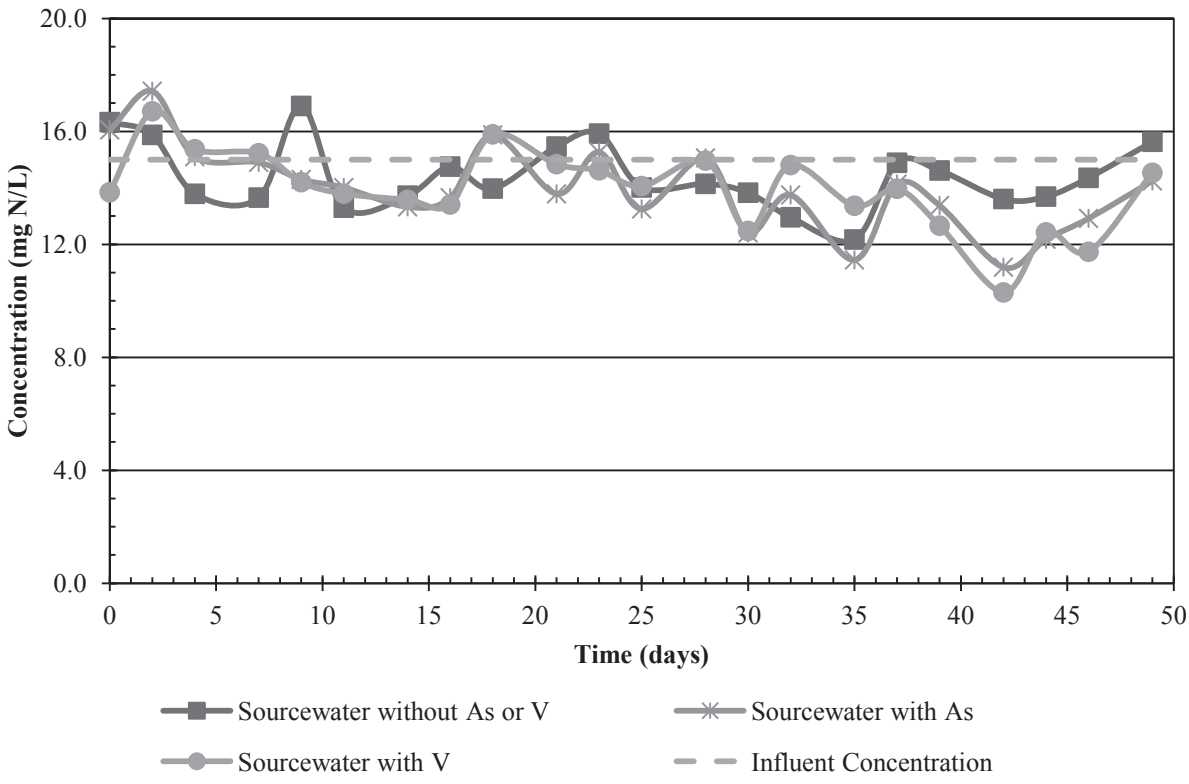

Figure 4: Effluent nitrate concentrations from biofilters inoculated with *P. denitrificans* without the addition of glycerol as a carbon source

An analysis of variance showed no significant difference between the effluent total nitrogen concentrations for filters with glycerol ($p=0.340$). The same was found for filters without glycerol ($p=0.425$). Figure 5 shows a comparison between the mean total nitrogen concentration and mean nitrate concentration after day nine. The dashed line shows the influent concentration of both nitrate parameters.

When glycerol was added to the filter, the nitrate concentration decreased below the guidelines while the total nitrogen concentration did not. Nitrate comprised 55.6% of the total nitrogen concentration. The remaining total nitrogen likely consisted of the

intermediate denitrification species. The intermediate species are nitrite, nitric oxide and nitrous oxide. It is also possible that ammonium was also present in the water.

In the absence of glycerol, the nitrate concentration did not meet guidelines. An analysis of variances showed that there was not a significance difference (p=0.240) between nitrate and total nitrogen. It is possible that there was a small concentration of intermediate species, but there is essentially no denitrification occurring in the absence of glycerol.

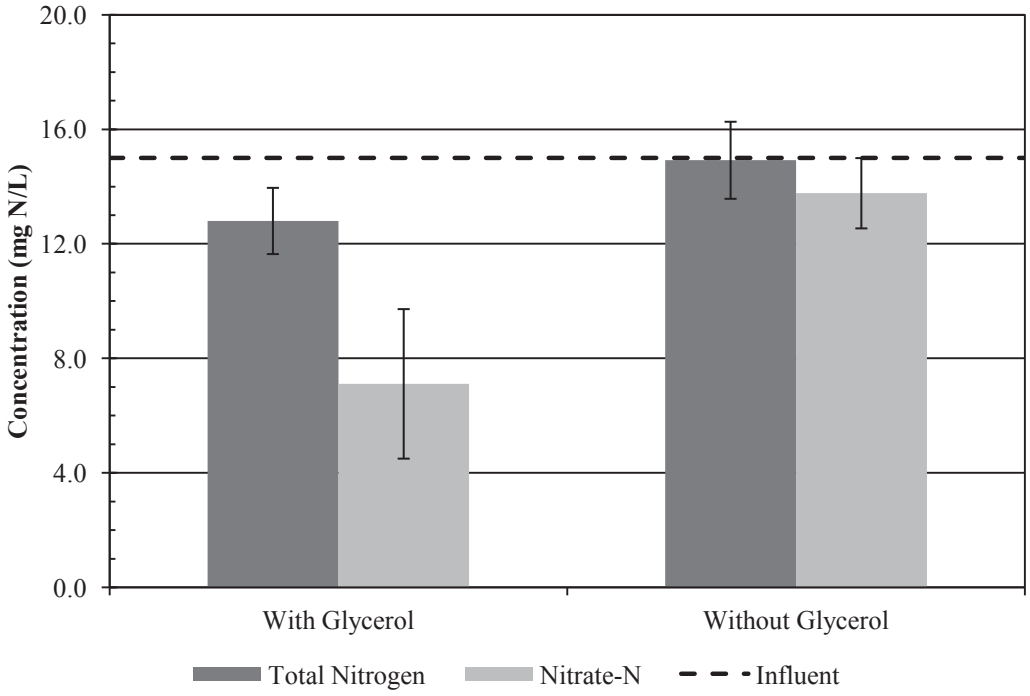

Figure 5: Comparison of the mean effluent total nitrogen and nitrate concentration coming from biofilters with and without glycerol addition

The importance of a carbon source for denitrification is shown in Figure 5. This trend is further demonstrated in Figure 6 which shows the effluent TOC concentration as well as the effluent total nitrogen and nitrate-N concentrations. The TOC follows very similar

trends as nitrate and total nitrogen. The amount of denitrification occurring is proportional to the amount of carbon being utilized. As more denitrification occurs, more carbon is removed from the effluent. The results shown in Figure 6 are from a filter which did not have any arsenic or vanadium in the source water. The filters which did have arsenic or vanadium had similar results.

The Pearson correlation between effluent TOC and effluent nitrate, as well as between effluent TOC and total nitrogen is seen in Table 14. There was a statistically significant positive correlation between TOC and nitrate for the filter that had no metal and the filter that had vanadium. The filter that was exposed to arsenic had a weaker positive correlation that was deemed to be not significantly different from zero. There was a statistically significant positive correlation between TOC and total nitrogen for the filter that had vanadium. The other filters had a weaker positive correlation, but again, the correlation was not significantly different from zero.

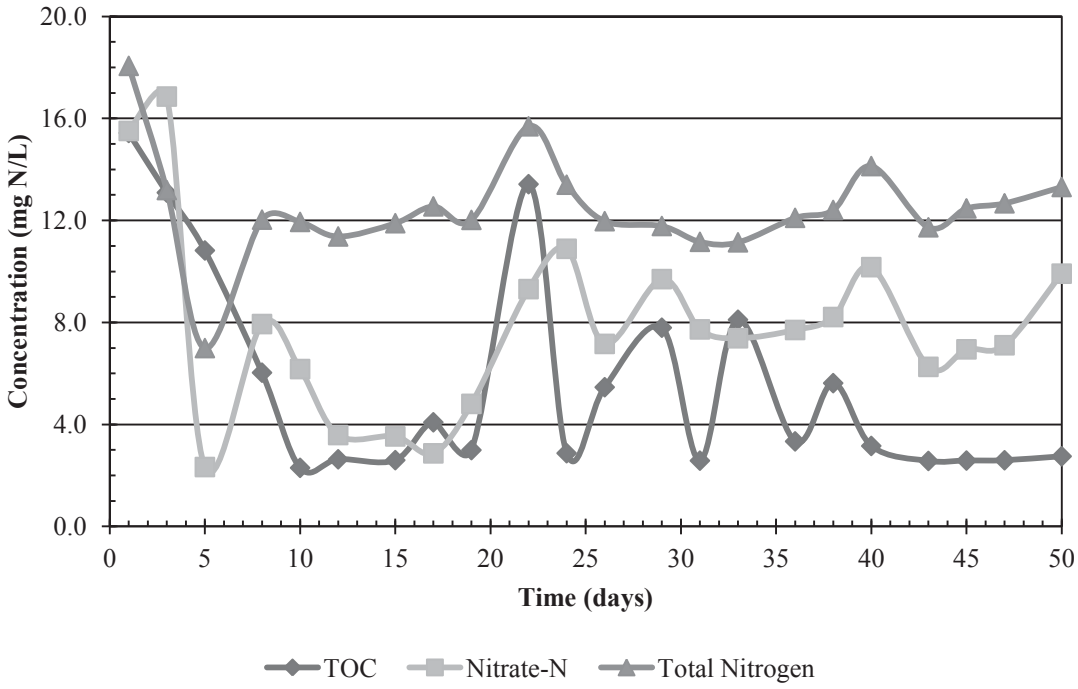

Figure 6: Effluent concentration of TOC, nitrate-N and total nitrogen for the filter which had no arsenic or vanadium

Table 14: Pearson correlation between effluent TOC and nitrate-N or total nitrogen

Parameters	Metals	Pearson Correlation	P-value
TOC and Nitrate-N	None	0.533	0.011*
TOC and Nitrate-N	Arsenic	0.276	0.214**
TOC and Nitrate-N	Vanadium	0.862	0.000***
TOC and Total Nitrogen	None	0.333	0.130**
TOC and Total Nitrogen	Arsenic	0.207	0.355**
TOC and Total Nitrogen	Vanadium	0.530	0.011*

* $p<0.05$, significant

** $p>0.05$, not significant

*** $p<0.001$, highly significant

4.2.2 Removal of Arsenic and Vanadium from biofilter inoculated with *P. denitrificans*

An analysis of variance indicated that there was no significant difference between the effluent arsenic concentrations from filters with varied nitrate and glycerol conditions (p=0.075). The same was true for vanadium (p=0.935)

The influent and mean effluent concentrations for arsenic and vanadium are shown in Table 15. There was little removal of arsenic and no removal of vanadium during the bench-scale denitrification experiment.

