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Chromium(VI) and Oxyanion Remediation Of Vadose Zone Soils With Zero Valent Iron (ZVI) and Biological Reduction

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CHROMIUM(VI) AND OXYANION REMEDIATION OF VADOSE ZONE SOILS WITH ZERO VALENT IRON (ZVI) AND BIOLOGICAL REDUCTION

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ABSTRACT

In areas with high industrial development, soil and groundwater are often heavily contaminated with hexavalent chromium [Cr(VI)], which commonly occurs as the oxyanions chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). By itself, Cr(VI) is a common contaminant in various industrial wastes, but other oxyanions such as nitrate [NO_3^-], chlorate [ClO_3^-], and perchlorate [ClO_4^-] can appear with Cr(VI) as co-contaminants based on the type of industrial waste. Cr(VI) and ClO_3^- occur as co-contaminants in areas where sodium chlorate is manufactured as a bleaching agent for the pulp and paper industry (ERCO Worldwide, 2012). ClO_4^- and Cr(VI) are common co-contaminants due to their shared applications in electroplating and leather tanning (Sorensen et al., 2006). ClO_4^-, NO_3^- and Cr(VI) can occur simultaneously in areas associated with the manufacture, use and disposal of rocket fuel (Rong, 2018). ClO_4^- and NO_3^- are also noted to be common co-contaminants in soil and groundwater. (Logan and Lapoint, 2002; Ziv-El and Rittman, 2009; Rong, 2018)

Prior to the implementation of RCRA regulations in 1986, wastes containing these contaminants were simply disposed of into the ground, resulting in the contamination of both vadose zone soils and groundwater. Technological options for remediation of vadose zone soils are limited in comparison to groundwater remediation due to lack of development and field testing, with very few options having been successfully implemented in vadose zone treatment (Dresel et al., 2011). This thesis focuses on bioremediation options for vadose zone soils, specifically on the remediation of Cr(VI), NO_3^-, and ClO_3^- using biological reduction.

The research objective of this study was to assess the viability of bioremediation as an alternative for the removal of Cr(VI) from vadose zone soils using bioremediation methods. Specifically, autotrophic removal through biotic contaminant removal under maintained anaerobic conditions and bio-augmented remediation using zero-valent iron [ZVI] were compared to determine which method of treatment was more effective at reducing Cr(VI) and its co-contaminants from vadose zone soils.

Microcosm experiments were performed using contaminated fine-grained soils taken from a site in the southwestern United States with high levels of Cr(VI), NO_3^-, and ClO_3^-. Biotic reduction tests comparing EOS-Pro and molasses as carbon sources were performed, where soil was divided, mixed with different carbon source and nutrients, prepared and placed in an anaerobic chamber to incubate. A second microcosm test was performed where contaminated soils were mixed with varying amounts of carbon source, nutrients, bacteria and stoichiometric ratios of ZVI to determine which combination of biological reduction and ZVI reduced the most contaminant in the least amount of time. Sample blanks were formed for both experiments to determine which soil amendment enhanced contaminant reduction, if any, and by how much.

During the biotic reduction experiments, it was determined that while molasses was more effective in stimulating Cr(VI) removal, neither carbon source had any significant effect on NO_3^- or ClO_3^- removal due to incomplete Cr(VI) reduction. Low soil moisture in the samples also inhibited Cr(VI) reduction, which in turn also inhibited soil denitrification and ClO_3^- reduction. In comparison, the ZVI remediation experiments showed that significant reduction of all three contaminants took place within 50 days of regular treatment of the vadose zone soils, with Cr(VI) and ClO_3^- being almost completely removed from the soil. As the ZVI experiments involved regular soil wetting to prevent desiccation, it raises the implication that a combination of soil flushing techniques with biological reduction using ZVI could be employed to treat highly contaminated vadose zone soils. Considerations for the use of either ZVI or biological reduction techniques in vadose zone treatment include the costs of using high stoichiometric ratios of ZVI to contaminant, the removal of potential byproducts like iron [Fe] and ammonia [NH_3], and the ambient soil conditions at the time of treatment.

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CHAPTER 1

INTRODUCTION

1.1 – Background

The presence of contaminants such as heavy metals in the environment is an ongoing problem in the modern world. Chromium in particular is a source of concern because unlike many contaminants, it is difficult to degrade, accumulating in living tissues and causing various disorders and diseases in living organisms (Gheju, 2011). Its health effects are well-documented and studied; Narayani and Shetty (2013) report that chromium is toxic, carcinogenic, mutagenic, and teratogenic; skin infection, contact dermatitis and chromium poisoning can result from direct skin contact, while direct inhalation can irritate the respiratory system, resulting in health issues such as asthma (Mohan and Pittman Jr., 2006). Chromium has been detected at numerous United States (U.S.) Department of Energy (DoE) and Department of Defense (DoD) sites as well as at numerous industrial facilities where chromium compounds are widely used, such as in electroplating, corrosion protection, wood preservation and leather tanning. Chromium's toxicity, prevalence and mobility have thus qualified it as a priority pollutant by the U.S. Environmental Protection Agency (EPA) (Chrysochoou et al., 2010; McLean et al., 2012; Narayani and Shetty, 2013). Depending on industry type, other compounds can also appear alongside chromium as co-contaminants; three of the most common are the oxyanions nitrate [NO_3^-], chlorate [ClO_3^-] and perchlorate [ClO_4^-], all of which can occur with chromium in various types of industrial wastes.

Numerous technologies and methods have been developed to treat chromium-contaminated soils and waters, with a full and exhaustive listing of these available at EPA's CLU-IN website (https://clu-in.org/contaminantfocus/default.focus/sec/chromium_VI/cat/Treatment_Technologies/). The methodology used to treat a given site is often dependent on the type of water and soil being treated, the initial chromium concentration, treatment of secondary effluent, and economic feasibility (Mohan and

Pittman Jr., 2006; Narayani and Shetty, 2013). Speciation is also a major control in how chromium is treated. In nature, trivalent chromium [Cr(III)] is the most dominant species of chromium in soil and groundwater – and in groundwater the concentrations typically range from 0.0005 to 0.21 mg/L (Richard and Bourg, 1991; Tokunaga et al., 2001; Pakzadeh and Batista, 2011). Hexavalent chromium [Cr(VI)] is largely absent in natural settings, though it can be oxidized from Cr(III) in the presence of manganese oxides and oxygen (Robles-Camacho and Armienta, 2000).

1.2 – Problem Definition

1.2.1 – Vadose Zone Remediation

Vadose zone soils are defined as unsaturated soils located in the subsurface between the ground surface and the water table (Hanson et al., 1993). Similarly, deep vadose zone refers to the subsurface region of soil above the water table that is below the zone of practicable excavation (Dresel et al., 2011). Vadose zone contamination is a significant problem in the United States, especially in arid and semi-arid regions, because contaminant transport is a gradual process and often a function of precipitation. In particular, vadose zone soils with mobile contaminants are treated as ongoing sources of pollution since rain and surface runoff can leach contaminant into the underlying groundwater (Hanson et al., 1993; Dresel et al., 2011). The nature of vadose zone hydrology and contaminant transport is such that remediation options are less developed than those for shallow soil or saturated zone contamination; and despite active research and development, very few processes have been field-tested, much less been successfully implemented for full Cr(VI) remediation (Dresel et al., 2011). One of the few potential alternatives for vadose zone treatment, soil flushing, has been used successfully at the United Chrome Products Superfund site in Oregon to remediate chromium contamination (National Risk Management Research Laboratory, 2000; Jacobs and Rouse, 2005).

Of particular interest is the application of vadose zone remediation technologies to sites where Cr(VI) in the vadose zone is a major source of groundwater contamination, such as the U.S. DoE sites at

Hanford in Washington and the Savannah River in South Carolina, where chromium contamination is associated with the use of sodium dichromate in nuclear reactors during the irradiation process (Ford et al., 2006; Dresel et al., 2008; Zhong et al., 2009). At Hanford, Cr(VI) concentrations in the groundwater range from 20 to 24,000 µg/L (CH2M-HILL, 2015), and soil concentrations are estimated to range from 10 to 40 mg/kg (Truex et al., 2012). Groundwater Cr(VI) concentrations at the Savannah River Site are reported to range from 50 to 2,700 µg/L (Cummins et al., 1990), and soil concentrations range from 38 to 175 mg/kg (Seaman et al., 2001). The NERT site in southern Nevada has Cr(VI) concentrations ranging from 10 to 25 mg/L, and soil concentrations as high as 22 mg/kg (Tetra Tech, Inc., 2018).

Aside from its applications in the energy industry as an anti-corrosion agent, Cr(VI) compounds are also used extensively in various industries such as electroplating, leather tanning, cement production, textiles, painting and pigment production, and automobile production (Mohan and Pittman Jr., 2006; Narayani and Shetty, 2013). Extensive Cr(VI) contamination is also associated with the manufacture of ammonium perchlorate, a type of rocket fuel, as chromium is used to prevent electrode corrosion during the electrochemical manufacturing of ClO_4^-. As a result, NO_3^- and ClO_4^- is also found with Cr(VI) as co-contaminants (Logan and Lapoint, 2002; Ziv-El and Rittman, 2009; Rong, 2018). Cr(VI) and ClO_3^- have also been found together in industrial wastes associated with the production of sodium chlorate as a bleaching agent for paper and pulp (Endrődi et al., 2017), and ClO_3^- shares industrial uses with Cr(VI) in leather tanning and the manufacture of explosives (Grant-Trusdale, 2005). In southern Nevada, extensive chromium contamination is associated with a number of sites like the Black Mountain Industrial Complex, with a full listing found on the Nevada Division of Environmental Protection website (https://ndep.nv.gov/environmental-cleanup/site-cleanup-program/active-cleanup-sites).

This thesis focuses on the removal of Cr(VI) and co-contaminants from vadose zone soils using biological reduction and zero-valent iron [ZVI]. ZVI has been successfully utilized in the treatment of Cr(VI)-contaminated waters (Gheju, 2011; Mitra et al., 2011). Because of the presence of multiple oxyanion co-contaminants in the soil, the feasibility of treating multiple co-contaminants along with

Cr(VI), particularly NO_3^- and ClO_3^-, is also analyzed. Two electron donor / carbon sources, along with ZVI, will be compared and contrasted for reduction of oxyanions.

1.2.2 – Cr(VI) Biotic Remediation and Abiotic Remediation with Zero-Valent Iron

Two papers by Oliver et al (2003) and Hunter (2005) also demonstrated the potential for vadose zone chromium bioremediation. Oliver et al. (2003) performed batch and column experiments using native microbial communities and an initial chromium concentration of 67 mg/L, and showed that with the addition of nutrients in the form of NO_3^- and organic carbon (here added as molasses) up to 87% of the initial chromium was reduced. Furthermore, after 45 days of column testing 10% of the total chromium had been immobilized as a result of nutrient amendments to the soil. Their report hypothesizes that 100% immobilization of Cr(VI) could also be achieved using longer flow paths through the vadose zone and longer contact times. Hunter (2005) also discusses the use of vegetable oil as a possible electron donor compared to molasses for microorganisms in contaminated zones, stimulating them and forming a permeable reactive barrier that could be used to remediate many contaminants – though his work focuses solely on oil injection as a means of providing nutrients for native bacterial communities, and not actual biological treatment of Cr(VI).

Another potential strategy to remove Cr(VI) from vadose zone soils is the use of ZVI to reduce it chemically. ZVI has been shown to reduce Cr(VI) under acidic conditions, and the accompanying pH increase during the reaction prevents the now-reduced Cr(III) from re-oxidizing back into its more toxic form. Gheju (2011) provides a comprehensive review of the current state of ZVI research over the last two decades with respect to Cr(VI) reduction with ZVI. More importantly, in recent full-scale applications, ZVI has been used in the formation of permeable reactive barriers (PRBs) through which Cr(VI)-contaminated waters pass through; the ZVI reacts with the Cr(VI) in the water and reduces into its less-mobile Cr(III) (Fruchter, 2002). One particular case study by Němeček et al. (2014) reports on the potential for full-scale application of advanced Cr(VI) bioremediation with ZVI. In the case study, nanoscale zero-valent iron [nZVI] was injected into the aquifers at the highly contaminated Kortan site

in the northern Czech Republic. As a result of the injection, Cr(VI) and total Cr concentrations in the groundwater decreased rapidly without substantially altering the groundwater's chemical properties, nor did it affect the indigenous bacteria population negatively. It was reported that the application of nZVI actually stimulated bacterial growth, which carries positive implications for its potential use in advanced bioremediation applications.

1.3 – Objectives

Fruchter (2002), Oliver et al. (2003) and Gheju (2011) have all demonstrated that bioremediation and ZVI reduction can potentially be used with soil flushing techniques for effective Cr(VI) removal from vadose zone soils, with the study by Němeček et al. (2014) also potentially demonstrating a case where bioremediation and chemical reduction with ZVI could be used on the same soil without adversely affecting the underlying groundwater table. However, to our knowledge, no direct study of these combined methods has been applied to vadose zone soils — only to saturated zone soils and water. Furthermore, though NO_3^- effects have been considered in Oliver et al.'s work, the effects of ClO_3^- as a co-contaminant on Cr(VI) bioremediation techniques have not been well-studied. The aim of the present investigation is to study the effectiveness of treating chromium and co-occuring oxyanions using the aforementioned soil flushing techniques. The specific questions to be addressed in this thesis are as follows:

- Which electron donor/carbon sources will be more suitable for biological Cr(VI) degradation, and how much will be sufficient for native microbial communities in vadose zone environments to effectively reduce Cr(VI)?
- What effects will the presence of co-contaminants [NO_3^- and ClO_3^-] have on the efficacy of significant Cr(VI) reduction in vadose zone soils, if any?
- How much contaminant reduction will we observe using biotic bioremediation by itself, and with ZVI-enhanced remediation?

- What ZVI dosage is required to produce the desired amount of contaminant removal from vadose zone soils?
- What are the implications for Cr(VI) bioremediation methods with respect to potential full-scale applications in the removal of Cr(VI) and other common co-contaminants from vadose zone soils?