Table 15: The influent and mean effluent concentration for arsenic and vanadium during the bench-scale denitrification experiment

Parameter	Influent Concentration (µg/L)	Mean Effluent Concentration (µg/L)
Arsenic	50.0	47.6
Vanadium	25.0	24.6

4.3 Bench-scale Enhanced Metal Removal

4.3.1 Effluent Nitrate Concentration from Biologically Active Filters

The mean effluent nitrate concentrations during the enhanced metal removal experiments are shown in Table 16. An analysis of variance showed that there was no significant difference between each condition (p=0.571). There was also no significant difference between the effluent concentrations during the bench-scale denitrification experiment and the enhanced metal removal experiment (p=0.157)

Table 16: Mean effluent nitrate concentration during the enhanced metal removal experiment

Metals Present	Nitrate Concentration (mg/L)
None	8.12
Fe	8.04
Fe, As	8.91
Fe, V	7.05

4.3.2 Effluent Metal Concentrations from Biologically Active Filters

The addition of iron drastically improved the removal of arsenic. The effluent arsenic concentrations during the enhanced metal removal experiment are shown in Figure 7. The dashed line represents the arsenic influent concentration. The filter with low nitrate consistently had a lower arsenic concentration than the filter with high nitrate, with the exception of one sample. The iron concentration remained at 1 mg/L until day 46. During this period the mean effluent arsenic concentration was 16.47 and 21.38 µg/L for low nitrate and high nitrate conditions, respectively. When the iron concentration was increased to 2 mg/L after day 46, the mean effluent arsenic concentration decreased to 6.34 and 8.43 µg/L for the low nitrate and the high nitrate conditions, respectively.

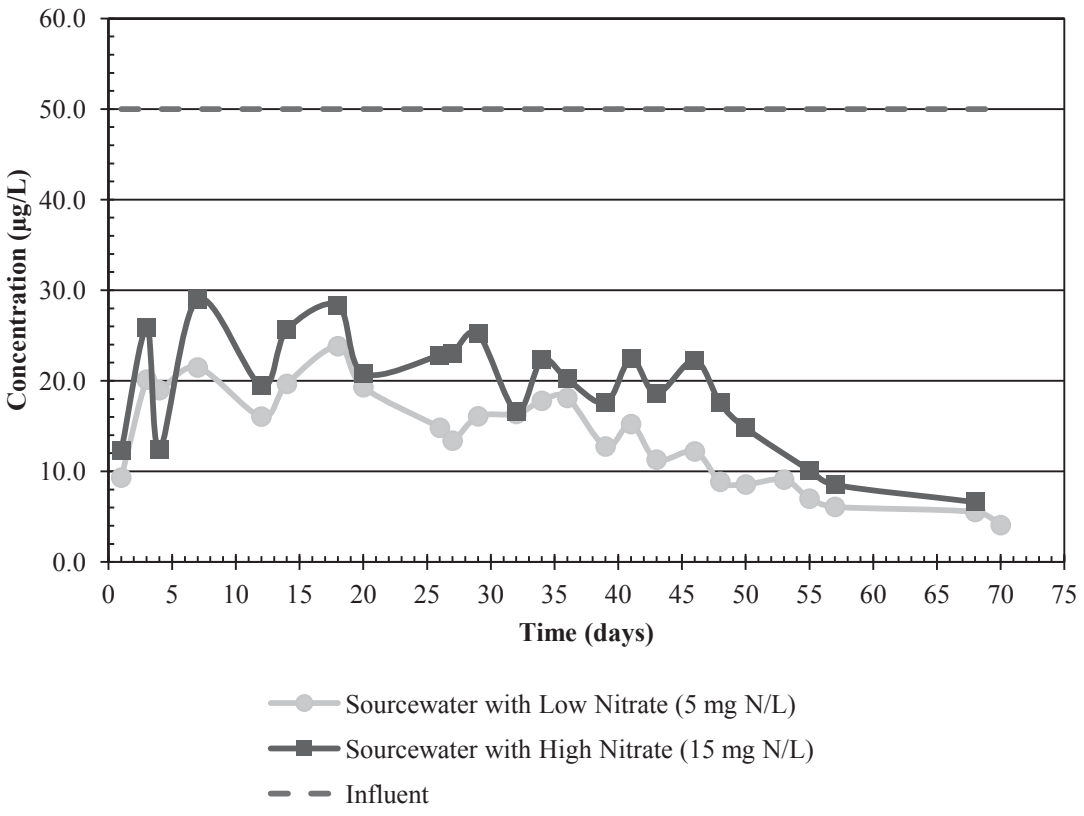

Figure 7: Effluent arsenic concentrations from biofilters with iron addition (Influent iron concentration: 1 mg/L, day 0-46; 2 mg/L, day 47-71)

Vanadium removal was also drastically improved by iron addition. The effluent vanadium concentrations during enhanced metal removal experiment are shown in Figure 8. The dashed line represents the vanadium influent concentration. The filter with the high nitrate concentration generally had effluent with a lower arsenic concentration than the filter with the low nitrate concentration. This trend reversed once the iron concentration was increased. The iron concentration remained at 1 mg/L until day 46. During this period the mean effluent vanadium concentration was 6.787 and 4.368 µg/L for the low nitrate and the high nitrate conditions, respectively. When the iron concentration was

increased to 2 mg/L after day 46, the mean effluent vanadium concentration decreased to 1.989 and 2.177 µg/L for the low nitrate and the high nitrate conditions, respectively.

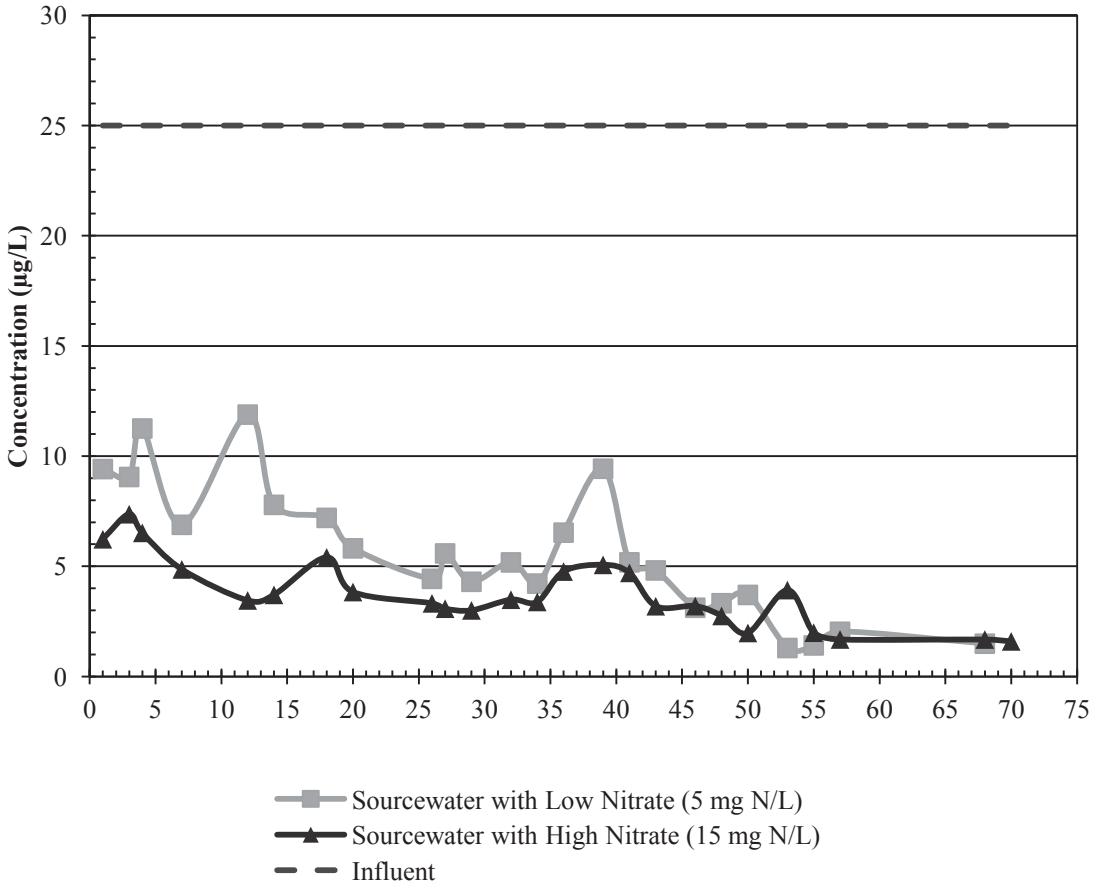

Figure 8: Effluent vanadium concentrations from biofilters with iron addition (Influent iron concentration: 1 mg/L, day 0-46; 2 mg/L, day 47-71)

The effluent iron concentrations from filters receiving iron are shown in Table 17. The percent removals were generally well above 90%, with effluent concentration below 100 µg/L. Iron was visually seen as brownish orange precipitate in the filters. Figure 9 shows the filters with and without iron in the source water. In the filters with iron the

schmutzdecke appeared brownish-orange, while in the filters without iron the schmutzdecke appeared white. In both filters the schmutzdecke was spread throughout the borosilicate balls.

Table 17: Effluent iron concentration of 6 filters with various source waters with two levels of iron input

Sourcewater Condition	1 mg/L Fe		2 mg/L Fe	
	Average Effluent Concentration (µg/L)	Removal (%)	Average Effluent Concentration (µg/L)	Removal (%)
Low Arsenic and Vanadium, High Nitrate	103.9 ± 93.9	89.6 ± 9.4	40.1 ± 20.4	98.0 ± 1.0
Low Arsenic and Vanadium, Low Nitrate	32.2 ± 36.9	96.8 ± 3.7	10.0 ± 1.5	99.5 ± 0.1
High Arsenic, High Nitrate	40.7 ± 26.4	95.9 ± 2.6	36.4 ± 26.8	98.1 ± 1.3
High Arsenic, Low Nitrate	24.8 ± 20.6	97.5 ± 2.1	17.0 ± 9.9	99.2 ± 0.5
High Vanadium, High Nitrate	56.8 ± 28.8	95.3 ± 2.9	47.1 ± 20.9	97.6 ± 1.0
High Vanadium, Low Nitrate	67.7 ± 65.3	93.2 ± 6.5	33.1 ± 33.8	98.3 ± 1.7

Figure 9: The top of the filters with and without iron in the sourcewater

Overall, as addition of iron to the sourcewater was increased, arsenic and vanadium decreased. Table 18 shows the Pearson correlation between iron addition and metal removal. The data from the Pearson correlation is a combination of the denitrification experiment, when iron addition was zero, and the enhanced metal removal experiment, when iron addition was 1 and 2 mg/L. All the filters had a very high positive correlation between the amount of iron added and the amount of arsenic and vanadium removed.

Table 18: Pearson correlation between iron addition and arsenic or vanadium removal

Parameters	Nitrate Influent	Pearson Correlation Coefficient	P-value
Iron Influent and Arsenic Removal	15 mg N/L	0.902	0.000*
Iron Influent and Arsenic Removal	5 mg N/L	0.935	0.000*
Iron Influent and Vanadium Removal	15 mg N/L	0.871	0.000*
Iron Influent and Vanadium Removal	5 mg N/L	0.859	0.000*

* $p<0.001$, highly statistically significant

4.3.3 Metal Removal with Inactive Filters

Filters that were not inoculated with *P. denitrificans* were found to have similar removal rates for arsenic and vanadium. A summary of the mean effluent concentrations for filters with and without bacteria is shown in Table 19. The mean effluents for the inoculated filters were from the enhanced metal removal experiments when 1 mg/L of iron was added.

The filter with no bacteria had the lowest arsenic concentration, but an analysis of variances showed it was not significantly different from the inoculated filter with low nitrate ($p=0.174$). The inoculated filter with high nitrate had the highest mean effluent arsenic concentration. An analysis of variance found that it was significantly different than the other two filters ($p=0.000$).

The inoculated filters with high nitrate had the lowest vanadium concentration. An analysis of variance showed that it was significantly different than the other two filters ($p=0.004$). The filter without bacteria and the inoculated filter with low nitrate were very similar. An analysis of variance showed that they are not significantly different ($p=0.794$)

A brownish-orange precipitate was seen in the filters, similar to what was seen in the enhanced metal removal filters. However, the iron was caught on the top of the sand layer in the inactive filter, as opposed to the schmutzdecke in the borosilicate balls in the inoculated filter. This is because there was no schmutzdecke built up in the inactive filter, so the iron precipitates were easily able to penetrate the filter to the sand layer. The iron precipitate in the inactive filer is shown in Figure 10.

Table 19: The mean effluent arsenic and vanadium concentrations for filters with and without *P. denitrificans*

	Arsenic Effluent Concentration (µg/L)	**Vanadium Effluent Concentration (µg/L)**
No Bacteria Added	14.3±3.3	6.5±2.5
Bacteria, Low Nitrate	16.5±3.8	6.8±2.5
Bacteria, High Nitrate	21.4±4.7	4.4±1.3

Figure 10: Brownish-orange precipitates an inactive filter

Chapter 5: Discussion

5.1 Toxicity

Results showed that there were no significant differences between plate counts with varying arsenic and vanadium concentration showing that *P. denitrificans* was tolerant to the tested metal concentrations. Similar metal resistance among other bacteria has been documented in nature. Various microbes which possess the *ars* operon have been shown to be arsenic resistant (Kaur *et al.*, 2011). There is also evidence that the *ARC1*, *ARC2* and *ARC3* genes are linked to arsenic resistance (Wysocki *et al.,* 1997). *Paracoccus* sp. was found to have the *ARC3* gene which is linked to arsenite resistance. The species was resistant to arsenite concentrations up to 262 mg/L and arsenate concentrations of 11987 mg/L (Achour *et al.*, 2007). Research into microbial resistance to vanadium has been limited, but Bell *et al*. (2004) did isolate vanadium resistant bacteria in soil contaminated with crude oil. The genes responsible for resistance were not identified. Based on the plate counts and previous studies, it is plausible that arsenic and vanadium are non-toxic to *P. denitrificans* at the examined concentration range.

5.2 Bench-scale Denitrification

There were two objectives during the bench-scale denitrification experiment. The first objective was to examine various denitrification parameters. These parameters included the necessity of an external carbon source (glycerol) and whether arsenic and vanadium would hinder denitrification. The second objective was to examine whether *P. denitrificans* are capable of removing arsenic and vanadium from a synthetic groundwater.

The first denitrification parameter examined was the necessity of an external carbon source. It is possible that *P. denitrificans* could perform denitrification without an external carbon source by utilizing organic matter from endogenous decay, but this pathway is limited by the growth and death of the biofilm bacteria. Glycerol had a significant impact on the amount of nitrate removed. With glycerol, the biofilters were able to remove an average of 52.7% of the nitrate. The resultant concentrations were generally below the drinking water guideline of 10 mg NO_3-N /L. Without glycerol, the nitrate concentration had a slight decreasing trend over time, but ultimately was only reduced by an average of 5.9%. This effluent concentration would not comply with the guidelines.

The effect of glycerol was also examined by measuring total nitrogen. Again, glycerol was found to increase the amount of total nitrogen removed. The total nitrogen effluent concentration with and without glycerol was above the nitrate guidelines of 10 mg NO_3-N /L. When glycerol was added, nitrate comprised 55.6% of the total nitrogen concentration. The remaining 44.4% of the total nitrate was assumed to be made up of the intermediate denitrification species. If the remaining total nitrogen was completely nitrite, then the effluent would not meet drinking water guidelines. Health Canada (2010) specifies that when nitrite is measured separately from nitrate, it must not exceed 3.2 mg/L. The decrease in total nitrogen proves that denitrification was occurring, but that it needs to be optimized. Increasing the EBCT of the filter may achieve greater nitrogen removal. However, increasing the EBCT was not explored due to laboratory constraints.

The relationship between carbon source and denitrification was also evident by examining the effluent TOC concentrations. There were statistically significant positive

correlations between effluent TOC and effluent nitrate for the filter that had no metals and the filter that had vanadium, as well as effluent TOC and effluent total nitrogen for the filter that had vanadium. This indicates that the use of carbon compounds was related to the removal of nitrogen from the water. Positive correlation coefficients found for other filters were deemed to be not significantly different from zero. It is possible that these weaker positive correlations could be found to be statistically significant with further research.

The second denitrification parameter examined whether arsenic and vanadium would affect denitrification. In the toxicity assessment it was found that arsenic and vanadium are non-toxic to *P. denitrificans* up to 10^2 times the proposed influent concentration. However, it was still necessary to prove that arsenic and vanadium were not interrupting the denitrification pathway. Nitrate removal was not affected by arsenic or vanadium. An analysis of variance showed that any difference between nitrate effluent concentrations was insignificant. This implies that arsenic and vanadium did not have a negative impact on denitrification.

The second objective was to examine the removal of arsenic and vanadium by the biofilters. The biofilters were ineffective at removing both arsenic and vanadium. The average removal for arsenic and vanadium was only 4.8% and 1.8%, respectively. Therefore while arsenic and vanadium are non-toxic to *P. denitrificans* and do not hinder its ability to perform denitrification, they cannot be removed by *P. denitrificans* alone.

Four possible removal mechanisms for arsenic and vanadium included undergoing a redox reaction resulting in a less mobile species (Valls and de Lorenzo, 2002), binding to

intra or extracellular proteins (Karanasios *et al.*, 2010), adsorbing to exopolymeric substances produced by the biofilm (Panwichian *et al.*, 2011) or adsorbing to other metal oxide-hydroxides bound to the biofilm (Burger, 2008). It is possible that the metals underwent redox reactions, similar to the results found by Sun *et al*. (2008), however there was an absence of a material that could bind or adsorb the less mobile species. The denitrification experiment implies that *P. denitrificans* may not have proteins with an affinity for arsenic or vanadium, because there was insignificant removal. Similarly, *P. denitrificans* may not produce exopolymeric substances capable of adsorbing arsenic and vanadium under the tested conditions in detectable amounts. The denitrification experiments did not show whether arsenic and vanadium can adsorb to biofilm bound metal oxide-hydroxides, as there were limited concentrations of other metals such as iron or manganese. This removal mechanism was explored further in the enhanced metal removal experiments.

5.3 Enhanced Metal Removal

The addition of iron significantly improved the removal of arsenic and vanadium. Over the course of the denitrification experiment and the enhanced metal removal experiment, there was a very strong positive correlation between the addition of iron and the removal of arsenic and vanadium. For the first 46 days of the enhanced metal removal experiment, when 1 mg/L of iron was added, arsenic and vanadium were removed at rates exceeding 57% and 73%, respectively. When the iron concentration was increased to 2 mg/L for the remainder of the experiment, the removal rates for arsenic and vanadium increased to 83% and 91%, respectively. Arsenic met the 10 µg/L guideline when 2 mg/L of iron was

present. There is no guideline for vanadium so comparisons with guidelines could not be made.

To better understand the removal mechanism for arsenic and vanadium, experiments had to be conducted without *P. denitrificans* inoculation. These experiments yielded arsenic and vanadium effluent concentrations which were not statistically different than the effluent concentration from the inoculated biofilters. Therefore the removal cannot be linked to the biofilm, but rather to the presence of iron. Based on adsorption and coagulation studies discussed in Sections 2.2.1 and 2.2.3, it is known that arsenic and vanadium adsorb to fixed iron, such as iron coated sand, and particulate iron, such as ferric hydroxide flocs. Images of the filters in the present study that received iron show a considerable build up of brownish-orange precipitate (see Figure 9). The color of the precipitate corresponds to ferric hydroxide.

Iron is extremely insoluble with a solubility product of $6x10^{-38}$ at 25°C (Sawyer *et al.*, 2003). The formation of ferric hydroxides is inevitable unless a system can be completely void of dissolved oxygen. There were three possible sources of oxygen which could have oxidized the iron. They are:

1. De-gassing with nitrogen depleted dissolved oxygen significantly, however it did not completely eliminate it
2. The synthetic groundwater dripped into the filtration columns which allowed the water to contact the air present in the headspace
3. The stock solution of ferric chloride used to make the synthetic groundwater was not prepared anoxically thus allowing for oxidation prior to use

The formation of ferric hydroxide is not unrealistic. An Eh-pH diagram for iron is shown in Figure 11. The data points on the diagram correspond to groundwater samples from a study conducted by Becking et al. (1960). The data suggests that ferric hydroxide and ferrous ions are the main forms of iron present in groundwater. Therefore the formation of iron hydroxides in the synthetic groundwater is consistent with the types of iron present in natural groundwater.