1.4 – Study Scope

This thesis will focus mainly on Cr(VI) removal from vadose zone soils. However, because of their presence in the soil as co-contaminants, NO_3^- and ClO_3^- will also be examined as a measure of the treatment efficiency. With respect to treatment methods, this thesis will focus solely on biotic and abiotic remediation. Though ClO_4^- is also mentioned as a potential co-contaminant, it will largely be ignored in favor of focusing on removing Cr(VI), NO_3^- and ClO_3^- due to the difficulty of removing it in the presence of high concentrations of total dissolved solids [TDS], which created analytical issues.

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CHAPTER 2

LITERATURE REVIEW

2.1 – Introduction to Chromium

In nature, chromium occurs in two common oxidation states: trivalent, denoted as Cr(III), and hexavalent, denoted as Cr(VI). Cr(III) is considered an essential micronutrient and trace element, aiding in the metabolism process. However, Cr(III) can be oxidized into Cr(VI), which is classified as a group A human carcinogen because it is toxic, carcinogenic, mutagenic, and teratogenic (Narayani and Shetty, 2013). McLean et al. (2012) describes at least two modes of action for the proposed effects of Cr(VI) in humans through ingestion, extrapolated from high-dosage mouse studies. Once Cr(VI) is ingested, some portion is reduced to Cr(III) in the digestive tract. However, the remaining Cr(VI) is absorbed and reduced to Cr(III), damaging cell DNA and resulting in mutagenesis, cell proliferation and tumors in the digestive tract. Alternatively, oxidative stress occurs, causing the gene expressions to change and spontaneously mutate the DNA.

Mohan and Pittman Jr. (2006) reports that acute exposure to Cr(VI) is linked to detrimental health effects like nausea, diarrhea, liver and kidney damage, dermatitis, internal hemorrhage, and respiratory problems. Direct inhalation can also result in acute toxicity, irritation and ulceration of the nasal septum, and asthma. Ingestion of Cr(VI) may affect kidney and liver functions. Skin contact may result in chromium poisoning, severe burns, and interference with the healing of cuts or scrapes – and can result in further skin infection if not treated properly. Permanent damage to the eyes may also result if eye exposure occurs.

Because of the numerous health effects associated with chromium ingestion and contact, the U.S. EPA regulates the total chromium levels in drinking water. Its maximum contaminant level [MCL] is set at 0.1 mg/L, while the World Health Organization places its total chromium MCL at 0.05 mg/L (U.S.

EPA, 2002; Mohan and Pittman Jr., 2006; Mills et al., 2011). Currently, there are no federal regulations that govern individual chromium species in drinking water, though in 2012 the U.S. EPA published the third Unregulated Contaminant Monitoring Rule [UCMR 3] which required all public water systems that serve more than 10,000 people and a statistical sample of smaller-scale system to monitor for both total Cr and Cr(VI) (U.S. EPA, 2012). In California, the state adopted a Cr(VI) MCL of 10 μg/L in 2014, though following a court order in 2017 invalidating the MCL, the standard has since reverted to 50 μg/L total Cr (California Water Boards, 2019).

Chromium discharges into waters and treatment requirements are currently regulated by a suite of statutes, which include the 1976 Resource Conservation and Recovery Act (RCRA); the 1977 Clean Water Act; and the 1980 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) – all of which classify chromium as a hazardous substance (Gilbert, 1996). On top of requiring pretreatment systems for chromium removal, the RCRA also required waste generators to keep a record of their estimated waste generation and discharge, and governed on-site treatment requirements and offsite disposal of chromium waste (Gilbert, 1996; Hawley and Jacobs R.G., 2005). CERCLA also published reportable release quantities for chromium, further placing responsibility onto chromium generators, transporters and disposers (Gilbert, 1996; Hawley and Jacobs R.G., 2005). Treatment and disposal requirements under these statutes, however, are largely applicable or relevant and appropriate requirements (ARARs) (Gilbert, 1996).

2.2 – Chromium Contamination

2.2.1 – Chromium Chemistry

Cr(III) hydrolysis produces the species $CrOH^{2+}$, $Cr(OH)^{2+}$, $Cr(OH)^{4-}$, $Cr(OH)_3$, $Cr_2(OH)_2$ and $Cr_3(OH)_4^{5+}$, while Cr(VI) hydrolysis produces the species CrO_4^{2-}, $HCrO_4^{2-}$, and $Cr_2O_7^{2-}$ (Mohan and Pittman Jr., 2006). Cr(III) is less toxic and is insoluble for pH greater than 5. Cr(VI) is more toxic than Cr(III) because of its high solubility and mobility. Its speciation is dependent on two factors: pH and

redox potential (Mohan and Pittman Jr., 2006; Barrera-Díaz et al., 2012). No Cr(VI) species can form insoluble precipitates; thus, Cr(VI) removal through direct precipitation is not possible. On the other hand, Cr(III) forms insoluble species which can be precipitated out of water (Barrera-Díaz et al., 2012). Under highly alkaline conditions, Cr(III) can reoxidize into Cr(VI). The distribution of various Cr species as a function of pH can be seen in Figure 2-1.

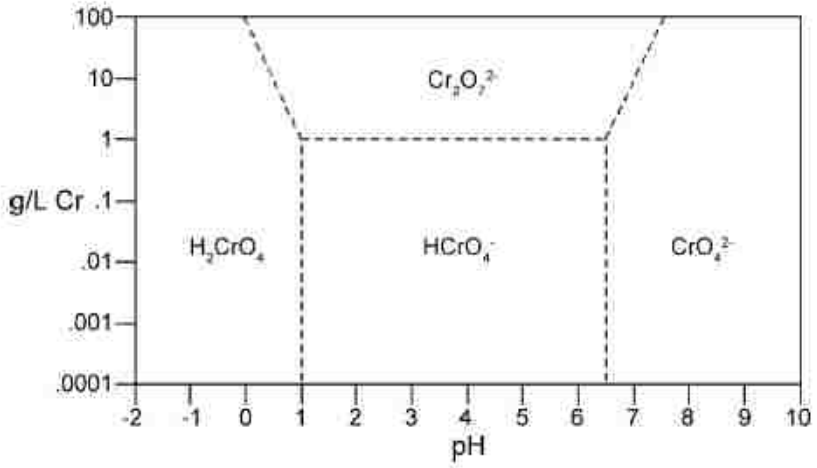

Figure 2-1: Speciation of Cr(VI) as a function of pH. (Source: Mohan and Pittman Jr., 2006)

The redox potential E_h-pH diagram seen in Figure 2-2 depicts the different oxidation states and forms of Cr that are stable at various E_h and pHs. In low E_h environments, aqueous chromium is in its trivalent form and is predominantly $Cr(OH)^{2+}$, $Cr(OH)^{4-}$, and $Cr(OH)_3$; with $Cr(OH)^{2+}$ becoming more prevalent as the pH increases (Eary, 1988; Richard and Bourg, 1991; Mohan and Pittman Jr., 2006). Cr(III) forms complexes with numerous ligands such as hydroxyls, sulfates, ammonium, cyanide, sulphocyanide, fluoride and chloride (Richard and Bourg, 1991). Its low solubility makes Cr(III) essentially immobile in most groundwaters (Mohan and Pittman Jr., 2006).

For high E_h environments, Cr(VI) is the predominant form of aqueous chromium and is present as CrO_4^{2-} and $HCrO_4^{2-}$, depending on pH (Richard and Bourg, 1991; Stanin and Pirnie, 2005; Mohan and Pittman Jr., 2006). Unlike Cr(III), Cr(VI) only exists as an oxide and not as a free ion (Stanin and Pirnie, 2005). As a result, Cr(VI) speciation depends significantly on pH and Cr concentration. $HCrO_4^{2-}$ only

exists at pH 1.0 to 6.0, and CrO_4^{2-} at pH greater than 6.0. If the Cr concentration is higher than 1000 mg/L, $Cr_2O_7^{2-}$ is the predominant form of Cr(VI) (Mohan and Pittman Jr., 2006).

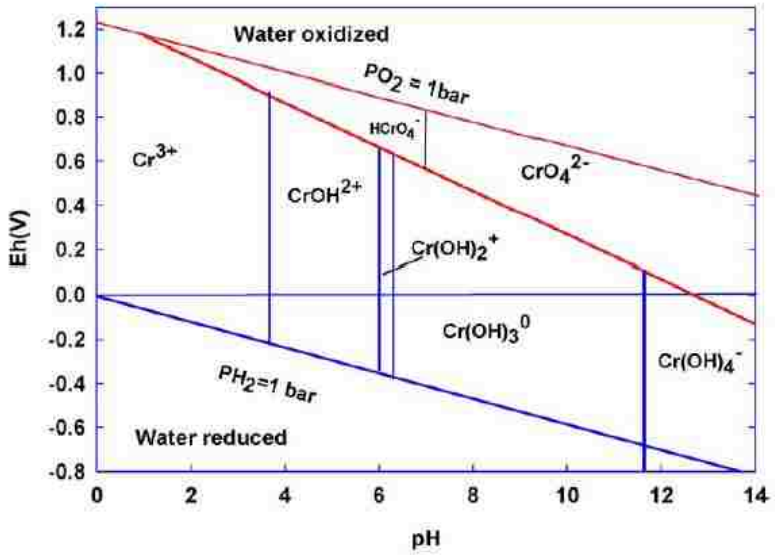

Figure 2-2: E_h-pH diagram for chromium. (Source: Mohan and Pittman Jr., 2006)

2.2.2 – Chromium in the Environment

Elevated concentrations of Cr in soils and water can occur naturally as a result of the alteration and weathering of ultramafic rocks. Several locations across the globe, including Zimbabwe, the western United States, Mexico, Tuscany in Italy, and Brazil, all have numerous exposures of ultramafic rocks that, when eroded, can potentially transport Cr into nearby soils and groundwater (Robles-Camacho and Armienta, 2000; Mills et al., 2011; Lelli et al., 2014). The U.S. EPA reports that natural Cr concentrations in soils and waters in the United States can range anywhere from 0.006 to 0.01 mg/L in groundwater, and in the range of 0.005 to 0.021 mg/L in surface water (U.S. EPA, 2002; Pakzadeh and Batista, 2011).

For the most part, chromium in water is anthropogenic in nature – generally the result of improper waste disposal and storage by various industries such as electroplating, wood preservation, leather tanning, stainless steel production, pigments, and anti-corrosion treatment of nuclear reactors (U.S. EPA, 2002; Narayani and Shetty, 2013; Qasim, 2013). This has resulted in widespread disposal of chromium wastes into the environment, making chromium contamination of soil and water a relatively

common occurrence. Narayani and Shetty (2013) cover some of the typical chromium concentrations discovered in various effluents, which have been summarized into Table 2-1 below.

TABLE 2-1: TYPICAL CHROMIUM CONCENTRATIONS IN VARIOUS TYPES OF WATERS

Soil/Water Type	*Typical Concentrations (mg/L)*
Domestic Wastewaters	150-2000
Industrial Waters	140-4800
Contaminated Soil	2650-8800
Electroplating Industry Effluent	140-49400
Leather Tanning Effluent	100-45000
Steel Production Effluent	> 40000
Pigment Production	90-7000
Mining and Ore Processing Residue	2500-4000

Of special interest is the consideration of Superfund sites – Hawley and Jacobs R. G. (2005) report that 306 Superfund sites list chromium as a major contaminant. Of these, two of the more well-known chromium-contaminated sites are the Hanford River site in Washington State and the Savannah River Site in South Carolina, where chromium contamination is localized to vadose zone soils and waters and is associated with the use of potassium dichromate as a nuclear reactor coolant (Ford, 2006). The range of Cr(VI) present in the soils at these sites – along with other sites of interest – is listed on Table 2-2. Unless noted otherwise, all site totals are specifically given for Cr(VI).

TABLE 2-2: CHROMIUM CONCENTRATIONS AT SELECTED SITES WHERE CR(VI) IS A MAJOR VADOSE ZONE CONTAMINANT

Site	Site Location	Contaminant Type	Concentration	Units	Source
Hanford Site	Columbia River, SE Washington State	Cr(VI) – Groundwater	20-24000	µg/L	CH2M-HILL (2015)
		Cr(VI) – Soil	10-40	mg/kg	Truex et al. (2012)
Savannah River Site	25 miles SE of Augusta, Georgia	Cr(VI) – Groundwater	50-2700	µg/L	Cummins et al. (1990)
		Cr(VI) – Soil	38-175	mg/kg	Seaman et al. (2001)
Los Alamos National Laboratory	Los Alamos, New Mexico	Cr(VI) – Groundwater	2.4-1000	µg/L	Heikoop et. al. (2014)
		Total Cr – Soil	5.6-3740	mg/kg	
Nevada Environmental Response Trust (NERT) Site	Henderson, Nevada	Cr(VI) – Groundwater	10-25	mg/L	Tetra Tech, Inc. (2018)
		Cr(VI) – Soil	≤ 22	mg/kg	

2.2.3 – Fate and Transport of Cr(VI) in Groundwater

Cr mobility in groundwater is highly dependent on both solubility and sorptivity. Both are, in turn, a function of the groundwater chemistry and the composition of the soil and aquifer material in contact with the Cr-contaminated water (Puls et al., 1994). Cr(III) is largely immobile in groundwater because as it precipitates out of groundwater, Cr(III) forms compounds that have low solubility in neutral and alkaline pH (Loyaux-Lawniczak et al., 2001; Tokunaga et al., 2001; Barrera-Diaz et al., 2012). Furthermore, as the pH increases, Cr(III) is adsorbed by a number of materials, including the soil fabric, clay minerals, sand, and Fe and Mn oxides. Thus, Cr(III) concentrations in water tend to be low in general, while Cr(III) concentrations in the soil itself are relatively high (Richard and Bourg, 1991; Tokunaga et al., 2001).

Cr(VI) in groundwater is significantly more mobile than Cr(III) due to a lack of solubility constraints and low sorption of Cr(VI) in neutral and alkaline waters (Stanin and Pirnie, 2005). However, Cr(VI) can be reduced by a number of subsurface materials. Tokunaga et al. (2001) demonstrated that Cr(VI) could be locally reduced at the mm scale in the presence of organic carbon and Cr(VI)-reducing microbes. In general, Cr(VI) reduction can occur in the presence of specific redox couples in soils – H_2O/O_2 (aq), Mn^{2+}/Mn^{4+}, NO_2/NO_3, Fe^{2+}/Fe^{3+}, S^{2-}/SO_4^{2-}, and CH_4/CO_2 are the most significant (Richard and Bourg, 1991). Cr(VI) reduction is enhanced by the presence of Fe^{2+} in solution or in minerals, which include iron oxides, biotite, hematite, pyrite, chlorite, and nontronite (Richard and Bourg, 1991; Puls et al., 1994; Loyaux-Lawniczak et al., 2001). However, Cr(III) can be reoxidized into Cr(VI) in the presence of manganese oxides through adsorption onto the active surface of MnO_2 (Eary, 1988; Richard and Bourg, 1991; Loyaux-Lawniczak et al., 2001).

Cr(VI) can also be reduced in the presence of soil organic matter, often in the form of humic and fulvic acids (Richard and Bourg, 1991; Puls et al., 1994; Loyaux-Lawniczak et al., 2001; Tokunaga et al., 2001). Organic matter can act as an electron donor for Cr(VI) reduction, and also provides a carbon source for microbial remediation of Cr(VI) under anaerobic conditions since organic matter also

decreases the level of O_2 in the soil (Losi et al., 1994). Jardine et al. (1999) showed that organic matter in particular can be used to reduce Cr(VI) in acidic soil conditions ($pH \leq 4$) even in the presence of competing hydrological and geochemical reactions, and can impede the amount of Cr(VI) mobilized into the environment. However, increasing the amount of organic matter present can also increase the pH through mineralization of the organic matter, which can adversely affect Cr(VI) reduction (Losi et al., 1994).

2.3 – Co-Contaminants

While Cr(VI) is the main focus of the research, it is usually not found by itself at contaminated sites; often, depending on the type of industrial site, it can be found in the presence of other common contaminants. Two of these are nitrate and chlorate, which will be discussed briefly in this section.

2.3.1 – Nitrate

Nitrate [NO_3^-] is a naturally occurring inorganic oxyanion that forms a key part of the nitrogen cycle, and is the stable form of nitrogen [N] in oxygenated systems (Bhatnagar and Sillanpää, 2011). In nature, NO_3^- is largely the result of soil-living bacteria converting soil ammonium [NH_4^+] into nitrite [NO_2^-] and then into NO_3^- through nitrification. NO_3^- can thus be used by plants as a nutrient through assimilation, or released back into the atmosphere as nitrogen gas through denitrification (Follett, 1995; Bhatnagar and Sillanpää, 2011).

NO_3^- is not generally dangerous by itself, being naturally present in vegetables such as spinach, leafy greens, celery, and beetroot (Adelana, 2005; Larsson et al., 2011; DellaValle et al., 2014). Several health organizations such as the World Health Organization and the European Union's Scientific Committee for Food even establish acceptable daily intake levels of 3.7 mg/kg of body weight for NO_3^- (Larsson et al., 2011). However, excessive amounts of NO_3^- in the body can lead to numerous disruptive health effects. The most serious of these is methemoglobinemia, which occurs when NO_3^- is converted into NO_2^- in the digestive tract. NO_2^- forms a stable complex with the hemoglobin in blood, which

prevents oxygen transport in the blood and results in symptoms of asphyxiation (Adelana, 2005). Infants in particular are most vulnerable to methemoglobinemia as a result of their digestive tracts being host to nitrate-reducing bacteria; as a result, methemoglobinemia is also commonly known as “blue-baby syndrome” (Adelana, 2005; Bhatnagar and Sillanpää, 2011). NO_2^- is also suspected to cause cancer in humans as a result of chemical reactions with amines, amides and other compounds in the digestive tract that form *N*-nitroso compounds [NOCs], which are known to be highly carcinogenic and are linked to gastric and colon cancer (Adelana, 2005; DellaValle et al., 2014). Other deleterious effects to the human body include increased infant mortality, cardiovascular issues, birth defects, abdominal problems, infectious disease outbreaks, and diabetes (Adelana, 2005; Bhatnagar and Sillanpää, 2011; Ebrahimi and Roberts, 2013; DellaValle et al., 2014).

Because of the numerous health effects associated with excess NO_3^- ingestion, the U.S. EPA set a MCL of 10 mg/L as N for NO_3^- , which is also the same standards used in Canada. The international community, including the World Health Organization and the European Union, have similarly set their standards as 11.4 mg/L as N (50 mg/L NO_3^-) (Adelana, 2005; Ebrahimi and Roberts, 2013; U. S. EPA, 2018). NO_3^- discharges into waters are regulated by both the 1974 Safe Drinking Water Act and the Phase II Chemical Contaminant Rules, the latter of which became effective in 1992 and not only lists the maximum allowable concentration of NO_3^- in waters, but also recommends technologies for its treatment (Inorganic contaminant MCLs and BATs (includes arsenic, nitrate, nitrite and asbestos), 1992).

Due to its negative ionic charge, NO_3^- is repelled by negatively-charged clay mineral surfaces in soil; as a result, NO_3^- does not readily bind to soil particles, making it highly susceptible to leaching (Follett, 1995; Bhatnagar and Sillanpää, 2011). Not only is it highly soluble in water, it's also highly mobile and easily displaced. NO_3^- is thus the primary form of nitrogen leached into groundwater supplies, making it a widespread contaminant in the global water supply (Follett, 1995; Almasri, 2007; Bhatnagar and Sillanpää, 2011). Leaching of NO_3^- into the groundwater supply is the primary means of NO_3^- groundwater contamination; the factors that influence its transport through the groundwater table largely

vary across locations mainly due to soil heterogeneity. Almasri (2007) reports that these factors include land usage, on-ground nitrogen loading, groundwater conditions, soil characteristics and soil-nitrogen dynamics, and water table depth.

NO_3^- occurs in soils and water as a part of the nitrogen cycle. NO_3^- can accumulate in perched water tables as a result of subsurface seepage, and can also be found in unweathered soils beneath the root zone of native vegetation, especially in semi-arid regions (Power and Schepers, 1989). In general, however, NO_3^- occurs in the environment anthropogenically as a result of contamination from various industries, including agricultural runoff, improper wastewater discharge, food processing and meat packing, and atmospheric deposition from nitrogen oxide emissions (Power and Schepers, 1989; Fanning, 2000; Almasri, 2007; Ebrahimi and Roberts, 2013).

Though agricultural processes are the main source of NO_3^- pollution in soil and groundwater, other industrial wastes have contributed to the global problem; Table 2-3 lists the typical NO_3^- concentrations of industrial discharges that utilize it in their practices.

TABLE 2-3: TYPICAL NITRATE CONCENTRATIONS IN VARIOUS TYPES OF WATERS

Water Type	*Typical Concentrations (mg/L)*	*Reported As*	*Source*
Natural Streams	< 1	NO_3^-	U.S. EPA (2012)
Wastewater Discharge	30		
Groundwater	0 - 800		Wick, Heumesser and Schmid (2012)
Explosives Manufacturing	1500 - 12500	NO_3^- — N	Bilanovic et al. (1999)
Food Processing	145 - 2700		
Fertilizers	200 - 6000		
Pharmaceuticals	145 - 475		
Oil and Gas Refining	24 - 865		

2.3.2 – Chlorate

Chlorate [ClO_3^-] is an inorganic oxyanion of chlorine that is known to be a powerful oxidizer ($E^0 = +0.62$ V). "Chlorate" can also refer to the collective group of chemical compounds that contain this

anion, such as the salts that form from chloric acid (U.S. EPA, 2016; Mastrocicco et al., 2017). ClO_3^- is generally not common in nature, though recent work by Rao et al. (2010) suggests that natural ClO_3^- deposits can occur in arid regions alongside perchlorate [ClO_4^-] as a result of atmospheric production and deposition into dry soils.

Alfredo et al. (2015) summarizes existing research with regards to current knowledge about ClO_3^- and its deleterious effects. ClO_3^- is toxic if ingested or inhaled; high concentrations of ClO_3^- in the bloodstream can rupture the blood cells, impairing the body's ability to transport oxygen. Methemoglobinemia then occurs as a result of oxidation of free hemoglobin in the bloodstream. ClO_3^- is also known to cause enlargement of the thyroid gland by decreasing iodide uptake through competitive inhibition.

At this time, there are no current federal regulations governing the presence of ClO_3^- in water, though ClO_3^- was monitored under the U.S. EPA's UCMR 3 from 2013 to 2015. The state of California currently lists ClO_3^- among its contaminants of interest, setting a notification level of 800 μg/L in 2007 (U.S. EPA, 2016). Health Canada set similar guidelines for ClO_3^- at 1 mg/L based on lifetime exposure and an 80% relative source combination from drinking water (Alfredo et al., 2015; U.S. EPA, 2016). The World Health Organization has also set limits on ClO_3^- in waters through provisional guidelines, at 0.7 mg/L (Alfredo et al., 2015; U.S. EPA, 2016).

van Ginkel, Plugge and Stroo (1995) report that the fate and transport of ClO_3^- is influenced by the presence of molecular oxygen [O_2] and NO_3^-. In particular, ClO_3^- can be reduced by microorganisms under anaerobic conditions provided that O_2 and NO_3^- levels are low, as both of these are more readily utilized by microorganisms before ClO_3^-. Outside of microbial reduction, ClO_3^- partitions into and is highly mobile in water; however, under typical environmental conditions ClO_3^- is subjected to extensive redox reactions that reduce it to chloride [Cl^-] species with lower oxidation states (U.S. EPA, 2016). ClO_3^- reduction is affected by temperature, pH, concentration, the presence of soil reductants and soil moisture, though in general ClO_3^- is stable under alkaline conditions (U.S. EPA, 2016).

ClO_3^- found in nature is generally limited to arid and semi-arid regions of the world (Rao et al., 2010; Mastrocicco et al., 2017). For the most part, ClO_3^- in the environment is the result of using chlorine dioxide and hypochlorite as a disinfectant in water treatment, which enters the water supply as a disinfection byproduct (Grant-Trusdale, 2005; Alfredo et al., 2015; U.S. EPA, 2016). Industries also known to generate ClO_3^- waste include the manufacture of bleaching agents for paper and pulp products, herbicides and defoliants for agricultural usage, and the production of explosives (Grant-Trusdale, 2005; Rao et al., 2010; Alfredo et al., 2015; U.S. EPA, 2016). ClO_3^- is also produced as a byproduct of the degradation of ClO_4^- into Cl^- by ClO_4^--reducing bacteria that use ClO_3^- as a terminal electron acceptor (Rao et al., 2010; Mastrocicco et al., 2017).

Very little information exists on ClO_3^- levels that are found in wastewaters, drinking water, and in industrial discharge. Table 2-4 collects what published data is available in the literature about ClO_3^- concentrations in various types of water and soil.

TABLE 2-4: TYPICAL CHLORATE CONCENTRATIONS IN VARIOUS TYPES OF SOILS AND WATERS

Water Type	*Typical Concentrations*	*Units*	*Source*
Untreated Water	0.01-0.081	mg/L	Bolyard (1993)
Treated Water	3.2-7	mg/L	Grant-Trusdale (2005)
Arid Region Soils	1.7-530	mg/kg	Rao et al. (2010)
Pulp Mill Discharges	10-70	mg/L	Lehtinen et al. (1988)
Agricultural Runoff	20000-40000	mg/L	Cheussard et al. (2009)

2.4 – Remediation Strategies

2.4.1 – Saturated Zone Treatment

Groundwater Cr(VI) remediation presents its own unique set of challenges. Though Cr(III) is found naturally in groundwaters in concentrations ranging from 0.0005 to 0.21 mg/L (Pakzadeh and Batista, 2011), natural materials in the subsurface can react with Cr(III) and oxidize it to Cr(VI), such as manganese dioxides. Other materials like carbonaceous materials and carbonates can in turn react with

Cr(VI), reducing it back to its less mobile form as a result of prevailing alkaline conditions (Eary, 1988; Thomasser and Rouse, 1999). The amount of Cr(III) and Cr(VI) in groundwaters can thus be affected by the surrounding mineralogy of the site and by water chemistry.

Thomasser and Rouse (1999) report at least three strategies for Cr(VI) removal from groundwater – soil excavation, pump-and-treat methods, and geochemical fixation using zero-valent iron [ZVI]. Soil excavation consists of removal of the contaminated soil, which is then either sent to a landfill or treated and replaced (Palmer and Wittbrodt, 1991). Though effective in groundwater treatment, soil excavation doesn't address the presence of Cr(VI) adsorbed onto soil particles and Cr(VI) already present within the soil at the time of deposition, and is unnecessary in cases where the Cr(VI) source is immobile or of limited solubility (Thomasser and Rouse, 1999). Furthermore, vertical flow in the soils can often carry Cr(VI) into the deeper soils while leaving surface soils untouched, thus forcing removal of uncontaminated soils to reach the contamination source (Palmer and Wittbrodt, 1991). In general, soil excavation is considered the least desirable option for treatment because of the costs and risk of exposure associated with landfill transport, and has been increasingly seen as simply moving the problem from one location to another (Palmer and Wittbrodt, 1991).

Pumping and treatment of groundwaters is effective, particularly for permeable aquifers and soils, and can be combined with reductants and reinjected throughout for in-situ reduction of residual Cr(VI) remaining in soil (Fruchter, 2002). However, this procedure doesn't work for contamination sources in low-permeability zones or in mobile sources. Furthermore, Cr(VI) can also be left behind in the aquifer during the drawdown process (Thomasser and Rouse, 1999; Fruchter, 2002).