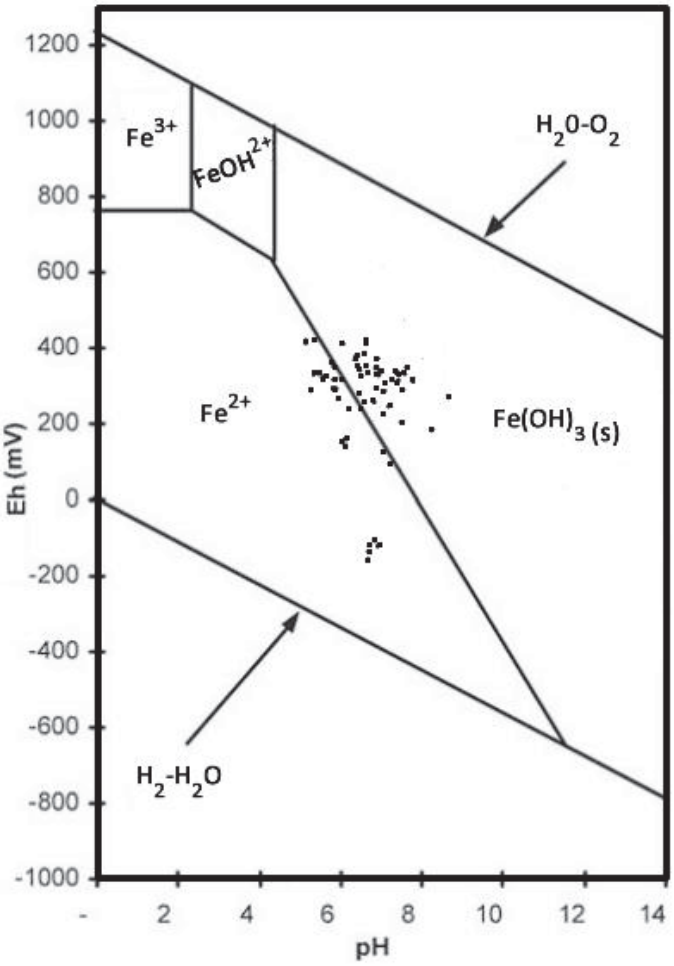

Figure 11: Eh-pH diagram from iron with groundwater samples (diagram adapted from Benzaazoua *et al.*, 2004, sample points adapted from Becking *et al.*, 1960)

The amount of arsenic removal by ferric hydroxide is consistent with the results found by McNeill and Edwards (1997). McNeill and Edwards (1997) developed a model which relates available iron precipitates to arsenic removal. The model was verified by sampling treatment plants that use ferric based coagulants and plants that used iron-manganese oxidation-precipitation processes. The model, based on the Langmuir adsorption isotherm, is shown in Equation 5.

$$As_{sorbed}\ (\%) = \frac{K[Fe]}{1 + K[Fe]} \times 100 \quad [5]$$

where:

As_{sorbed} (%) = Percentage of arsenic sorbed to iron particulate

K = Unknown constant

[Fe] = Particulate iron formed in mM

McNeill and Edwards (1997) found that K values ranging from 80 to 120 were appropriate for the data found. Since the Langmuir adsorption isotherm model was verified with data from treatment plants that use ferric based coagulants and plants using iron-manganese oxidation-precipitation processes, it clearly demonstrates that arsenic is removed adsorptively by iron hydroxides. Figure 12 shows the data collected by McNeill and Edwards (1997), their model for K=80 and K=120 and the average arsenic removal for each iron concentration during the enhanced metal removal bench-scale experiment. The average arsenic removal during the enhanced metal removal bench-scale experiment was well represented by the model when K=120. The percent different between the

models predicted removal and the actual removal was only 1.3% when 1 mg/L of iron was added and 3.3% when 2 mg/L of iron was added. The model shows that as iron doses increase, the amount of arsenic removal does not increase proportionally. To achieve 99% arsenic removal, an iron dose of 46 mg/L would be needed. The dose would need to be further increased to 462 mg/L to achieve a removal of 99.9%.

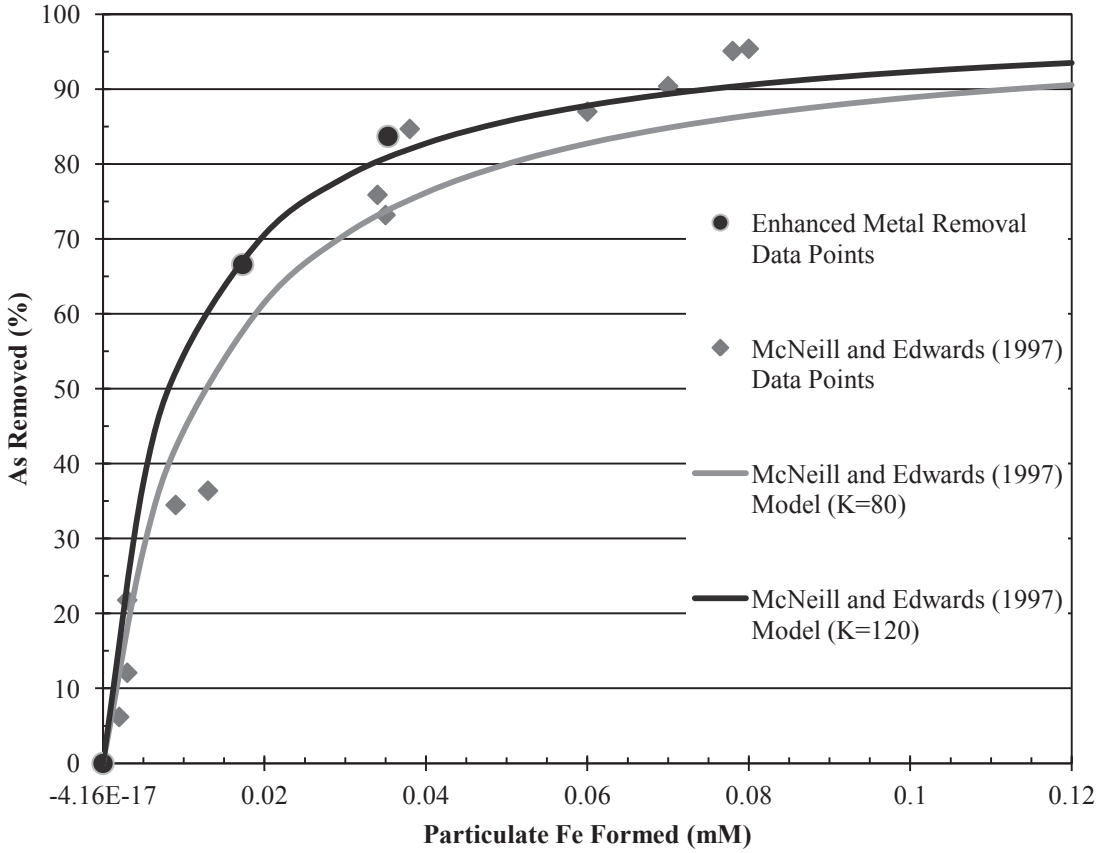

Figure 12: Overlay of enhanced metal removal results with a model developed by McNeill and Edwards (1997)

Importantly, the addition of iron did not affect the amount of denitrification. Therefore iron could be used as a treatment pathway in conjunction with *P. denitrificans* to remove nitrate, arsenic and vanadium simultaneously. The ability to perform both mechanisms in one vessel adds to the eco-friendliness as well as the cost effectiveness.

5.4 Proposed Removal

A schematic of the proposed removal pathways is seen in Figure 13. The removal pathway for nitrate is well known. Biological denitrification involves the transformation of nitrate to nitrogen gas by denitrifying bacteria. In the experimental filter *P. denitrificans* utilized glycerol to achieve denitrification. Evidence that suggests biological denitrification is occurring in the filters involves the positive correlation between the amount of TOC utilized and the amount of nitrate reduced. Furthermore, based on the available treatment techniques for nitrate, there is a lack of other possible removal mechanisms in a biologically active sand filter. Exact biofilm kinetic parameters, such as biofilm thickness and substrate flux, were not examined in this study.

Arsenic and vanadium removal is directly linked to iron removal. When ferric ions are oxidized, they form ferric hydroxide precipitates which can be removed by the filter and act as adsorbents. The presence of iron hydroxide was evident by the high iron removal and visual inspection of the filter sand. The high positive correlation between iron addition and arsenic and vanadium removal is evidence that adsorption to iron hydroxide is the main removal mechanism for arsenic and vanadium. A model relating iron availability to arsenic removal developed by McNeill and Edwards (1997) provided

further evidence that arsenic removal during the enhanced metal removal experiments was due to the presence of iron precipitates. There was little difference between the arsenic removal from the enhanced metal removal experiments and the models predicted removal.

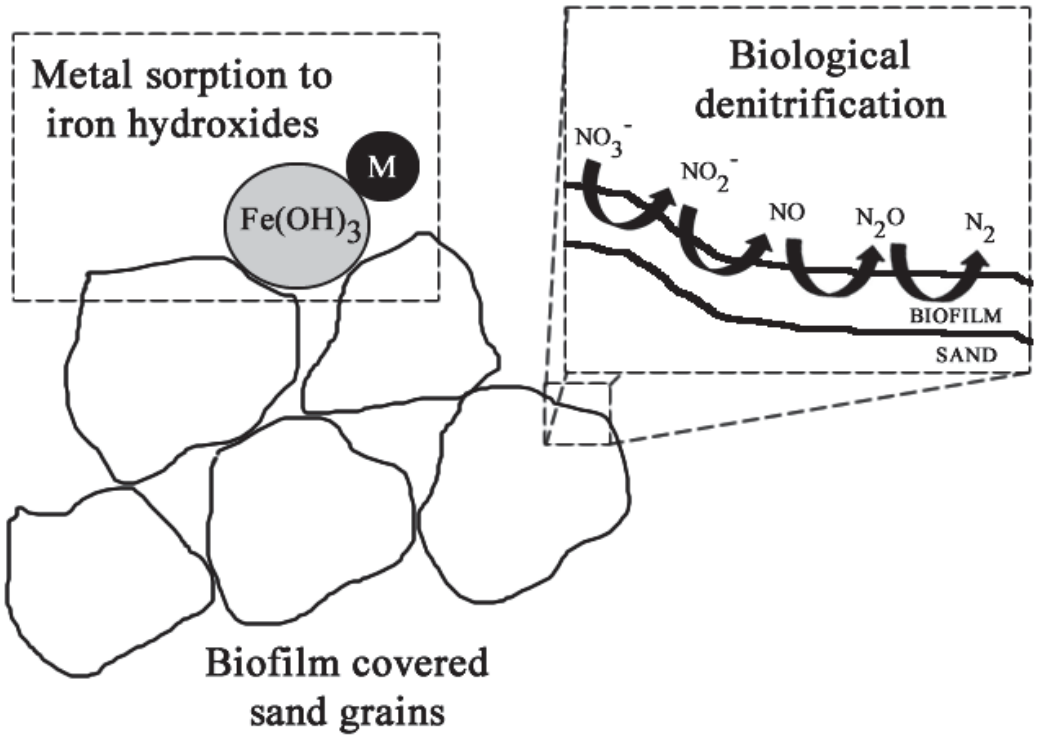

Figure 13: Proposed removal mechanism for arsenic, vanadium (both represented as M) and nitrate

Chapter 6: Conclusions

An experimental treatment method was successfully designed to remove nitrate, arsenic and vanadium simultaneously. Effluent nitrate concentrations were below the guidelines, but further optimization with a higher EBCT is needed to increase total nitrogen removal. In the presence of 2 mg/L of iron, effluent arsenic concentrations were well below the guidelines. As previously stated, there are no vanadium guidelines, but >90% reduction was considered to be very promising.