More recently, ZVI has gained interest as a Cr(VI)-reducing agent in groundwaters during the last two decades – Gheju (2011) discusses ZVI usage in Cr(VI) removal in great depth in their research, especially with respect to types of ZVI available, its mechanisms and kinetics of Cr(VI) removal, and the various parameters that influence its reduction capacity. In-situ methods of Cr(VI) treatment that use ZVI include the use of reactive barriers in boreholes, where reductive solution is placed in barriers

downgradient of the Cr(VI) plume (Fruchter, 2002). Another method includes the use of direct "push grid" injection of reductants in a grid pattern to induce hydrofracturing of low-permeability soils, diffusing and reducing Cr(VI) to Cr(III) (Thomasser and Rouse, 1999). The use of ZVI barriers is generally limited to depths of 10 meters below the surface or less; at greater depths barrier placement becomes more difficult, and due to high pH formation in such barriers, minerals can precipitate out and plug the barrier, decreasing its Cr(VI)-reducing capacity (Fruchter, 2002).

2.4.2 – Vadose Zone Treatment

Treatment of vadose zone waters and soils is less known and less researched than saturated zone waters, but in general treatment tends to be more complex. Dresel et al. (2011), Shen et al. (2011) and Zhong et al. (2009) describes these unique conditions, which are also shown in Figure 2-3.

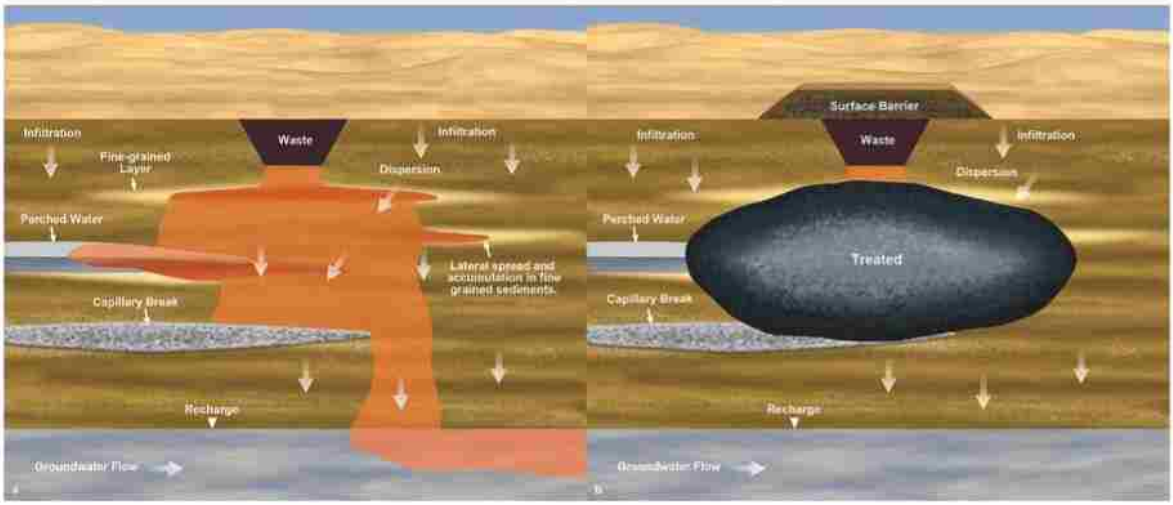

Figure 2-3: Conceptual diagram of fluid flow through the vadose zone (a) following waste discharge and moisture redistribution and (b) following remediation. (Source: Dresel et al., 2011)

Water movement through the vadose zone is typically a function of moisture distribution across the soil, surface infiltration, and hydraulic conductivity (Dresel et al., 2011). In general, vadose zone flow is controlled more by preferential gravitational flow through the soil and through capillary forces. Under unsaturated conditions, water is held in tension within the capillary zone, and flow is controlled through pressure gradients (Dresel et al., 2011). Coarse sediments within this zone allow water to flow laterally

into finer-grained sediments, where they accumulate until the soil becomes saturated, at which point water flows vertically through the coarse sediments once pressure is overcome (Dresel et al., 2011).

Due to these flow conditions and their effects on contaminant transport into the underlying saturated zone, vadose zone contaminants are often treated as ongoing sources of contamination. Progressive contamination of the underlying groundwater as a result of precipitation and runoff can result (Hanson et al., 1993; Dresel et al., 2011). Gravitational flow and preferences towards high-permeability pathways lower the overall lateral transport of contaminant reactants through the vadose zone, bypassing low permeability zones containing the contaminations. Furthermore, flushing solutions can easily mobilize contaminants, which can produce a Cr(VI) moving front that can contaminate the underlying aquifer (Hanson et al., 1993; Zhong et al., 2009; Shen et al., 2011). However, unsaturated flow through the vadose zone is often slow and incremental, increasing flow times of fluid through the sediments before it reaches the saturated zone – all positive aspects for the remediation of vadose zone soils. As a result, most vadose zone remediation strategies focus on both limiting contaminant transport into the groundwater and in-situ treatment of the contaminated water and soil to maintain groundwater and surface water safety standards (Dresel et al., 2011).

Two proposed remediation strategies for Cr(VI)-contaminated vadose zone waters are direct injection of reductants into the vadose zone, and microbial reduction of Cr(VI) using indigenous bacterial communities. Calcium polysulfide [CaS_5] is the most widely-used reducing agent in vadose zone treatment, and reacts with Fe(III) in sediments to form a reactive barrier enhancing Cr(VI) reduction and immobilization (Zhong et al., 2009; Dresel et al., 2011). Because of preferential fluid flow, foam emulsions have been proposed as a means to deliver the reductant to the vadose zone. Foam is a non-Newtonian liquid in nature, which can be spread more uniformly across heterogeneous soil systems, transporting reductant in the lateral direction and increasing treatment efficacy. Foam flow is dominated by pressure gradients, which allows for better control over reductant injection, reducing the chances of contamination escaping into the groundwater (Zhong et al., 2009; Shen et al., 2011). Zhong et al. (2009)

demonstrated that by adding CaS_5 as foam into vadose zone waters, the total Cr(VI) present was reduced to less than 10%, compared to at least 77% Cr(VI) mobilization when aqueous Cr(VI) solution was injected into the system.

In-situ bioremediation of vadose zone systems is far simpler and less expensive than conventional methods such as pumping and treating. Like direct injection, bioremediation employs the use of a permeable reactive barrier for contaminant removal and reduction. However, substrate is directly injected into the soil as a carbon source to encourage microbial degradation of compounds. Examples of substrates that can be added into the soil include vegetable oil, Tween 80, molasses, and NO_3^- (Oliver et al., 2003; Hunter, 2005). Performing batch experiments to simulate vadose zone conditions and using both molasses as an organic carbon source and nitrate as substrates, Oliver et al. demonstrated that with significantly high concentrations of NO_3^- and molasses, up to 87% Cr(VI) removal was observed in vadose zone soils. Reduction of Cr(VI) under these conditions was primarily limited to the coarse-grained soils in comparison to fine-grained soils, where it was hypothesized that the longer residence time for treatment would result in oxygen depletion and anoxic conditions. It has been suggested by Han et. al. (2000) that Cr(VI) reduction can be favorable under anoxic conditions, though the presence of NO_3^- plus the preferential flow through the vadose zone could possibly inhibit Cr(VI) bioremediation since one of the degradation byproducts of NO_3^-, NO_2^-, has been known to inhibit Cr(VI) reduction (Oliver et al., 2003).

2.5 – Cr(VI) Remediation and Removal Technologies

2.5.1 – Soil Flushing

Soil flushing is the process through which Cr is removed from unsaturated zone soils using a solvent – which is usually water-based because of Cr(VI) solubility. This solvent percolates through the contaminated soil, where it is either recovered and treated for reuse – or, if the solvent is water by itself, discharges into the underlying water table, raising it and allowing for conventional ex-situ treatment

(Hanson et al., 1993; National Risk Management Research Laboratory et al., 2000; Hawley et al., 2005; Dresel et al., 2011).

The effectiveness of soil flushing is largely site-specific, and is dependent on soil hydraulic properties that influence contaminant collection with and recovery of the flushing solution (National Risk Management Research Laboratory et al., 2000). Other factors that affect its feasibility include the depth to water and both the initial and target Cr(VI) concentrations (Hawley et al., 2005). Another factor to consider is the potential for lateral spreading and bypass of the contaminated zone through preferential flow paths (Dresel et al., 2011). Soil flushing can potentially accelerate Cr(VI) removal through rapid mobilization as a result of the provided hydraulic push, which raises the hydraulic gradient (National Risk Management Research Laboratory et al., 2000). However, this rapid mobilization of Cr(VI) can potentially produce a front that can spread the contaminant both laterally and vertically (Hanson et al., 1993; National Risk Management Research Laboratory et al., 2000). Furthermore, this process of treatment creates a liquid waste stream that requires treatment, which can increase treatment costs (Hanson et al., 1993).

One well-documented case study of chromium removal through soil flushing is that of the United Chrome Products Superfund site in Corvallis, Oregon (Jacobs and Rouse, 2005). A former chromium-plating facility, it discharged an unknown amount of Cr-plating wastewater into a dry well near the site from 1960 to 1977, significantly contaminating the local soil and groundwater. At its worst, total chromium levels were as high as 60,000 mg/kg for soil and up to 19,000 mg/L for the groundwater (National Risk Management Research Laboratory, 2000). Implementation of an *in-situ* pumping strategy helped to contain the Cr(VI) plume, decreasing Cr(VI) concentrations from 5000 to 50 mg/L during the first two and a half years of operation. (National Risk Management Research Laboratory, 2000; Jacobs and Rouse, 2005). As of December 2004, groundwater extraction operations have been ceased, and groundwater monitoring has been ongoing since, to identify any changes in the current concentrations (Opalski, 2011).

2.5.2 – Physicochemical Remediation of Chromium in Waters

Physicochemical methods are the most conventional methods used in the removal of Cr(VI) from waters. The most common is oxidation-reduction and precipitation, in which a reducing agent is added to waters, facilitating the reduction process from Cr(VI) to Cr(III). The Cr(III) is then precipitated out of waters through an increase in pH, either from the redox reaction or through addition of NaOH (Duncan et al., 2007; Barrera-Díaz et al., 2012). The most common reducing agents used include ferrous sulfate [$FeSO_4$], sodium metabisulfite [$Na_2S_2O_5$], and ZVI (Mitra et al., 2011; Celajes and Hilario, 2015). $FeSO_4$ is the most widely used reducing agent in Cr(VI)-contaminated waters, and has been used in its heptahydrate and monohydrate forms to reduce Cr(VI) in Portland cement as well as to indirectly treat workers with contact dermatitis from Cr(VI) (Eary, 1988; Guertin, Jacobs, and Avakian, 2005; Chou et al., 2008; Sharma and Sharma, 2015).

CaS_5, another chromium reducing agent, has been successfully used in a number of applications to treat chromium-contaminated waters, including groundwater (Ford et al., 2006), wastewaters (Yahikozawa et al., 1978), chromite ore processing residue (Graham et al., 2006), and ion-exchange brines (Pakzadeh and Batista, 2011). CaS_5 has also seen some application in Cr(VI) removal from the Hanford site (Ford et al., 2006). The reduction reactions between Cr(VI) and the various reducing agents are dependent on a number of factors, including pH and Cr(VI) concentration. A summary of these reactions can be seen in Table 2-5.

TABLE 2-5: CR(VI) REDUCTION REACTIONS FOR VARIOUS REDUCING AGENTS

Reducing Agent	*Chemical Reaction*	*Notes*	*Source*
$FeSO_4$	$14H^+ + 6Fe^{2+} + Cr_2O_7{}^{2-} \rightarrow 6Fe^{3+} + 2Cr^{3+} + 7H_2O$	Acidic conditions	Jacobs and Rouse (2005)
	$3Fe^{2+} + CrO_4{}^{2-} + 4H_2O \rightarrow 3Fe^{3+} + Cr^{3+} + 8OH^-$	Neutral / alkaline conditions	
$Na_2S_2O_5$	$Na_2S_2O_5 + H_2O \rightarrow 2NaHSO_3$ $3NaHSO_3 + 2H_2CrO_4 + 3H_2SO_4 \rightarrow Cr_2(SO_4)_3 + 5H_2O + 3NaHSO_4$	Acidic conditions; $Na_2S_2O_5$ used to produce Cr(VI)-reducing $NaHSO_3$	Duncan et al. (2007)
ZVI	$2Fe^0 + Cr_2O_{7(aq)}{}^{2-} + 14H^+ \rightarrow 2Fe_{(aq)}{}^{3+} + 2Cr_{(aq)}{}^{3+} + 7H_2O$	Acidic conditions	Mitra et al. (2011)
CaS_5	$2CrO_4{}^{2-} + 3CaS_5 + 10H^+ \rightarrow 2Cr(OH)_3(s) + 15S(s) + 3Ca^{2+} + H_2O$	All conditions	Pakzadeh and Batista (2011)

Because Cr(III) can re-oxidize into Cr(VI) under highly alkaline conditions, physical processes such as sorption and ion-exchange can also be employed to remove Cr(VI) from water and bypass chemical reduction completely (Eary, 1988; Han et al., 2000).

2.5.3 – Biological Remediation of Chromium in Waters

Narayani and Shetty (2013) report that conventional techniques are often economically expensive and offer numerous disadvantages such as incomplete metal removal, high energy costs, and toxic sludge generation. Furthermore, many of the current chromium treatment strategies are also limited by the initial chromium concentration; most technologies are only economically viable for high to moderate levels of chromium, and not for low levels (1-100 mg/L). Cervantes et al. (2001) reports that Cr(III) is considered to be less toxic than Cr(VI) due to its inability to cross cell membranes. Cr(III) insolubility below pH 5 allows for its precipitation and removal. Thus, for these reasons, bioremediation and biodegradation have emerged as appealing and cost-effective alternatives for treatment of heavily contaminated waters (Cheung and Gu, 2007).

Most microbes in the environment are sensitive to Cr(VI); however, bacteria that are isolated from sites contaminated with Cr(VI) are reported to be highly resistant. In their review on chromium-resistant bacteria, Narayani and Shetty (2013) reports that of all the bacterial strains studied for their

tolerance of Cr(VI), gram-positive bacteria are predominant over gram-negative bacteria for their Cr(VI) resistance. Of these, the *Bacillus* genus is the most prominent of the gram-positive bacteria, while the *Pseudomonas* genus is the most prominent of the gram-negative bacteria. Bacterial Cr(VI) tolerance varies greatly, ranging from as low as 52 mg/L to 49,400 mg/L.

Cr(VI) can be used by microorganisms as an electron acceptor, becoming reduced as part of their metabolic processes – the specific mechanism for reduction is dependent on the type of environment. (Cervantes et al., 2001; Cheung and Gu, 2007). Under aerobic conditions, Cr(VI) reduction is associated with soluble chromium intermediates being used as enzymes to aid in the breakdown of NADH and NADPH (Cervantes et al., 2001). Under anaerobic conditions, Cr(VI) reduction occurs due to Cr(VI) being used as an electron acceptor, terminal or otherwise, in the respiratory electron transport chain. Cr(VI) can also be reduced anaerobically by sulfur-reducing bacteria using H_2S, and has been used by such bacteria to provide energy for growth (Cervantes et al., 2001; Cheung and Gu, 2007). Cr(VI) reduction may also occur as a result of chemical reactions with compounds such as amino acids, nucleotides, sugars, vitamins, organic acids, or glutathione. Ascorbate, FMN and FAD – riboflavin derivatives – have all been shown to reduce Cr(VI) (Cervantes et al., 2001). Jacobs and Rouse (2005) report that most biological Cr(VI) remediation strategies assume that that Cr(VI) is reduced metabolically in the presence of large amounts of electron donors, using the aerobic and anaerobic mechanisms postulated by Cervantes et al. (2001) and Cheung and Gu (2007).

2.5.3.1 – Bacterial Growth in Soils

Several factors must be accounted for in regards to effective Cr(VI) bioremediation. For wastewater treatment, these factors are well-known and include biomass density, initial Cr(VI) concentration, the carbon source, pH, temperature, redox potential, and the presence of competing oxyanions and metal cations (Chen and Hao, 1998; Narayani and Shetty, 2013) However, for bioremediation in soils, the factors affecting bacterial growth differ significantly as a result of the medium. Boopathy (2000) and Iovieno and Bååth (2008) point out several important factors that control

soil bioremediation, but the three most important are moisture content, substrate availability (nutrient content / presence of organic matter) and soil temperature.

The content of organic material is a key control in bacterial growth – surface soils typically have high organic matter due to regular input from plants, typically associated with high microbial numbers and a great diversity of microbial populations. Deeper soils like subsurface soils and groundwater decrease in organic content present, thus lowering microbial numbers and population diversity. As a result, nutrients or the lack thereof can limit bacterial growth significantly. Demoling et al. (2007) demonstrated this in a study where they studied 28 different soils and measured the bacterial growth rates after 48 hours using thymidine and leucine incorporation techniques. The study was performed under the assumption that carbon was the most common limiting nutrient in bacterial growth – and their results confirmed their assumption. In particular, bacterial growth in soils with low organic matter content could be significantly enhanced with additional carbon input. Losi et. al. (1994) also reports that for aerobic Cr(VI) reduction of Cr(VI), increasing the organic matter in the soil created the most optimal conditions for Cr(VI) reduction since organic matter acts as an electron donor; under aerobic, field-moist conditions, 96% of the Cr(VI) added to their organic-rich soil was reduced.

Soil moisture content is also an important factor in bacterial growth – microbial activity is typically low in dry soils, and generally increases with an increase in moisture content (Howard and Howard, 1993; Iovieno and Baath, 2008). Microbial respiration rates are often used as an estimation of the bacterial growth rate – Cook and Orchard (1983) determined that the respiration rate has a linear relationship with the soil moisture content. In general, for adequate microbial growth, the moisture content has to be over a minimum of 5%; below this moisture content, the microbial decay rate increases. Depending on the soil type, a higher moisture content may be needed to sustain microbial growth. For example, Howard and Howard (1993) give ranges of optimal moisture contents for microbial growth ranging from 30% for stagnopozollonic soil to 67% for humic alluvial clayey soils. As a general rule, as the moisture content increases, microbial respiration associated with growth increases – however, Iovieno

and Baath (2008) make a point to note that the respiration rate is a poor estimation of the microbial growth rate, demonstrated through their drying and rewetting experiments on bacteria. Though the respiration rate immediately increased as a result of the rewetting, the growth rates only recovered gradually and linearly – even with additional glucose – because of dormant bacteria becoming active following the rewetting phase.

Temperature is one factor that remains important in Cr(VI) treatment of both soil and water, and has been well-documented for both water and soils. At low temperatures, membrane fluidity decreases, preventing substrates from entering the cell and thus increasing the incubation time needed for bacterial growth (Demoling et al., 2007; Narayani and Shetty, 2013). At higher temperatures, irreversible thermal denaturation occurs, which affects Cr(VI) reductase function – and by extension, the Cr(VI) reduction process itself (Narayani and Shetty, 2013). The optimal temperature for Cr(VI) reduction depends mainly on species, but in general 37°C is considered the optimal temperature for most bacteria (Narayani and Shetty, 2013). In soils, the relationship between soil temperature and bacterial growth rates can be described using a square root model, in which the square root of the bacterial growth rate is linear to the temperature below the optimal growth temperature (Ratkowsky et al, 1982). In general, soil temperature changes with depth – at the surface, temperature varies the most due to exposure to solar radiation, and equilibrates into a more constant temperature with depth (Brady and Weil, 2010). Based on the work of both Rinnan et al. (2009) and van Gestel et al. (2013), soil temperature sensitivity for bacterial growth fluctuates globally. The minimum temperature for bacterial growth ranges from -15°C to 0°C, while the optimal temperature ranges from 25°C to 45°C, providing a much larger range for soil in comparison to the 20°C – 30°C temperature range for bacterial growth in water (Narayani and Shetty, 2013).

2.5.4 – Co-Contaminant Remediation

Multiple technologies exist for the removal of NO_3^-; including ion exchange, reverse osmosis, ZVI, zero-valent magnesium, activated carbon adsorption, electrodialysis, and biological treatment (Bhatnagar and Sillanpää, 2011; Ebrahimi and Roberts, 2013). On the other hand, ClO_3^- treatment

strategies generally focus on either minimizing ClO_3^- generation in treated water due to the lack of full-scale technologies that can be used to remove it, or encouraging conditions under which it can reduce into Cl^- (Grant-Trusdale, 2005; Alfredo et al., 2015; U.S. EPA, 2016; Mastrocicco et al., 2017).

With respect to simultaneous removal of Cr(VI), NO_3^- and ClO_3^- together, recent literature discusses the potential for both NO_3^- and ClO_3^- being reduced in the presence of ZVI at near-neutral pH (Westerhoff, 2003; Su and Puls, 2004; Suzuki et al., 2012). NO_3^- is directly reduced by ZVI through electron transfer as a result of ZVI corrosion instead of being reduced indirectly through hydrogen [H_2] gas, thus producing ammonia [NH_3] in the form of the ammonium ion [NH_4^+] (Suzuki et al., 2012). According to Su and Puls (2004), NO_3^- reduction follows the spontaneous reaction below:

$$NO_3^- + 4Fe^0 + 10H^+ \rightleftharpoons NH_4^+ + 4Fe^{2+} + 3H_2O \quad (1)$$

As Equation 1 illustrates, NO_3^- reduction is the most favorable under acidic conditions. NH_4^+ is also shown to occur in nearly equal amounts as NO_3^- in the final aqueous solution, though some studies also report NO_2^- occurring in solution as an intermediate species (Su and Puls, 2004; Suzuki et al., 2012). Equation 1 also depicts NO_3^- reduction as a highly corrosive process; high-valence oxides can form on and remain stable on the ZVI surface, forming a film that prevents further reactions along the ZVI surface in a process known as passivation (Luo et al., 2010). This film is composed of hematite [Fe_2O_3], goethite [FeOOH], and other oxyhydroxide mineral phases, and can inhibit reduction mechanisms via reducing surface contact between the ZVI and the contaminants of concern (Luo et al., 2010; Chen et al., 2013). Passivation of ZVI by NO_3^- is thus one of the major concerns in the long-term stability of in-situ ZVI treatment, though it can potentially be remedied by providing an electron source via electrically-induced reduction to rejuvenate passivated ZVI (Luo et al., 2010).

Similar to NO_3^-, ClO_3^- is also directly reduced electrochemically to Cl^- in the presence of ZVI (Westerhoff, 2003). As ZVI treatment of NO_3^- and ClO_3^- produces other byproducts left behind in the treated water, however, further research needs to address how these and other reaction byproducts will be

treated before full implementation into water treatment procedures (Westerhoff, 2003). This is especially true if there are other contaminants in the water that can sorb onto the ZVI, forming complexes with the iron oxides and decreasing NO_3^- and ClO_3^- reduction (Su and Puls, 2004).

It is also possible to remove both NO_3^- and ClO_3^- biologically; several papers already exist in which simultaneous reduction of both NO_3^- and ClO_4^- have been investigated (Logan and Lapoint, 2002; Ucar et al., 2017). Furthermore, NO_3^- and ClO_3^- bioremediation are already natural processes — NO_3^- occurs naturally as a result of the oxidation of NH_4^+ by bacteria and is part of the nitrogen cycle (Follett, 1995), while ClO_3^- is also readily utilized by bacteria during ClO_4^- degradation, acting as a terminal electron acceptor (Rao et al., 2010; Mastrocicco et al., 2017). In soils where Cr(VI), NO_3^- and ClO_3^- are all present, based on standard reduction potentials Cr(VI) is expected to be reduced first (E_h = 1.232 V), followed by NO_3^- (E_h = 0.934 V), with ClO_3^- being the last to be completely reduced (E_h = 0.62 V) (Vanýsek, 2011).

Denitrification processes for the removal of NO_3^- are already commonplace in the treatment of wastewater, and soil denitrification is a major part of the earth's nitrogen cycle, on top of being a major issue in agricultural management of soils and livestock (Follett, 1995). With respect to NO_3^- reduction in soil, the biggest controlling factor is soil moisture. At higher soil moisture contents (which correspond to > 60% of soil pore space being filled with water), NO_3^- undergoes more complete denitrification and degrades into nitrous oxide [N_2O], and eventually into nitrogen gas [N_2] itself (Bouwman, 1998).

Biological soil denitrification was utilized in one study involving a NO_3^--contaminated site in Arizona, where the addition of moisture through irrigation and direct injection of carbon source (ethanol) helped to lower NO_3^- levels in the source area by stimulating the native microbial community to reduce NO_3^- (Jordan et al., 2007). In another study, sequential heterotrophic and autotrophic bioremediation were utilized to remove NO_3^-; groundwater with NO_3^- levels as high as 83.22 mg/L was treated to 19 mg/L in the heterotrophic portion of the treatment system, and almost completely removed in the autotrophic party

of the system. This study also demonstrated the removal of ClO_4^- using this same system, with reduction levels hitting 15 mg/L · d (Ucar et al., 2017).

Though very little research has been published with respect to ClO_3^- bioremediation, it is implied to be possible due to its utilization by perchlorate-reducing bacteria in removing ClO_4^- from soils and waters. Mastrocicco et al. (2017) suggests in his research about the temporal variations of ClO_3^- levels at the Po River floodplain in Italy that ClO_3^- cycles between appearances and disappearances as a result of biological activity related to the reduction of ClO_3^- by perchlorate-reducing bacteria. van Ginkel, Plugge and Stroo (1995) further explains that ClO_3^- biodegradation often occurs under anaerobic conditions, and that ClO_3^- degradation by bacteria is inhibited in the presence of O_2 and NO_3^- since both are more preferable electron acceptors thermodynamically compared to ClO_3^-, which was confirmed in the results of Mastrocicco et al.'s and Ucar et al.'s studies.

The potential for contaminant removal with a combination of bioremediation techniques and ZVI reduction was also explored in one recent paper by Zhang et al. (2019), where NO_3^- was biologically reduced in the presence of ZVI under anoxic conditions. In this study, the combination of ZVI and biological reduction methods resulted in complete removal of NO_3^- within 80 hours, compared to 10% and 82% reduction in samples with ZVI-only treatment and biological treatment, respectively. Under these conditions, pH, initial NO_3^- concentration and ZVI dosage affected NO_3^- removal; optimal conditions for NO_3^- removal were achieved at near-neutral pH, low initial NO_3^- concentrations (< 25 mg/L), and large ZVI doses (> 0.2 g/L). Furthermore, it was observed that bacterial growth was enhanced by ZVI through providing at least four different electron donors which could have further enhanced biological denitrification: electrons from the ZVI itself, H_2, Fe^{2+} ions released from the ZVI, and biogenic acetate.

CHAPTER 3

METHODOLOGY

For this thesis, two types of experiments were performed to investigate the feasibility of remediating vadose zone soils contaminated with Cr(VI) and common co-contaminant oxyanions NO_3^- and ClO_3^-. In the first experiment set, two organic electron donor/carbon sources, emulsified oil and molasses, were compared for their efficiency in biological contaminant degradation under anaerobic conditions. The second experiment set analyzed contaminant removal using ZVI, both by itself, and in combination with electron donor/carbon sources.

Contaminant reduction using ZVI is an abiotic process. In cases where ZVI is combined with microbial reduction for bio-augmented contaminant removal [bio-ZVI] , two scenarios can occur. The first is that ZVI reduction produces H_2, which microbes can utilize as an electron donor for anaerobic degradation (Gheju, 2011). In the second scenario, abiotic reduction with ZVI occurs with contaminants fast enough that microbial participation in contaminant removal is minimal at best (Gheju, 2011).

The following nomenclature was thus developed to distinguish the experiments performed for this thesis: *biotic reduction* refers to microbial degradation using organic electron donors/carbon sources, *abiotic reduction* refers to contaminant removal with ZVI by itself, and *bio-ZVI reduction* refers to contaminant removal using ZVI and microbial degradation with organic electron donor/carbon source. Furthermore, *anaerobic experiments* refer to the first set of biotic reduction experiments with emulsified oil and molasses performed under maintained anaerobic conditions, and *ZVI remediation experiments* refer to the second suite of biotic, abiotic and bio-ZVI experiments performed to analyze contaminant removal using ZVI.