Factors considered when designing the treatment option included cost effectiveness, ability to treat multiple contaminants, waste creation and utilization of green technology. The biofilters ability to reduce the content of nitrate, arsenic and vanadium simultaneously in one filter column is a very important outcome. This shows that the biofilters are diverse and more eco-friendly compared to treatment options which would require multiple treatment steps to remove multiple contaminants.

There were a few additives that could reduce the cost effectiveness. A carbon source needs to be added for denitrification and iron may need to be added if background concentrations are not sufficient. There are some novel methods that could be used to introduce these chemicals. Agricultural by-products such as corn cobs or rice husks can be used as carbon sources (Xu *et al.,* 2009; Shao *et al.,* 2009). Trickling water over iron fillings prior to biofiltration could also provide the necessary iron. Both options would increase operation ease as liquid additives would not be needed, and would decrease costs.

Waste creation was not significant during the bench-scale experiments, but would have to be considered as the scale increases. Backwash water would have to be dealt with.

Further development of this technology could be very promising, especially for rural Canadians.

Chapter 7: Recommendations

The next steps for this research should focus on three parts. The first involves understanding the biofilm kinetics of the denitrifying bacteria. Parameters such as substrate flux, biofilm thickness, and active biomass density would need to be considered to get a clearer understanding of the biofiltration mechanism. The next part involves studying and modelling vanadium adsorption to iron hydroxides. These studies have been done for arsenic (McNeill and Edwards, 1997), but not for vanadium. Finally, operational parameters will have to be considered for larger scale experiments. The bench-scale biofilters did not need to be backwashed often, but larger scale filters would likely required more frequent backwashing. Therefore the frequency of backwashing and the effect backwashing has on the biofilm will need to be studied. The backwash water will contain high concentrations of metals, so a disposal plan must be developed for those residuals.

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Appendix A: Bench-scale Denitrification Results

The columns in Table A2, A3, A4, A5 and A6 correspond to the sourcewater conditions shown in Table A1. Table A2, A3, A4, A5 and A6 show the raw data for total nitrogen, total organic carbon, nitrate-N, arsenic and vanadium, respectively.

Any missing values were due to sampling or instrument errors.

Table A1: The sourcewater conditions for each of the 12 filters

	NO_3-N	Glycerol	As	V
1	High	Low	Low	Low
2	Low	High	Low	Low
3	Low	Low	High	Low
4	High	High	Low	Low
5	High	Low	High	Low
6	Low	High	High	Low
7	Low	Low	Low	Low
8	High	High	High	Low
9	Low	Low	Low	High
10	High	Low	Low	High
11	Low	High	Low	High
12	High	High	Low	High

Table A2: Total nitrogen effluent concentrations in mg/L during the benchscale denitrification experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	17.39	5.60	5.86	18.06	17.16	5.72	5.87	17.14	5.87	17.25	5.90	17.09
2	11.87	2.46	5.17	13.19	13.78	4.39	4.04	11.63	4.59	14.20	4.87	13.71
4	12.63	4.14	4.10	6.99	6.78	2.81	1.83	12.01	1.92	13.22	3.68	11.65
7	13.86	1.77	3.74	12.03	13.07	4.04	4.68	11.60	4.56	13.93	4.04	12.57
9	13.65	3.65	4.31	11.94	12.15	3.82	4.20	11.19	4.71	13.30	4.05	11.72
11	12.68	4.01	4.53	11.37	12.89	4.01	4.06	11.92	4.41	13.05	4.12	11.91
14	13.93	4.68	4.70	11.89	14.15	4.68	4.71	12.53	4.67	13.66	4.96	12.53
16	14.22	4.66	4.95	12.55	14.67	4.81	4.93	13.33	4.88	14.75	4.96	12.39
18	13.48	4.08	5.04	12.02	14.88	4.48	5.13	12.90	5.02	15.47	4.74	11.98
21	15.38	4.76	5.05	15.69	14.79	4.87	5.18	13.31	5.31	15.24	5.04	13.27
23	14.98	4.85	4.81	13.40	12.89	4.88	5.06	13.99	5.13	15.04	4.95	12.76
25	14.07	4.31	4.54	11.97	14.03	4.42	4.57	11.95	4.82	13.85	4.28	11.68
28	13.45	3.82	4.61	11.78	13.73	4.38	4.40	11.93	4.74	13.59	4.56	12.35
30	13.88	3.99	4.56	11.16	14.04	4.35	4.43	11.23	4.76	13.92	4.28	10.91
32	13.87	3.60	4.67	11.14	13.91	3.89	5.02	12.49	5.26	15.72	4.83	12.62
35	13.87	4.19	4.36	12.10	13.77	4.46	4.94	13.48	5.40	15.57	5.04	13.74
37	15.71	4.72	4.82	12.41	15.49	4.72	4.51	12.95	5.36	15.71	4.90	11.72
39	14.02	4.81	5.26	14.13	15.49	5.12	4.20	15.35	5.37	15.40	5.11	14.23
42	16.26	4.78	5.07	11.73	15.90	5.07	5.12	13.87	5.32	15.77	5.01	13.66
44	17.44	5.22	5.62	12.47	17.01	5.53	5.56	14.81	5.87	17.41	5.64	13.06
46	17.38	5.35	5.57	12.67	17.48	5.57	5.70	15.66	6.01	17.66	5.64	13.05
49	16.37	5.13	5.57	13.31	16.69	5.28	5.48	14.87	5.70	16.39	5.26	12.70

Table A3: Total organic carbon effluent concentrations in mg/L during the benchscale denitrification experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	1.35	5.57	1.27	15.43	1.35	6.20	1.37	16.38	1.39	1.35	6.02	16.06
2	1.19	2.97	1.41	13.09	1.62	5.15	11.38	5.07	1.73	1.60	5.78	15.44
4	1.36	1.87	1.50	10.82	1.60	2.16	3.71	7.98	1.51	1.77	2.28	9.33
7	1.43	1.99	1.39	6.03	1.55	1.93	1.61	7.74	1.64	1.61	2.04	7.02
9	1.51	1.77	1.42	2.30	1.44	1.79	1.52	5.78	1.50	1.53	2.22	2.72
11	2.14	1.97	1.65	2.63	1.77	2.17	2.32	4.11	1.66	1.62	2.22	2.49
14	4.26	2.64	1.88	2.60	1.77	2.56	3.04	3.24	2.43	1.66	2.54	2.63
16	2.20	2.68	2.01	4.08	1.88	2.54	2.41	3.43	2.16	1.74	2.64	2.83
18	2.21	2.62	1.95	2.99	1.79	2.39	1.99	2.72	1.86	1.68	2.16	3.19
21	1.89	2.28	2.06	13.42	2.30	2.12	1.88	4.30	1.76	1.85	2.00	5.10
23	1.83	2.38	2.41	2.87	2.38	2.15	1.91	2.90	1.81	2.14	2.14	3.10
25	1.74	1.95	2.25	5.46	2.03	1.92	2.03	4.76	1.73	1.97	1.88	4.18
28	1.73	2.05	2.08	7.79	1.96	1.70	2.06	8.05	1.73	1.73	2.30	9.76
30	1.73	2.27	2.02	2.58	1.97	2.05	2.18	2.65	1.92	1.88	2.33	2.26
32	1.75	1.99	1.74	8.10	1.69	2.93	2.13	7.46	2.02	1.96	2.24	6.79
35	1.49	2.50	2.16	3.33	1.65	2.29	2.17	3.06	1.91	2.00	2.84	3.58
37	1.60	2.42	2.38	5.62	1.96	2.18	2.23	3.85	1.87	1.97	2.68	4.53
39	1.71	2.68	2.07	3.16	1.92	2.58	2.16	3.03	2.05	2.02	2.77	3.28
42	1.90	2.49	2.06	2.57	2.49	2.51	2.01	2.68	2.21	1.84	2.91	2.51
44	1.81	2.68	1.99	2.58	2.18	2.61	2.47	2.80	2.02	2.10	2.83	3.35
46	1.77	2.95	2.12	2.59	2.15	2.55	2.62	3.19	1.97	2.11	2.89	2.63
49	1.75	2.72	1.86	2.75	1.69	2.46	2.08	5.08	1.74	1.89	2.66	2.52

Table A4: Nitrate effluent concentrations in mg NO_3^--N/L during the benchscale denitrification experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	16.33	5.18	5.39	15.51	16.05	4.51	4.52	14.95	5.24	13.84	4.89	15.03
2	15.87	5.13	5.66	16.86	17.42	5.07	5.01	14.88	4.72	16.71	5.70	16.03
4	13.79	4.04	4.69	2.33	15.10	4.01	4.18	14.98	4.75	15.37	3.99	11.64
7	13.65	3.67	4.91	7.93	14.90	4.05	4.89	9.48	5.01	15.23	4.58	8.68
9	16.90	4.20	4.90	6.17	14.30	4.33	4.79	4.12	5.04	14.20	4.25	6.21
11	13.30	4.28	4.92	3.58	14.01	4.56	3.99	5.88	4.86	13.79	4.11	5.76
14	13.72	3.43	4.22	3.53	13.33	4.38	4.17	8.92	4.26	13.58	3.72	4.10
16	14.75	3.86	4.57	2.86	13.65	4.54	4.47	12.45	4.72	13.41	4.04	4.50
18	13.97	4.25	4.74	4.80	15.88	4.48	4.96	7.62	4.91	15.89	4.57	3.94
21	15.46	4.32	4.59	9.30	13.79	4.52	4.49	7.63	5.16	14.84	4.79	6.22
23	15.92	4.96	4.95	10.89	15.26	4.86	5.13	12.32	5.14	14.62	4.72	6.59
25	14.01	4.04	4.63	7.16	13.25	4.60	4.41	6.03	4.78	14.06	4.99	7.18
28	14.14	3.21	4.86	9.70	15.06	4.51	4.48	5.82	5.16	14.97	4.18	7.76
30	13.83	3.58	4.65	7.73	12.41	4.03	4.19	5.49	4.51	12.47	3.70	4.14
32	12.96	1.51	4.38	7.38	13.75	1.90	4.80	4.82	5.03	14.81	3.99	5.48
35	12.17	3.36	1.62	7.70	11.45	3.57	4.33	8.09	4.59	13.37	4.24	8.96
37	14.89	4.13	4.37	8.21	14.13	3.00	3.66	0.43	4.76	13.97	3.89	4.01
39	14.61	4.18	4.38	10.17	13.38	3.88	3.42	12.61	4.39	12.66	3.61	9.20
42	13.60	3.38	3.96	6.26	11.19	3.14	3.63	8.83	3.89	10.30	3.75	55.91
44	13.69	3.83	4.07	6.94	12.18	3.42	3.68	8.21	3.78	12.42	3.59	5.45
46	14.35	4.12	4.34	7.10	12.92	4.01	4.20	11.20	4.15	11.74	3.89	5.51
49	15.63	4.36	4.81	9.91	14.24	4.12	4.68	10.89	4.87	14.53	4.62	6.52

Table A5: Arsenic effluent concentrations in µg/L during the benchscale denitrification experiment

Days	1	2	3	4	5	6	7	8	9	10	11	12
0	0.14	0.31	49.76	0.41	53.26	55.22	0.36	51.95	0.42	0.31	0.31	0.37
2	0.31	0.19	49.32	0.27	49.23	48.72	0.32	45.66	0.28	0.19	0.16	0.31
4	0.31	0.22	47.81	0.24	46.61	46.05	0.28	47.31	0.30	0.22	0.17	0.25
7	0.19	0.19	45.39	0.24	45.78	46.58	0.16	44.19	0.19	0.18	0.24	0.18
9	0.16	0.14	45.89	0.15	46.54	46.85	0.16	46.70	0.21	0.16	0.12	0.18
11	0.16	0.23	59.89	0.19	59.02	60.49	0.25	55.14	0.12	0.14	0.13	0.14
14	0.13	0.06	49.29	0.16	49.01	51.46	0.20	47.77	0.19	0.11	0.15	0.18
16	0.13	0.16	50.44	0.15	50.67	53.33	0.20	47.45	0.11	0.11	0.08	0.21
18	0.21	0.16	45.36	0.29	46.89	46.66	0.24	45.99	0.14	0.13	0.14	0.14
21	0.17	0.13	39.47	0.29	43.94	47.71	0.19	38.63	0.20	0.18	0.21	0.17
23	0.12	0.13	39.60	0.15	44.56	47.01	0.12	40.24	0.19	0.18	0.32	0.21
25	0.23	0.14	43.95	0.79	50.21	50.05	0.22	42.28	0.26	0.20	0.24	0.23
28	0.18	0.36	48.27	0.24	49.52	48.08	0.26	47.25	0.28	0.24	0.19	0.34
30	0.19	0.13	46.13	0.17	47.20	45.14	0.24	46.28	0.16	0.08	0.16	0.22
32	0.26	0.23	42.79	0.32	46.44	44.72	0.24	44.58	0.23	0.21	0.30	0.19
35	0.13	0.32	44.38	0.17	47.95	46.19	0.20	45.85	0.22	0.19	0.20	0.16
37	0.20	0.19	44.85	0.18	44.71	46.35	0.18	43.53	0.15	0.14	0.15	0.16
39	0.14	0.15	45.37	0.18	45.24	47.03	0.15	44.13	0.17	0.16	0.15	0.17
42	0.18	0.15	44.79	0.19	50.04	46.96	0.32	45.45	0.18	0.26	0.19	0.27
44	0.14	0.15	43.92	0.14	45.94	45.75	0.16	45.56	0.18	0.16	0.17	0.22
46	0.24	0.19	49.50	0.23	52.83	52.23	0.22	51.21	0.17	0.20	0.23	0.24
49	0.26	0.18	51.38	0.25	52.70	52.33	0.22	51.56	0.23	0.20	0.28	0.29

Table A6: Vanadium effluent concentrations in µg/L during the benchscale denitrification experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
7	0.11	0.06	0.05	0.04	0.04	0.04	0.04	0.04	24.08	23.71	24.36	23.39
9	0.03	0.02	0.02	0.01	0.01	0.02	0.02	0.02	24.40	24.68	25.36	24.24
11	0.06	0.04	0.04	0.03	0.04	0.10	0.03	0.04	31.98	31.93	32.40	32.05
14	0.06	0.04	0.02	0.00	0.01	0.02	0.05	0.00	25.35	26.78	26.63	24.76
16	0.03	0.01	0.03	0.00	0.01	0.02	0.03	0.03	24.44	25.33	25.77	24.33
18	0.06	0.03	0.03	0.00	0.00	0.00	0.00	0.00	23.79	25.03	25.01	24.49
21	0.06	0.03	0.05	0.06	0.03	0.02	0.01	0.00	21.97	21.82	22.19	22.56
23	0.05	0.03	0.03	0.02	0.02	0.01	0.01	0.01	22.98	22.27	23.31	23.90
25	0.17	0.14	0.13	0.14	0.12	0.11	0.12	0.13	24.00	25.01	24.10	23.16
28	0.18	0.17	0.15	0.13	0.13	0.14	0.15	0.15	24.39	24.81	23.10	24.68
30	0.11	0.11	0.09	0.08	0.06	0.09	0.08	0.06	23.37	23.33	23.80	23.74
32	0.17	0.11	0.10	0.06	0.05	0.06	0.04	0.03	24.75	26.07	25.09	23.82
35	0.04	0.04	0.03	0.01	0.00	0.03	0.00	0.01	24.75	25.95	26.71	26.95
37	0.12	0.12	0.09	0.10	0.09	0.09	0.05	0.08	26.00	26.28	27.04	26.30
39	0.06	0.06	0.04	0.03	0.03	0.03	0.01	0.02	26.44	26.42	27.69	26.79
42	0.00	0.03	0.03	0.03	0.05	0.01	0.08	0.06	25.87	25.86	26.41	26.71
44	0.03	0.02	0.02	0.02	0.06	0.05	0.04	0.07	27.59	26.99	27.20	25.67
46	0.13	0.11	0.10	0.09	0.10	0.11	0.10	0.10	21.05	21.54	21.19	20.07
49	0.11	0.11	0.10	0.10	0.10	0.11	0.10	0.14	21.29	21.55	20.97	21.33

Appendix B: Bench-scale Enhanced Metal Removal Results

The columns in Table B2, B3, B4, B5 and B6 correspond to the sourcewater conditions shown in Table B1. Table B2, B3, B4, B5, B6 and B7 show the raw data for total nitrogen, total organic carbon, nitrate-N, arsenic, vanadium and iron, respectively.

Any missing values were due to sampling or instrument errors.

Table B1: The sourcewater conditions for each of the 12 filters

	NO_3-N	Iron	As	V
1	High	Low	Low	Low
2	Low	High	Low	Low
3	Low	Low	High	Low
4	High	High	Low	Low
5	High	Low	High	Low
6	Low	High	High	Low
7	Low	Low	Low	Low
8	High	High	High	Low
9	Low	Low	Low	High
10	High	Low	Low	High
11	Low	High	Low	High
12	High	High	Low	High

Table B2: Total nitrogen effluent concentrations in mg/L during the enhanced metal removal experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	9.37	3.91	4.37	11.18	12.16	4.36	3.92	9.30	4.79	10.78	4.38	10.97
3	12.25	4.13	4.12	11.45	12.56	4.62	4.28	11.45	4.83	12.44	4.27	12.04
4	7.88	4.11	4.28	10.09	12.50	4.20	4.06	9.21	4.51	10.14	3.71	9.33
7	9.82	3.77	4.17	11.07	12.02	4.22	4.18	10.98	4.45	11.10	4.17	10.90
12	9.53	3.89	3.80	8.74	10.12	4.20	4.33	8.88	3.90	9.92	3.58	10.29
14	10.46	4.13	4.23	10.36	9.15	0.06	4.37	9.86	4.02	10.07	3.52	11.19
20	10.10	4.19	4.24	11.89	11.05	4.18	4.29	10.41	4.25	10.79	4.15	11.11
23	10.04	4.54	4.54	10.64	9.81	4.86	4.39	10.50	4.52	11.18	4.19	11.52
26	11.85	4.29	4.34	12.29	14.65	4.92	4.02	12.87	5.03	12.47	4.65	14.53
27	11.05	0.14	4.35	11.95	17.10	5.13	4.34	11.71	4.39	10.74	4.25	11.18
29	11.17	0.39	4.54	12.81	17.55	4.82	4.48	12.05	4.58	14.12	4.18	12.24
32	9.87	4.24	4.72	11.03	12.95	4.41	4.79	11.77	4.62	12.34	4.65	11.74
34	11.90	4.27	4.82	12.74	13.03	4.56	4.34	12.67	4.86	12.93	4.66	12.92
36	10.77	4.18	4.63	12.51	12.91	4.32	4.04	12.88	4.64	12.59	4.60	12.73
39	12.09	4.53	4.85	12.58	11.22	27.65	3.77	12.00	4.22	12.07	2.85	11.84
41	12.40	4.52	4.48	8.31	10.86	4.96	3.85	12.26	4.45	12.05	5.10	12.50
43	8.94	4.29	4.09	11.68	11.85	4.85	4.20	13.75	4.38	12.00	4.72	12.84
46	11.13	5.00	3.70	12.86	12.47	4.70	4.02	13.60	4.27	12.68	4.82	13.20
48	9.63	3.61	3.60	10.69	9.21	3.85	3.68	11.47	2.98	9.41	3.97	11.82
50	8.97	3.58	3.56	12.28	12.16	4.04	3.11	10.89	2.77	10.29	3.63	7.20
53	10.10		3.84	11.80	11.43	4.32	0.16	9.60	0.30	10.61	2.82	6.51
55			4.27	11.80	12.18	4.52	4.12	13.70	4.74	13.01	4.84	15.89
68	11.80	5.51	4.34	11.19	13.74	5.44	4.60	15.15	4.62	14.73	15.10	5.18
70	11.42	5.24	4.51	10.07	11.51	5.05	4.34		4.33	12.40	12.88	4.94

Table B3: Total organic carbon effluent concentrations in mg/L during the enhanced metal removal experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	2.01	1.88	1.94	3.61	4.86	1.81	1.97	5.63	2.78	4.90	1.89	2.97
3	2.80	2.37	2.33	3.69	4.03	2.35	2.46	2.81	3.31	4.75	2.28	3.15
4	2.40	2.17	2.32	2.81	7.50	1.96	2.17	2.97	2.52	5.13	2.26	4.36
7	2.29	2.21	2.21	2.67	3.10	2.17	2.42	2.41	2.44	2.50	1.95	2.69
12	2.54	1.97	2.39	2.63	2.76	2.10	2.20	3.03	2.30	3.50	2.06	3.26
14	3.96	2.66	3.68	3.03	2.89	0.12	2.51	2.72	3.59	6.22	2.16	3.71
18	3.42	2.25	2.46	2.71	2.86	2.14	2.27	2.76	2.39	2.72	2.27	2.86
20	3.12	2.10	2.17	1.99	2.15	2.05	1.98	1.95	2.00	2.14	2.03	2.09
26	3.15	2.20	2.25	2.51	2.62	2.00	2.47	2.45	2.17	2.52	2.12	2.59
27	2.44	1.94	2.26	1.89	2.65	1.81	2.51	1.83	1.70	2.58	1.93	3.21
29	3.64	3.57	2.61	2.66	3.68	1.96	1.98	2.34	1.82	2.57	2.06	2.96
32	3.02	2.08	2.28	2.81	2.83	2.39	2.32	2.78	2.18	2.57	2.09	3.63
34	3.78	2.18	2.26	2.37	3.31	2.25	2.35	2.47	2.83	2.51	2.18	2.24
36	4.96	2.36	2.33	3.01	3.33	2.42	2.45	2.96	2.22	2.67	2.31	3.02
39	3.53	2.16	3.02	2.48	2.47	2.03	1.95	2.10	2.00	2.40	1.96	1.89
41	3.60	2.27	7.84	3.90	3.96	2.21	2.27	2.76	2.16	2.80	1.96	2.55
43	2.81	1.83	2.07	3.07	2.47	1.94	2.07	2.11	1.92	2.56	1.82	3.13
46	5.42	2.32	6.84	3.43	4.26	2.05	2.45	3.29	2.19	5.30	2.03	3.32
48	1.96	1.49	4.70	1.83	3.10	1.63	2.28	2.09	1.90	1.83	1.69	1.87
50	2.43	1.45	1.91	2.21	3.21	1.66	39.78	3.70	33.57	2.49	34.01	2.43
53	2.79		2.14	2.47	4.95	1.72	18.76	3.28	24.64	3.65	27.60	6.10
55			2.12	1.90	3.95	1.66	4.77	1.80	3.65	2.35	2.35	2.25
57	2.31	1.63	1.83	3.15	2.89	1.74	2.54	2.21	2.40	2.42	1.93	5.46
68	2.41	1.91	2.45	2.81	2.73	2.08	2.71	2.30	2.23	2.66	4.46	2.18
70	2.31	1.77	2.18	2.17	2.23	2.01	2.77		2.06	2.15	2.07	2.11