3.1 – Soil Characterization

The contaminated soils used for the experiment, labeled soil A and soil B, were collected from a heavily contaminated site in the southwestern U.S. from between 90 and 110 feet below the ground surface. Prior to experimental testing, contaminant levels, pH and the gravimetric moisture contents (soil moisture) of both soils were determined. As depicted in Table 3-1, these soils were not only heavily contaminated with Cr(VI), NO_3^-, ClO_3^-, and ClO_4^-, but also had higher moisture contents than those of typical vadose zone soils. Thus, the soils were dried to the desired moisture content to be used in the experiments. Comparison with the expected ranges of bacterial tolerance reported by Narayani and Shetty (2013) [52 mg/L to 49,400 mg/L] determined that the Cr(VI) levels in the soil were not toxic to the bacterial seed utilized for this experiment.

TABLE 3-1: SOIL MOISTURE AND CONTAMINANT LEVELS IN SOIL SAMPLES

Average Values in Soil		
Soil Name	**A**	**B**
Moisture content (%)	49.9%	51.5%
Contaminant Levels (mg/kg)		
Cr(VI)	87.9	13.4
NO_3^- (as mg/kg N)	46.3	16.2
ClO_3^-	16356.0	2823.0
ClO_4^-	2093.8	1464.9

Since the soils used in the experiments largely consisted of silt and clay materials, both samples were further characterized using X-ray diffraction (XRD). These samples were prepared and sent to the University of British Columbia for XRD analysis by fellow graduate student Nicole Martin, who was working with the same soils for a different purpose. Both soil samples were prepared for XRD by grinding in a vibratory Mill McCrone (The McCrone Group, Westmont, IL) under ethanol for ten minutes. The actual analysis was performed using a D8 ADVANCE diffractometer with Bragg-Brentano geometry (Bruker Co., Billerica, MA) equipped with a Fe monochromator foil, a 0.6 mm divergence slit

at 0.3°, incident- and diffracted-beam Soller slits, and a LynxEye-XE detector. The diffractogram was operated at 35 kV and 40 mA with a takeoff angle of 6°, and data was collected from 3 to 80°2θ using CoKα radiation. Scans were processed using the TOPAS 4.2 software and further refined using Rietveld structure refinement (Bruker AXS, Kaklsruhem, Germany). Mineral composition was determined by comparing published XRD data provided by the International Center for Diffraction Data (Newtown Square, PA) and Bruker's Search-Match software.

3.2 – Electron Donors / Carbon Sources and Nutrient Amendments

As Cr(VI) and its oxyanionic co-contaminants act as electron acceptors for microorganisms, electron donors must be provided to facilitate biological reduction (Jacobs and Rouse, 2005). Biodegradation falls under two categories: *heterotrophic* degradation, where organic compounds are utilized by microorganisms as electron donors and carbon sources, or *autotrophic* degradation, where inorganic compounds like H_2 are utilized as electron donors and CO_2 is used as the carbon source.

Two carbon sources were selected for comparison in the anaerobic tests: enriched emulsified vegetable oil [EOS-Pro] (EOS Remediation, LLC; Raleigh, NC) and blackstrap molasses (Golden Barrel; Good Food, Inc., Honey Brook, PA). Both of these carbon sources were selected as they both are readily biodegraded, have been used extensively in bioremediation projects, and in the case of molasses is relatively inexpensive (Oliver et al., 2003; Hunter, 2005). Dilute solutions of both EOS-Pro and molasses were prepared by measuring 100 mL of carbon source and mixing them with 900 mL of deionized water to produce a 10× diluted solution. The properties of both diluted solutions are shown in Table 3-2.

TABLE 3-2: CARBON SOURCE PROPERTIES AND SOLUTION CONCENTRATIONS

Carbon Source	Density (g/mL)	COD (mg oxygen/L)	Dilution Factor	mL C in 1 L of solution	Concentration (mg C/L solution)
EOS-Pro	0.98	2000000	10	100	98000
Molasses	1.4	1000000	10	100	140000

In order to compare between carbon sources, the COD of both carbon sources was also measured; this provided an equivalent carbon source dose, a COD equivalent, which allows for the same amount of carbon equivalent to be added to both sets of samples. This COD measurement was also used to determine how much of both EOS-Pro and molasses would be needed to completely remove Cr(VI) and its co-contaminants from soil.

Along with carbon source, nitrogen and phosphorus were provided in the form of a 39% diammonium phosphate/urea blend. The bacterial seed used in the experiments was a sludge consortium taken from a fluidized bed reactor currently used to treat water contaminated with Cr(VI), NO_3^-, and ClO_3^- — thus making it suitable for use in both sets of experiments. Vitamin B12 was also added since it is known to stimulate anaerobic processes (Lee et al., 2012).

3.3 – Microcosm Tests: Biotic Contaminant Reduction

Microcosm tests were performed to compare how efficiently the microbes utilized EOS-Pro and molasses in removing contaminants from the contaminated soil. Soil A was used because of its higher contaminant concentration.

The contaminated soil was air-dried for eight hours until it had a soil moisture of 35%, considered a typical value of deep vadose zone soil moisture for the southwestern U.S. based on U.S.G.S. soil moisture data from the Amargosa Desert region in Nye County (Kauble et al., 2018). The soil moisture of 35% utilized for this experiment was also determined to be comparable to similar vadose zone sites where Cr(VI) was a major contaminant, such as the Hanford River site, where soil moisture ranged from 10% to 45% (U.S. Department of Energy, 2011), and the Savannah River site, where soil moisture levels ranged from 9% to 38% (Subramanian, 2007). Four total batches of soil — two weighing 750 grams, and two weighting 250 grams — were weighed and measured, with one 250-gram batch of soil being split further into two 125-gram batches. These were mixed with varying amounts of carbon source, vitamin B12, nutrient mix, and the fluidized bed reactor sludge.

Once well-mixed, the soils were molded into cylinders using zinc soil samples rings 1” in height and 2 ⅜” in diameter, compacted, carefully removed and placed into containers made of Shelby tube end caps sealed with aluminum tape to assist with establishing anaerobic conditions. Each soil ring was determined to hold roughly 125 grams of soil, thus forming sixteen total samples: of these, six with diluted EOS-Pro, and six with diluted molasses. The remaining four samples were utilized as sample blanks as a means of comparing addition of only one set of nutrients or amendments. Two samples were designated as carbon blanks, where only the bacterial seed was added; and the other two were designated biomass blanks, one dosed with EOS-Pro and the other with molasses. It is important to note that the bacterial seed utilized in this experiment contained residual ethanol from the fluidized bed reactor it was collected from.

Table 3-3 shows the specific amounts of soil and amendments mixed for each type of sample; carbon dosages were calculated in terms of the total amount of carbon source required to treat the total milligrams of contaminant [Cr(VI), NO_3^-, and ClO_3^-] found in 125 grams of saturated soil. COD equivalents were used to convert between EOS-Pro and molasses. A more detailed explanation behind these calculations for the carbon source dosage is included in Appendix A. Calculations for soil moisture are included since sample moisture was to be maintained as close to 35% as possible for the experiment duration.

TABLE 3-3: SAMPLE DESIGN MATRIX – ANAEROBIC EXPERIMENTS

Sample Type	*Anaerobic Experiment Design Matrix*				
	Carbon Blank	**Biomass Blank**		**Biotic Degradation Samples**	
Soil Weight (g)	250	125	125	750	750
Ind. Sample Weight (g)	125				
Number of Samples	2	1	1	6	6
Soil Moisture (%)	34.9%				
Water Mass (g)	87.25	43.63	43.63	261.75	261.75
Carbon Source Used	None	EOS-Pro	Molasses	EOS-Pro	Molasses
Nutrients Added					
Carbon Source (mL)	0	1	2	6	12
Sludge (mL)	1	0	0	3	3
39% Urea (mL)	2	1	1	6	6
Vitamin B12 (mL)	2	1	1	6	6
Total Liquid (mL)	5	3	4	21	27
New Liquid Mass (g)	92.25	46.63	47.63	282.75	288.75
New Soil Moisture (%)	36.9%	37.3%	38.1%	37.7%	38.5%

Figure 3-1: Molding of prepared soil samples to be placed in the anaerobic chamber.

Figure 3-2: Finished soil samples placed in labelled Shelby tube end caps. These were wrapped in aluminum tape to prevent any air from reaching the samples, effectively sealing them completely. The lines in each sample indicate how much to take for contaminant analysis.

Following sample sealing, they were placed into an anaerobic chamber prepared with Gaspak anaerobic CO_2 indicators (BD, Franklin Lakes, NJ) (Figure 3-3) and left alone to allow for the samples to incubate under anaerobic conditions. Roughly one half of each soil sample, weighing 50-60 grams, was taken every seven days to determine contaminant degradation, soil moisture and pH. After 84 days, the experiment was terminated; on the final day, the sample blanks were analyzed for contaminants, soil moisture and pH along with the regular samples to determine if any reduction had taken place.

Figure 3-3: Soil samples left in the anaerobic chamber to incubate. More samples were previously in this chamber; the time elapsed for the experiment at the time this picture was taken was at least 35 days.

3.4 – Microcosm Tests: Contaminant Reduction Using Zero-Valent Iron and Organic Electron Donors

Further microcosm tests were also performed to compare biotic reduction with EOS-Pro, abiotic reduction, and bio-ZVI reduction using EOS-Pro. Like the anaerobic experiments, soil A was used and then measured out into batches for mixing. However, in order to encourage ZVI oxidation, the soil was not air dried prior to mixing.

Five batches of soil were set aside: two 130-gram batches for carbon and biomass controls, two 650-gram batches for bio-ZVI and abiotic reduction, and one 260-gram batch for biotic reduction for

comparison. Like the anaerobic experiments, appropriate amounts of nutrient mix, carbon source and fluidized bed sludge were added to the soil batches. ZVI was also added to both the bio-ZVI and abiotic samples based on ratios of ZVI to contaminants, and the appropriate soil weight was removed to maintain the ratios. Two ratios were used: 1:1 and 10:1. Individual samples weighing 65 grams each were measured into plastic seedling pots 2 ¼" high and 2 ½" in diameter and lightly compacted with a spoon, to model ambient ground conditions. 28 total sample plots were formed: of these, four were biotic reduction with EOS-Pro sample plots, ten bio-ZVI sample plots total with five 1:1 ratio and five 10:1 ratio samples, and ten abiotic reduction sample plots with five 1:1 ratio and five 10:1 ratio samples. Like the anaerobic experiments, two carbon blank samples dosed only with bacterial seed and two biomass blanks dosed only with EOS-Pro were formed to compare potential reduction if only one set of nutrient amendments were added.

Table 3-4 shows the amounts of soil and amendments mixed for each sample type, with detailed calculations and calculation explanations for carbon source and ZVI dosage included in Appendix B. The initial ZVI dosage was calculated based on the number of moles of ZVI required to treat the contaminant of interest based on stoichiometry. For example, in Table 2-5, it can be seen that two moles of ZVI are required for every mole of Cr(VI) to be reduced. The exact number of moles of ZVI required for contaminant treatment can be seen in Appendix B. Carbon source calculations were carried out in much the same manner as those for the anaerobic experiments, with the carbon source dose based on the total milligrams of contaminants in 65 grams of soil (Appendix A).

TABLE 3-4: SAMPLE DESIGN MATRIX – ZVI REMEDIATION EXPERIMENTS

Sample Type	*ZVI Remediation Experiment Design Matrix*						
	Carbon Blank	**Biomass Blank**	**Biotic Degradation**	**Bio-ZVI Reduction**		**Abiotic Reduction**	
Soil Weight (g)	130	130	260	325	325	325	325
Ind. Sample Weight (g)	65						
Number of Samples	2	2	4	5	5	5	5
Nutrients Added							
EOS-Pro (mL)	0	1.04	2.08	2.6	2.6	—	—
Sludge (mL)	0.52	0	1.04	1.3	1.3	—	—
39% Urea (mL)	1.04	1.04	2.08	2.6	2.6	—	—
Vitamin B12 (mL)	1.04	1.04	2.08	2.6	2.6	—	—
ZVI Added Per Individual Sample (g)	—	—	—	1.38	13.77	1.38	13.77
ZVI/Contaminant Ratio	—	—	—	1	10	1	10
Total ZVI Added (g)	—	—	—	6.89	68.87	6.89	68.87
Final Soil Mass (g)	—	—	—	318.12	256.14	318.12	256.14

Figure 3-4: Soil being mixed with bacteria and nutrients for the advanced bioremediation sample preparation.

Samples were regularly monitored for soil moisture using soil moisture sensors (Jellas Corporation, Hong Kong) and periodically sprayed with water to prevent them from drying out. Microcosms were then tightly covered with aluminum foil and incubated.

Based on standard redox potentials, ClO_3^- was expected to take the longest to degrade in the presence of Cr(VI) and NO_3^-. As very few samples modeling abiotic, biotic and bio-ZVI treatment were

molded for analyzing contaminant reduction, it was decided that initially, only one set of samples would be analyzed to ensure that ClO_3^- reduction had occurred. After seven days, one half-plot each of the abiotic 1:1 and 10:1 ZVI / contaminant ratio samples were taken and analyzed to determine if ClO_3^- removal had occurred. No other samples were collected for analysis.

The biotic reduction and bio-ZVI samples were not collected due to the expectation that degradation would occur slowly; a more frequent sampling rate would have used up all of the sample microcosms before ClO_3^- degradation was observed. Following confirmation of ClO_3^- reduction, samples were left alone again to incubate until 21 total days had elapsed, at which point one half-plot each of the biotic reduction, abiotic reduction, and the bio-ZVI samples were taken in rough two-week intervals to monitor for contaminant degradation, soil moisture and pH. The experiment was terminated after 100 days of treatment; like the anaerobic experiments, sample blanks were analyzed on the final day for contaminants, soil moisture and pH along with the regular samples for potential contaminant reduction.