Table B4: Nitrate effluent concentrations in mg NO_3^--N /L during the enhanced metal removal experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	9.48	3.63	3.82	7.35	8.45	3.63	2.98	6.35	3.74	6.46	2.69	5.08
3	11.10	3.63	3.92	6.84	10.85	3.59	3.42	7.16	3.79	9.30	3.58	7.89
4	22.21	25.58	18.31	5.77	8.18	3.04	2.90	4.22	5.26	2.77	2.58	2.92
7	11.21	5.04	5.01	8.24	15.54	5.19	23.03	2.23	26.98	48.54	39.82	28.76
41	8.74	2.84	1.30	5.64	7.21	3.99	2.53	8.33	3.54	7.84	4.50	7.66
43	6.65	3.93	2.50	7.70	5.87	3.68	1.71	10.46	2.79	4.38	4.02	8.40
46	7.47	4.33	1.68	8.53	9.18	4.51	3.33	11.21	3.13	4.56	5.12	7.52
48	7.33	4.65	2.96	12.63	5.97	4.38	4.46	8.14	3.64	3.36	4.75	8.76
50	10.46	4.78	4.67	11.28	10.35	5.14	0.02	7.92	0.04	4.58	0.31	4.58
53	7.72	0.00	2.11	6.03	8.61	4.43	0.21	5.18	0.38	3.75	0.00	0.79
55	4.09	5.03	5.42	6.32	0.00	9.45	5.18	15.40	5.10	6.53	5.82	12.75
57	12.58	3.76	5.87	9.91	10.19	3.96	0.63	13.61	2.30	5.77	2.95	13.31

Table B5: Arsenic effluent concentrations in µg/L during the enhanced metal removal experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	0.34	0.19	54.78	0.25	51.66	9.30	0.27	12.29	0.52	0.62	0.25	0.24
3	0.46	0.19	54.32	0.27	54.65	20.12	0.30	25.83	0.56	0.51	0.32	0.35
4	0.39	0.24	51.93	0.27	53.32	18.96	0.32	12.44	0.50	0.68	0.30	0.33
7	0.17	0.04	52.71	0.05	55.89	21.46	0.09	29.01	0.33	0.47	0.14	0.15
12	0.40	0.00	54.21	0.05	49.63	16.04	0.16	19.47	0.34	0.38	0.13	0.22
14	0.66	0.41	55.07	0.21	56.14	19.65	0.30	25.63	0.53	0.63	0.23	0.32
18	0.36	0.14	56.12	0.17	56.12	23.81	0.25	28.34	0.66	1.06	0.26	0.32
20	0.21	0.09	55.83	0.15	52.95	19.28	0.21	20.82	0.45	0.43	0.17	0.17
26	0.31	0.10	54.98	0.16	55.53	14.80	0.26	22.81	0.47	0.46	0.12	0.49
27	0.26	0.09	56.86	0.14	57.49	13.38	0.21	23.05	0.48	0.61	0.10	0.24
29	0.29	0.27	55.12	0.18	56.15	16.07	0.24	25.18	0.50	0.62	0.24	0.32
32	0.27	0.16	53.97	0.17	54.28	16.33	0.18	16.65	0.46	0.47	0.16	0.27
34	0.29	0.13	54.49	0.27	55.99	17.78	0.28	22.31	0.50	0.56	0.20	0.24
36	0.18	0.04	52.70	0.13	52.29	18.10	0.13	20.20	0.38	0.42	0.16	0.15
39	0.18	0.00	53.78	0.19	50.34	12.74	0.12	17.56	0.34	0.52	0.19	0.13
41	0.16	0.00	74.51	0.09	52.17	15.21	0.12	22.42	0.32	0.49	0.03	0.12
43	0.17	0.00	55.31	0.00	53.64	11.29	0.17	18.58	0.36	0.36	0.03	0.12
46	0.36	0.20	62.55	0.21	53.31	12.19	0.29	22.28	0.51	0.50	0.18	0.29
48	0.43	0.07	66.52	0.10	46.75	8.84	0.28	17.55	0.36	0.17	0.30	0.17
50	0.26	0.08	50.80	0.08	51.92	8.54	0.49	14.79	0.61	0.39	0.17	0.23
53	0.22		54.38	0.12	51.78	9.08	0.51	24.63	0.55	0.42	0.28	0.52
55	0.24	0.12	52.33	0.15	49.29	6.98	0.21	10.14	0.45	0.52	0.10	0.09
57	0.29	0.13	53.44	0.10	52.97	6.07	0.30	8.52	0.46	0.50	0.12	0.14
68	0.25	0.04	0.08	0.10	51.10	5.51	0.37	6.62	0.49	0.46	0.16	0.10
70	0.33	0.08	52.47	0.09	50.79	4.06	0.31		0.49	0.47	0.20	0.18

Table B6: Vanadium effluent concentrations in µg/L during the enhanced metal removal experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	0.20	0.12	0.16	0.10	0.16	0.09	0.17	0.19	26.14	25.92	9.42	6.23
3	0.15	0.11	0.20	0.12	0.17	0.10	0.18	0.16	26.24	27.27	9.06	7.37
4	0.20	0.13	0.20	0.14	0.21	0.13	0.23	0.16	25.78	26.05	11.26	6.52
7	0.06	0.00	0.01	0.00	0.00	0.00	0.00	0.00	24.90	24.11	6.89	4.87
12	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	24.48	23.49	11.89	3.46
14	0.14	0.10	0.12	0.06	0.12	0.06	0.13	0.07	22.07	21.69	7.79	3.71
18	0.14	0.06	0.15	0.05	0.22	0.06	0.14	0.08	22.29	23.17	7.20	5.41
20	0.25	0.18	0.28	0.17	0.28	0.18	0.29	0.19	22.25	23.39	5.82	3.84
26	0.28	0.18	0.28	0.18	0.30	0.19	0.27	0.19	23.17	23.81	4.44	3.32
27	0.33	0.22	0.31	0.23	0.29	0.24	0.28	0.21	23.14	22.31	5.59	3.08
29	0.24	0.23	0.22	0.13	0.22	0.13	0.23	0.13	21.57	21.18	4.30	3.01
32	0.24	0.15	0.25	0.18	0.24	0.16	0.24	0.16	20.25	20.31	5.18	3.49
34	0.34	0.18	0.31	0.18	0.30	0.20	0.29	0.19	22.39	22.76	4.22	3.39
36	0.36	0.30	0.35	0.20	0.34	0.23	0.33	0.20	29.05	29.35	6.53	4.77
39	0.33	0.23	0.35	0.22	0.34	0.23	0.37	0.23	26.59	27.35	9.43	5.07
41	0.37	0.24	0.41	0.27	0.37	0.24	0.41	0.27	27.93	28.37	5.19	4.71
43	0.36	0.23	0.38	0.23	0.42	0.27	0.38	0.26	28.01	26.91	4.82	3.20
46	0.23	0.09	0.25	0.08	0.21	0.10	0.21	0.10	24.66	23.49	3.13	3.20
48	0.18	0.14	0.25	0.16	0.78	0.12	0.19	0.19	18.17	1.39	14.97	2.75
50	0.19	0.13	0.19	0.14	0.27	0.14	0.25	0.20	21.52	9.42	3.33	1.98
53	0.20		0.21	0.16	0.27	0.16	0.21	0.29	21.02	13.27	3.71	3.93
55	0.21	0.16	0.22	0.20	0.25	0.17	0.23	0.18	23.97	19.36	1.30	1.99
57	0.24	0.21	0.27	0.21	0.29	0.20	0.25	0.21	24.90	22.09	1.42	1.69
68	0.32	0.21	1.66	0.20	0.33	0.21	0.44	0.24	23.71	26.06	2.03	1.68
70	0.32	0.22	0.34	0.22	0.34	0.22	0.37		24.50	24.38	1.49	1.60

Table B7: Iron effluent concentrations in µg/L during the enhanced metal removal experiment

Day	1	2	3	4	5	6	7	8	9	10	11	12
0	8.5	50.7	12.8	287.1	4.8	24.8	7.5	96.9	4.7	4.8	105.9	61.9
3	5.9	38.2	17.1	280.1	6.5	9.3	4.7	72.4	5.2	5.3	135.9	47.2
4	11.9	54.4	10.9	233.8	7.3	45.3	9.4	83.7	11.5	9.7	137.1	89.8
7	3.1	21.2	0.0	147.9	0.0	3.0	3.2	37.3	0.0	21.6	81.0	32.9
12	0.0	8.6	0.0	28.0	0.0	0.0	0.0	24.6	0.0	0.0	267.1	62.6
14	0.0	0.7	2.7	143.6	0.1	0.2	1.1	12.6	4.9	0.7	76.2	67.5
18	4.1	58.2	1.9	98.0	2.6	5.5	0.9	60.2	8.6	35.3	69.0	107.1
20	9.1	16.2	10.0	31.3	11.1	62.0	12.2	58.0	12.2	10.4	34.4	60.7
26	13.6	11.7	8.3	16.9	9.3	30.7	10.6	13.6	8.5	9.7	16.2	21.4
27	15.9	83.9	15.4	51.8	14.4	20.0	12.4	31.0	12.0	18.8	37.5	68.9
29	5.3	143.4	6.5	14.4	4.2	7.0	2.1	10.2	0.8	2.4	12.3	10.2
32	6.2	7.9	6.7	58.0	6.4	61.9	6.9	53.3	5.6	7.5	49.1	86.0
34	10.7	11.2	6.7	20.0	10.1	15.7	9.4	17.4	7.3	7.0	32.7	26.0
36	7.9	8.8	5.9	26.9	7.5	26.7	8.2	15.6	4.9	3.1	18.2	18.6
39	8.1	10.4	8.9	90.4	6.6	36.5	4.2	46.9	11.5	7.0	41.8	99.4
41	6.7	10.5	13.2	193.5	6.1	34.9	5.4	29.6	10.8	5.7	18.7	61.0
43	7.6	13.9	15.5	44.8	10.5	43.4	13.5	29.2	17.5	8.5	18.0	44.7
46	10.0	10.4	9.6	29.9	7.8	20.5	6.9	14.4	11.7	6.5	10.6	29.8
48	7.8	10.3	9.8	42.0	15.6	11.1	16.8	14.6	18.0	30.8	23.1	33.0
50	10.1	9.4	13.6	20.9	19.9	10.2	35.8	32.6	44.2	40.7	17.2	84.2
53	9.5		15.7	19.8	19.1	12.5	56.6	51.6	29.5	24.0	108.3	1692
55	8.6	9.7	27.4	52.8	24.8	10.4	26.0	21.4	27.6	35.1	12.6	59.7
57	15.7	12.9	22.9	35.2	19.8	11.8	14.2	30.2	19.3	27.4	11.3	48.9
68	14.7	9.9	42.8	83.0	16.6	39.0	49.4	89.8	11.9	53.6	56.1	51.0
70	11.1	7.8	11.8	37.5	13.8	20.3	17.8		13.6	13.0	25.7	23.1