Figure 3-5: Two finished soil samples prepared for incubation at ambient conditions. The water visible on the pot is from spraying to prevent premature drying of the sample.

Figure 3-6: The samples for the advanced bioremediation with ZVI being left to incubate. The aluminum foil in the background was prepared to cover the samples and keep them from drying out completely during incubation.

3.5 – Soil Sample Laboratory Analysis

Soil sample analysis procedures were largely the same for both experiment sets. For the anaerobic experiments, two 20-gram soil samples were set aside for contaminant analysis while the remaining 10-20 grams were set aside to determine soil moisture using gravimetric methods. Similarly, for the ZVI remediation experiments, two 10-gram soil samples were set aside and the rest used for gravimetric measurement of soil moisture.

The samples that were set aside were subjected to multiple extractions using deionized [DI] water in a Sorvall Legend RT Centrifuge (Kendro Laboratory Products, Newtown, CT). Anaerobic experiment samples were extracted with 50 mL of DI water per 20 grams of soil, and ZVI remediation experiment samples were extracted with 40 mL of DI water per 10 grams of soil. Centrifugation for soil extraction was performed at 3500 rpm for 30 minutes at a temperature of 23°C. The aqueous solution was then filtered through 0.22 μm-nylon syringe filters to remove any remaining impurities from the centrifugation. This procedure was repeated three more times for a total of four rinsates per soil sample.

ClO_3^- is highly soluble in water and NO_3^- is easily leached into water, making extraction relatively simple (Follett, 1995; U.S. EPA, 2016), while Cr(VI) solubility is highly dependent on sorptivity and solubility (Puls et al., 1994). However, based on preliminary tests for these experiments, four rinses were considered sufficient to extract most, if not all, contaminants of interest from the soil. The soil sample rinsates were analyzed for Cr(VI), NO_3^-, ClO_3^- , and other contaminants where desired. Following extraction, the first set of extraction rinses for all samples were also analyzed for rinsate pH. For the ZVI remediation experiment samples, additional analyses for Fe and NH_3 were performed. Fe was measured in the rinsate pre-filtering, and NH_3 was measured post-filtering.

As the goal of soil flushing is to prevent contaminant mobilization from soil, the contaminant analysis focuses specifically on the leached contaminants, not those adsorbed onto the soil particles themselves.

3.6 – Analytical Methods

Cr(VI) was measured using colorimetric methods. Total Cr(VI) was measured using EPA 7196, a colorimetric method where Cr(VI) reacts with a 1,5-diphenylcarbohydrazide reagent to produce a complex with measurement wavelength of 560 nm (Hach DOC316.53.01033). This method has a detection limit of 0.01 mg/L. For NO_3^-, samples were sent to the Utah State University Analytical Laboratories (USUAL; Utah State University, North Logan, UT) for NO_3^- as N analysis using a Lachat QuikChem 8000 Series Flow Injection Analyzer System (Lachat Instruments, Hach Company, Loveland, CO). Their method detection limit is listed as 0.1 mg/L.

ClO_3^- was measured with ion chromatography. Samples were processed using a Dionex ICS-2000 RFIC-EG System with an AS50 Autosampler, and then analyzed using the Chromeleon 7 Chromatography Data System, program version 7.2.7 (Thermo Fisher Scientific, Waltham, MA). Calibration tests performed on the system itself produced a detection limit of 0.5 mg/L for ClO_3^-. Additional samples were sent to TestAmerica (TestAmerica, Irvine, CA) for analysis.

As the reactions between ZVI/Cr(VI) and ZVI /NO_3^- are known to produce iron hydroxide and NH_4^+ as end products (Su and Puls, 2004; Mitra et al, 2011), both Fe and NH_4^+ were also measured in the bio-ZVI and abiotic reduction samples. Fe analysis was performed using Hach method 8008 (Hach DOC316.53.01053). Fe present in the sample reacts with a 1-10 phenanthroline reagent to produce a complex with a measurement wavelength of 520 nm. This method has a detection limit of 0.02 mg/L. NH_3 as N was measured using Hach method 10031 for ammonia, as NH_4^+ is largely present in water as NH_3 under neutral conditions (Hach DOC316.53.01079). In this method, NH_3 compounds react with with chlorine to form monochloramine, which then react with salicylate to form 5-aminosalicylate. This compound oxidizes in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. As the catalyst is present in excess, the resultant sample solution is colored green and measured using a wavelength of 655 nm. This method has a detection limit of 0.4 mg/L.

Rinsate pH was measured using an Orion Star A111 benchtop pH meter (Thermo Fisher Scientific, Waltham, MA). Its relative accuracy is ± 0.01 pH unit.

3.7 – Statistical Analyses

The one-factor analysis of variance (ANOVA) method was used in the anaerobic experiments to assess differences between the diluted EOS-Pro and diluted molasses solutions in reducing Cr(VI), NO_3^- and ClO_3^- in the soil cylinders and in the control samples. Furthermore, the two-factor ANOVA method with replicates was used in the ZVI remediation experiments to assess the effects of treatment type and treatment time on contaminant levels. An additional one-way ANOVA was performed to determine if there were any differences in ZVI dosage on contaminant removal, and for comparison of bio-ZVI and abiotic reduction. For both experiments, one-way ANOVA methods with Tukey's post-hoc HSD tests were utilized to assess the effects of treatment method on soil moisture and pH. Results were considered statistically significant if $p < 0.05$ for each analysis.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 – Soil Characterization

Visual inspection revealed that both soils are largely fine-grained clayey soils. The XRD analyses determined that both soils are predominantly composed of montmorillonite (> 40%), followed by quartz (18 - 20%) and andesine (14 - 17%). Other trace minerals found in the soils include calcite, dolomite, mica minerals, microcline, hematite and kaolinite. The results of this thesis research thus apply to clayey soils; other types of soils were not investigated.

The full results summary of the soil characterization can be seen in Table 4-1.

TABLE 4-1: SOIL MINERALOGY OF SELECTED SOIL SAMPLES

Mineral Constituent	Chemical Formula	Percentage of Soil	
		A	*B*
Montmorillonite	$(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$	46.9	42.0
Quartz	SiO_2	18.6	21.3
Andesine	$(Ca,Na)(Al,Si)_4O_8$	14.6	17.4
Mica Minerals	—	7.5	10.0
K-Feldspar	$KAlSi_3O_8$	5.0	3.8
Calcite	$CaCO_3$	3.7	1.1
Kaolinite	$Al_2Si_2O_5(OH)_4$	1.9	1.4
Dolomite	$CaMg(CO_3)_2$	1.1	1.7
Hematite	Fe_2O_3	0.7	0.5
Actinolite	$Ca_2(Mg_{4.5\text{-}2.5}Fe^{2+}_{0.5\text{-}2.5})Si_8O_{22}(OH)_2$	—	1.0
	TOTAL	100	100

4.2 – Microcosm Tests: Biotic Contaminant Reduction

Two sets of contaminant measurements were taken at each sampling event. The average of both was graphed as a function of time, and standard deviations were graphically expressed as vertical error

bars over each individual data point. For every two data points on the graph, one complete soil cylinder from the anaerobic chamber was consumed; thus, at least five total soil cylinders from each biotic degradation sample set were used in data collection. However, all samples used were mixed and formed using the same soil mix (Table 3-3).

Soil moisture and pH were regularly monitored throughout the experiment to ensure adequate conditions for biotic degradation of contaminants. Figures 4-1 and 4-2 depict the change in water content and pH with time during the experiments.

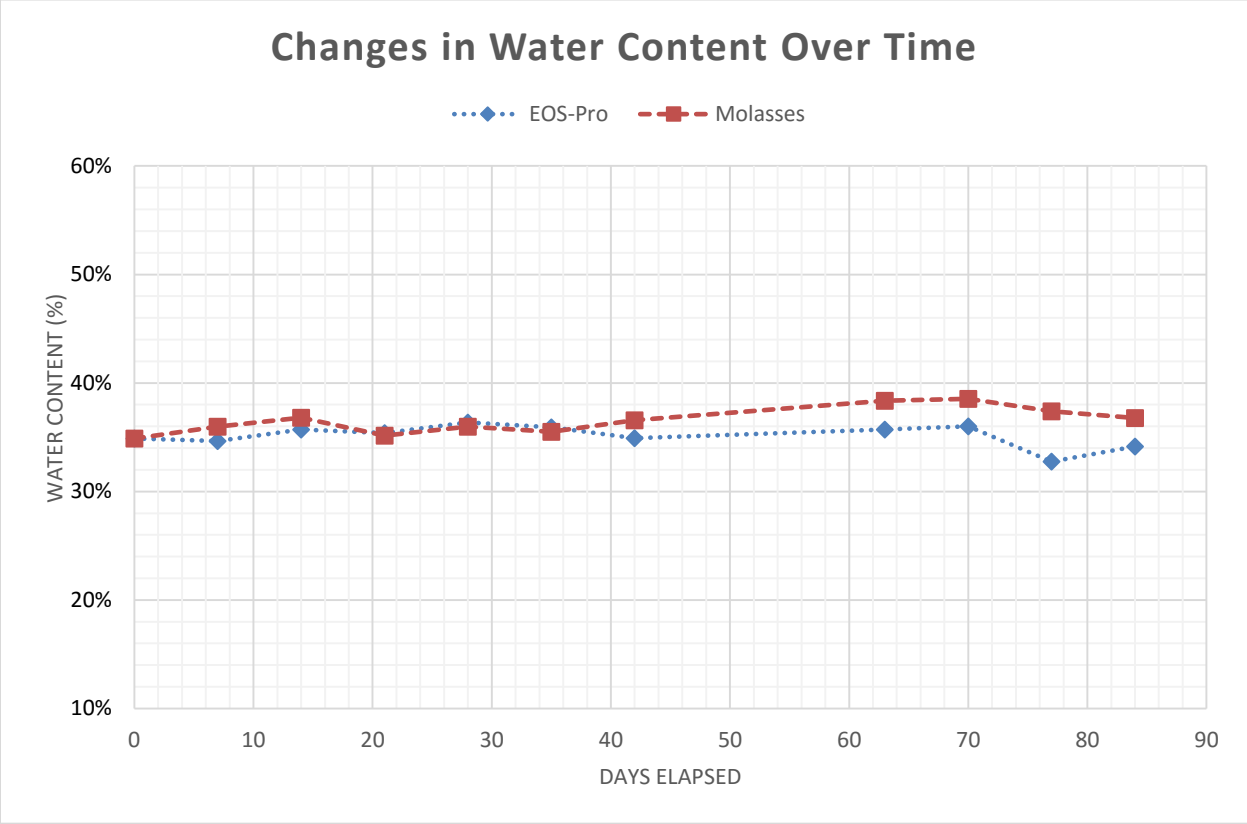

Figure 4-1: Soil moisture readings in anaerobic experiment microcosms amended with nutrients and diluted EOS-Pro and molasses as an electron donor/carbon source for biotic reduction. Data points represent a single measurement of the soil moisture taken at the time of sample collection.

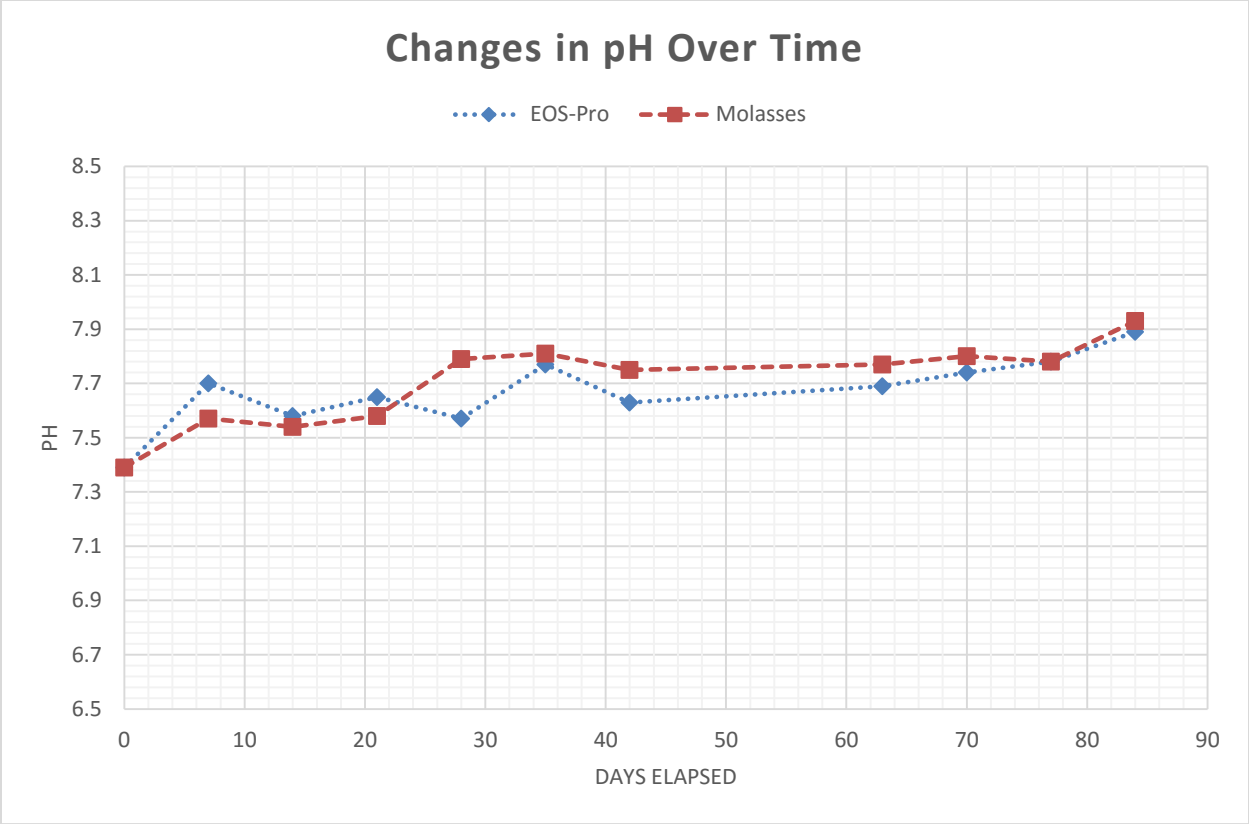

Figure 4-2: pH measurements of anaerobic experiment microcosms amended with nutrients and diluted EOS-Pro and molasses as an electron donor/carbon source for biotic reduction. Data points represent a single measurement of the pH taken at the time of sample collection.