Appendix C: Analysis of Variance Data

Section 4.1 Statistics

Arsenic Plate Counts - Toxicity

One-way ANOVA: C1 versus C2

```
Source  DF          SS         MS     F      P
C2       3   670500000  223500000  1.16  0.428
Error    4   771000000  192750000
Total    7  1441500000

S = 13883   R-Sq = 46.51%   R-Sq(adj) = 6.40%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean  StDev  -----+---------+---------+---------+----
1      2  82500   3536         (-----------*-----------)
2      2  91500  26163             (-----------*-----------)
3      2  81000   1414        (-----------*-----------)
4      2  66000   8485  (-----------*-----------)
                        -----+---------+---------+---------+----
                         50000     75000    100000    125000

Pooled StDev = 13883
```

Vanadium Plate Counts - Toxicity

One-way ANOVA: C1 versus C2

```
Source  DF           SS          MS     F      P
C2       3   5945500000  1981833333  1.12  0.440
Error    4   7086000000  1771500000
Total    7  13031500000

S = 42089   R-Sq = 45.62%   R-Sq(adj) = 4.84%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
1      2  157500  60104             (-------------*-------------)
2      2   93500   4950   (-------------*------------)
3      2  128500  58690         (------------*-------------)
4      2   91500   2121  (-------------*-------------)
                         ---------+---------+---------+---------+
                              60000    120000    180000    240000

Pooled StDev = 42089
```

Section 4.2.1 Statistics

Nitrate – With Glycerol

One-way ANOVA: C1 versus C2

```
Source  DF     SS    MS     F      P
C2       2   30.1  15.0  1.16  0.319
Error   63  813.7  12.9
Total   65  843.8

S = 3.594   R-Sq = 3.56%   R-Sq(adj) = 0.50%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  --+---------+---------+---------+-------
1      22  7.819  3.607      (------------*------------)
2      22  8.893  3.848                (------------*------------)
3      22  7.268  3.305  (------------*-----------)
                         --+---------+---------+---------+-------
                         6.0       7.2       8.4       9.6

Pooled StDev = 3.594
```

Nitrate – Without Glycerol

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       2    3.27  1.64  0.86  0.429
Error   63  120.15  1.91
Total   65  123.42

S = 1.381   R-Sq = 2.65%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev  ---+---------+---------+---------+------
1      22  14.435  1.175            (-----------*----------)
2      22  13.984  1.503   (-----------*----------)
3      22  13.944  1.443  (-----------*-----------)
                          ---+---------+---------+---------+------
                          13.50     14.00     14.50     15.00

Pooled StDev = 1.381
```

Total Nitrogen – With Glycerol

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       2    5.94  2.97  1.10  0.340
Error   63  170.39  2.70
Total   65  176.33

S = 1.645   R-Sq = 3.37%   R-Sq(adj) = 0.30%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev  ----+---------+---------+---------+-----
1      22  12.455  1.995  (-----------*----------)
2      22  13.188  1.589             (-----------*----------)
3      22  12.786  1.267       (-----------*-----------)
                          ----+---------+---------+---------+-----
                           12.00     12.60     13.20     13.80

Pooled StDev = 1.645
```

Total Nitrogen – Without Glycerol

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       2    5.48  2.74  0.87  0.425
Error   63  198.85  3.16
Total   65  204.34

S = 1.777   R-Sq = 2.68%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev  ----+---------+---------+---------+-----
1      22  14.563  1.593      (------------*-----------)
2      22  14.307  2.240  (-----------*------------)
3      22  15.005  1.384             (------------*------------)
                          ----+---------+---------+---------+-----
                           13.80     14.40     15.00     15.60

Pooled StDev = 1.777
```

Total Nitrogen compared to Nitrate (without glycerol)

One-way ANOVA: C1 versus C2

```
Source  DF     SS    MS     F      P
C2       1   2.79  2.79  1.42  0.240
Error   42  82.48  1.96
Total   43  85.27

S = 1.401   R-Sq = 3.28%   R-Sq(adj) = 0.97%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level   N    Mean  StDev     +---------+---------+---------+---------
1      22  14.625  1.606               (-----------*-----------)
2      22  14.121  1.162     (-----------*-----------)
                             +---------+---------+---------+---------
                          13.50     14.00     14.50     15.00

Pooled StDev = 1.401
```

Section 4.2.2 Statistics

Arsenic – Denitrification Experiments

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       3   109.6  36.5  2.39  0.075
Error   84  1285.8  15.3
Total   87  1395.4

S = 3.912   R-Sq = 7.85%   R-Sq(adj) = 4.56%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev   --+---------+---------+---------+-------
1      22  46.707  4.344     (----------*----------)
2      22  48.559  3.605                (----------*----------)
3      22  48.860  3.877                  (----------*----------)
4      22  46.305  3.785   (----------*----------)
                           --+---------+---------+---------+-------
                          45.0      46.5      48.0      49.5

Pooled StDev = 3.912
```

Vanadium – Denitrification Experiments

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       3    2.59  0.86  0.14  0.935
Error   84  517.17  6.16
Total   87  519.76

S = 2.481   R-Sq = 0.50%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev  -------+---------+---------+---------+--
1      22  24.348  2.393  (--------------*--------------)
2      22  24.689  2.419       (--------------*--------------)
3      22  24.761  2.676        (--------------*--------------)
4      22  24.434  2.427   (--------------*--------------)
                          -------+---------+---------+---------+--
                              23.80     24.50     25.20     25.90

Pooled StDev = 2.481
```

Section 4.3.1 Statistics

Nitrate during Enhanced Metal Removal

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       3   15.65  5.22  0.68  0.571
Error   32  245.74  7.68
Total   35  261.39

S = 2.771   R-Sq = 5.99%   R-Sq(adj) = 0.00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N   Mean  StDev  ------+---------+---------+---------+---
1      9  8.115  2.129         (-----------*-----------)
2      9  8.036  2.412        (-----------*-----------)
3      9  8.906  3.066              (-----------*-----------)
4      9  7.048  3.312  (-----------*-----------)
                         ------+---------+---------+---------+---
                             6.0       7.5       9.0      10.5

Pooled StDev = 2.771
```

Nitrate – Denitrification experiment vs. enhanced metal removal experiment

One-way ANOVA: C1 versus C2

```
Source  DF      SS     MS     F      P
C2       6   64.28  10.71  1.60  0.157
Error   86  575.82   6.70
Total   92  640.10

S = 2.588   R-Sq = 10.04%   R-Sq(adj) = 3.77%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N   Mean  StDev  -------+---------+---------+---------+--
1       9  8.115  2.129           (----------*-----------)
2       9  8.036  2.412          (-----------*----------)
3       9  8.906  3.066                (----------*-----------)
4       9  7.048  3.312    (----------*----------)
5      19  7.227  2.305        (-------*-------)
6      19  7.939  3.198             (-------*-------)
7      19  6.168  1.672  (-------*-------)
                          -------+---------+---------+---------+--
                               6.0       7.5       9.0      10.5

Pooled StDev = 2.588
```

Section 4.3.2 Statistics

Arsenic Comparison – No bacteria and Low Nitrate

One-way ANOVA: C1 versus C2

```
Source  DF     SS    MS     F      P
C2       1   26.1  26.1  1.96  0.174
Error   24  319.4  13.3
Total   25  345.5

S = 3.648   R-Sq = 7.55%   R-Sq(adj) = 3.70%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev   --+---------+---------+---------+-------
1       8  14.302  3.252   (-------------*------------)
2      18  16.473  3.800                  (--------*--------)
                           --+---------+---------+---------+-------
                          12.0      14.0      16.0      18.0

Pooled StDev = 3.648
```

Arsenic Comparison – All 3 filters

One-way ANOVA: C1 versus C2

```
Source  DF      SS     MS      F      P
C2       2   356.9  178.4  10.47  0.000
Error   41   698.7   17.0
Total   43  1055.6

S = 4.128   R-Sq = 33.81%   R-Sq(adj) = 30.58%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level   N    Mean  StDev   --+---------+---------+---------+-------
1       8  14.302  3.252   (---------*---------)
2      18  21.382  4.723                         (-----*------)
3      18  16.473  3.800            (------*-----)
                            --+---------+---------+---------+-------
                           12.0      15.0      18.0      21.0

Pooled StDev = 4.128
```

Vanadium Comparison – All 3 filters

One-way ANOVA: C1 versus C2

```
Source  DF      SS     MS     F      P
C2       2   58.25  29.12  6.48  0.004
Error   41  184.18   4.49
Total   43  242.42

S = 2.119   R-Sq = 24.03%   R-Sq(adj) = 20.32%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N   Mean  StDev   --+---------+---------+---------+-------
1       8  6.503  2.532              (-----------*-----------)
2      18  6.787  2.537                    (--------*-------)
3      18  4.368  1.325  (-------*--------)
                          --+---------+---------+---------+-------
                          3.6       4.8       6.0       7.2

Pooled StDev = 2.119
```

Vanadium Comparison – No bacteria and Low Nitrate

One-way ANOVA: C1 versus C2

```
Source  DF      SS    MS     F      P
C2       1    0.45  0.45  0.07  0.794
Error   24  154.33  6.43
Total   25  154.78

S = 2.536   R-Sq = 0.29%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N   Mean  StDev  ---+---------+---------+---------+------
1       8  6.503  2.532  (-----------------*-----------------)
2      18  6.787  2.537           (-----------*-----------)
                          ---+---------+---------+---------+------
                           5.0       6.0       7.0       8.0

Pooled StDev = 2.536
```