Soil moisture remained relatively constant for the experiment duration, with many of the data points falling within ± 3% of the initial soil moisture of 35%. Furthermore, the overall pH increased during the contaminant biological reduction from 7.4 to 7.8 — 7.9 after 84 days, indicating that reductive activity took place during the experiment (Figure 4-2). The soils amended with diluted molasses are seen to have slightly higher soil moisture and pH with time, compared to those amended with EOS-Pro.

The results of the one-way ANOVA for soil moisture and pH are seen in Table 4-2.

TABLE 4-2: RESULTS OF THE ONE-WAY ANOVA FOR SOIL MOISTURE AND PH EFFECTS ON ANAEROBIC MICROCOSMS

Parameter	One-Way ANOVA Results	
	Soil Moisture	pH
Sample Size (n)	22	22
F-value	8.61	0.22
p-value	0.008	0.64

It can be seen that there's a statistically significant difference at the 5% level between EOS-Pro and molasses in affecting soil moisture; however, no such statistically significant differences were observed with the pH data ($p = 0.64$), implying that neither carbon source has an effect on significantly altering pH, most likely as a result of the soil mineralogy bolstering its buffering capacity (Table 4-1).

4.2.1 – Contaminant Removal Rates, Kinetic Parameters, and Percent Removal

Contaminant removal rates were calculated for Cr(VI), $NO_3^- \cdot N$ and ClO_3^- using a combination of linear regression and calculation via the rate law. It was observed during the anaerobic experiments that biotic degradation of these contaminants slowed down past a certain time point — 7 days for both Cr(VI) and $NO_3^- \cdot N$. Removal rates were thus calculated as follows:

- *Overall rates* measure the total contaminant removed during the entire experimental period. These were calculated by taking the difference between the initial and final contaminant readings and dividing by the experiment duration, which was 84 days.
- *Maximum instantaneous rates* were calculated by determining the instantaneous rate of contaminant removal after seven days, during which reduction was at a maximum.
- *Long-term rates* were calculated by performing a linear regression on the remaining contaminant data by excluding the first data point, which was used in calculating the maximum instantaneous rate. These rates were calculated assuming zero, first, and second-order kinetics to determine which order contaminant removal most closely followed.

The contaminant removal rates and associated kinetic rate constants are summarized by contaminant in Tables 4-3 through 4-5 and are calculated per kilogram of contaminated soil. Positive values for the overall and maximum instantaneous removal rates indicate contaminant removal. R-values were calculated for long-term rates to determine how closely the assumed kinetic model fitted the data.

TABLE 4-3: CHROMIUM(VI) KINETICS AND REMOVAL RATES OVER TIME – ANAEROBIC EXPERIMENTS

Sample Type	Cr(VI)			
	Removal Rate (mg / d)	**Rate Constant *k***	***k* Units**	**R^2**
EOS-Pro				
Overall Rate	0.43	—	—	—
Max. Inst.	2.96	—	—	—
Long-Term				
Zero Order	—	-0.21	mg / kg · d	0.933
First Order	—	-4.2E-03	d^{-1}	0.929
Second Order	—	8.4E-05	kg / mg · d	0.923
Molasses				
Overall Rate	0.62	—	—	—
Max. Inst.	3.23	—	—	—
Long-Term				
Zero Order	—	-0.33	mg / kg · d	0.903
First Order	—	-8.4E-03	d^{-1}	0.942
Second Order	—	2.2E-04	kg / mg · d	0.962

TABLE 4-4: NITRATE KINETICS AND REMOVAL RATES OVER TIME – ANAEROBIC EXPERIMENTS

Sample Type	NO_3^- (as N)			
	Removal Rate (mg / d)	**Rate Constant *k***	***k* Units**	**R^2**
EOS-Pro				
Overall Rate	0.08	—	—	—
Max. Inst.	0.93	—	—	—
Long-Term				
Zero Order	—	0.0212	mg / kg · d	0.153
First Order	—	5.0E-04	d^{-1}	0.153
Second Order	—	1.0E-05	kg / mg · d	0.157
Molasses				
Overall Rate	0.03	—	—	—
Max. Inst.	0.69	—	—	—
Long-Term				
Zero Order	—	0.04	mg / kg · d	0.463
First Order	—	1.1E-03	d^{-1}	0.463
Second Order	—	-3.0E-05	kg / mg · d	0.464

TABLE 4-5: CHLORATE KINETICS AND REMOVAL RATES OVER TIME – ANAEROBIC EXPERIMENTS

Sample Type	ClO_3^-			
	Removal Rate (mg / d)	**Rate Constant *k***	***k* Units**	**R^2**
EOS-Pro				
Overall Rate	3.76	—	—	—
Max. Inst.	—	—	—	—
Long-Term				
Zero Order	—	-6.3498	mg / kg · d	0.026
First Order	—	-0.0004	d^{-1}	0.021
Second Order	—	-0.00001	kg / mg · d	0.157
Molasses				
Overall Rate	-5.58	—	—	—
Max. Inst.	—	—	—	—
Long-Term				
Zero Order	—	12.425	mg / kg · d	0.072
First Order	—	8.0E-04	d^{-1}	0.076
Second Order	—	-3.0E-05	kg / mg · d	0.464

Overall, contaminant degradation was initially rapid during the first week of treatment before slowing down during the remainder of the experimental period. As Cr(VI) degradation did not vary significantly during the experiment, the long-term data fit zero and first-order models with very high correlation coefficients, with the molasses data even closely fitting a second-order model ($R^2 = 0.962$). In comparison, NO_3^- did not degrade much, with reduction rates not correlating well with time due to contaminant levels stabilizing after seven days. Similarly, the ClO_3^- data does not correlate well with any kinetic model due to insubstantial degradation. For Cr(VI) analysis, it will be assumed that Cr(VI) reduction follows zero-order kinetics after the initial rapid reduction, regardless of carbon source used.

Another measure of the treatment efficiency, the percent removal, was calculated using the initial and final contaminant concentrations. Table 4-6 depicts the percent removal of each contaminant from the samples treated with EOS-Pro and molasses.

TABLE 4-6: PERCENT REMOVAL – ANAEROBIC EXPERIMENTS

Sample Type	Cr(VI)	NO_3^- (as N)	ClO_3^-
	Percent Removal		
EOS-Pro	45.1%	7.2%	2.1%
Molasses	65.4%	11.2%	-3.1%

4.2.2 – Statistical Analysis

The results of the one-way ANOVA for the anaerobic microcosms are summarized below in Table 4-7, with F-values and *p*-values listed by each contaminant.

TABLE 4-7: RESULTS OF THE ONE-WAY ANOVA FOR THE ANAEROBIC EXPERIMENT MICROCOSMS

Parameter	One-Way ANOVA Results		
	Cr(VI)	NO_3^-	ClO_3^-
Sample Size (n)	44	44	44
F-value	7.12	0.45	0.93
p-value	0.011	0.51	0.34

Based on the results, there is a statistically significant difference at the 5% level between groups based on Cr(VI) reduction from vadose zone soils. ($p < 0.05$). However, there were no statistically significant differences between either EOS-Pro and molasses regarding soil denitrification ($p = 0.51$) and ClO_3^- degradation ($p = 0.34$).

4.2.3 – Biotic Reduction of Cr(VI)

Cr(VI) initially degrades at a rapid rate regardless of carbon source used. As seen in Table 4-3, Cr(VI) degrades at an initial rate of 2.96 mg/d in the EOS-Pro samples, and 3.23 mg/d in the molasses samples. However, after the first seven days, both samples degrade at a much slower rate, at a rate of 0.21 mg/d in the EOS-Pro samples and to 0.33 mg/d in the molasses samples. The samples dosed with diluted molasses degraded more Cr(VI) over time compared with the diluted EOS-Pro samples (Figure 4-3).

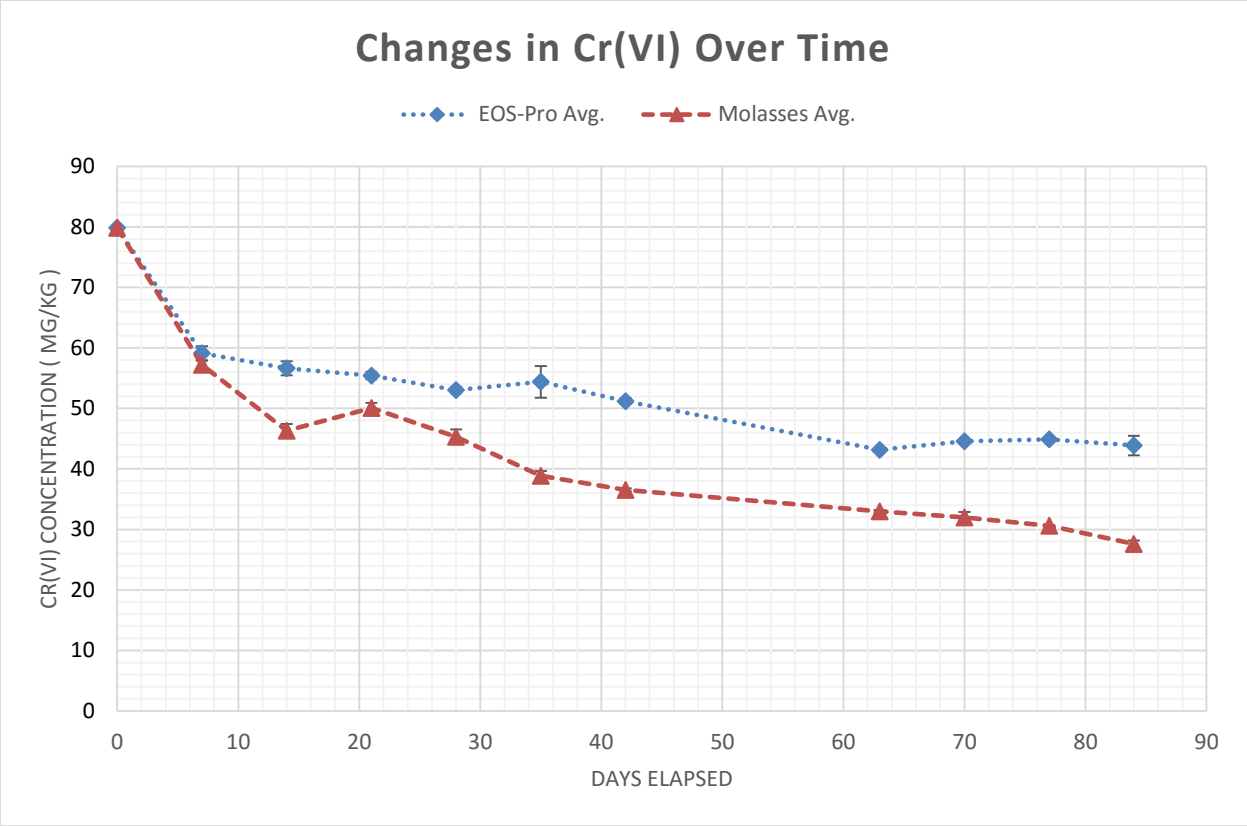

Figure 4-3: Biotic reduction of Cr(VI) [in mg Cr(VI)/kg soil] observed in the anaerobic experiment microcosms treated with nutrients and EOS-Pro and molasses as an electron donor/carbon source. Data points represent the means of duplicate Cr(VI) analysis ± 1 standard deviation.

Cr(VI) reduction was much higher in the soil samples treated with molasses compared to the soils treated with EOS-Pro (65.4% > 45.1%; see Table 4-6). Compared to Oliver et al.'s work, the percent removal of Cr(VI) is much lower than the observed maximum percent removal of 87%. However, this maximum % removal was only observed at 2000 mg/L C and 34,600 mg/L NO_3^- in soil. In comparison, the carbon source dosages used in the experiment were 10× diluted solutions with concentration of roughly 100 — 300 mg C/L of solution per soil cylinder. In the presence of excess NO_3^- and a dosage of 200 mg C/L in each soil sample, Oliver et al. reports anywhere from 22 to 66% removal of Cr(VI).

Furthermore, Oliver et al.'s experiment was performed in 35 days with coarse-grained soils; in comparison, this experiment was performed in 84 days using fine-grained clayey soils. As coarse-grained soils are more permeable than fine-grained soils, these results can be considered valid if soil type is taken into account. These results are thus in line with expected values based on these observations and

comparison with Oliver et al.'s previous work. It can also be inferred that the same dosage of a less diluted carbon source solution could be used to increase the microbial degradation rate of Cr(VI) in fine-grained vadose zone soils.

Oliver et al. (2003) reports that longer contact times and longer flow paths would be required for 100% Cr(VI) reduction in thick vadose zone soils. Based on the removal rate calculations of Table 4-3 and assuming zero-order kinetics applies to both sets of data, Cr(VI) would be 100% immobilized within six months of the initial application of the nutrient amendments if molasses were used as the carbon source, or 10 and a half months if EOS-Pro is instead used. These findings would be consistent with Oliver et al.'s report based on the type of soil used in the experiment.

4.2.4 – Biotic Reduction of NO_3^-

Like Cr(VI), NO_3^- were initially removed at relatively rapid rates from the soil, at 0.93 mg/d for EOS-Pro samples and 0.69 mg/d for molasses samples (Table 4-4). After seven days, however, NO_3^- reduction plateaued and contaminant concentrations remained stable until the end of the experiment. In particular, the long-term rate calculations for NO_3^- produced negative values (-0.02 mg/d for EOS-Pro, and -0.04 mg/d for molasses); this and a visual inspection of the $NO_3^- \cdot N$ data indicate that NO_3^- stopped reducing entirely (Figure 4-4).

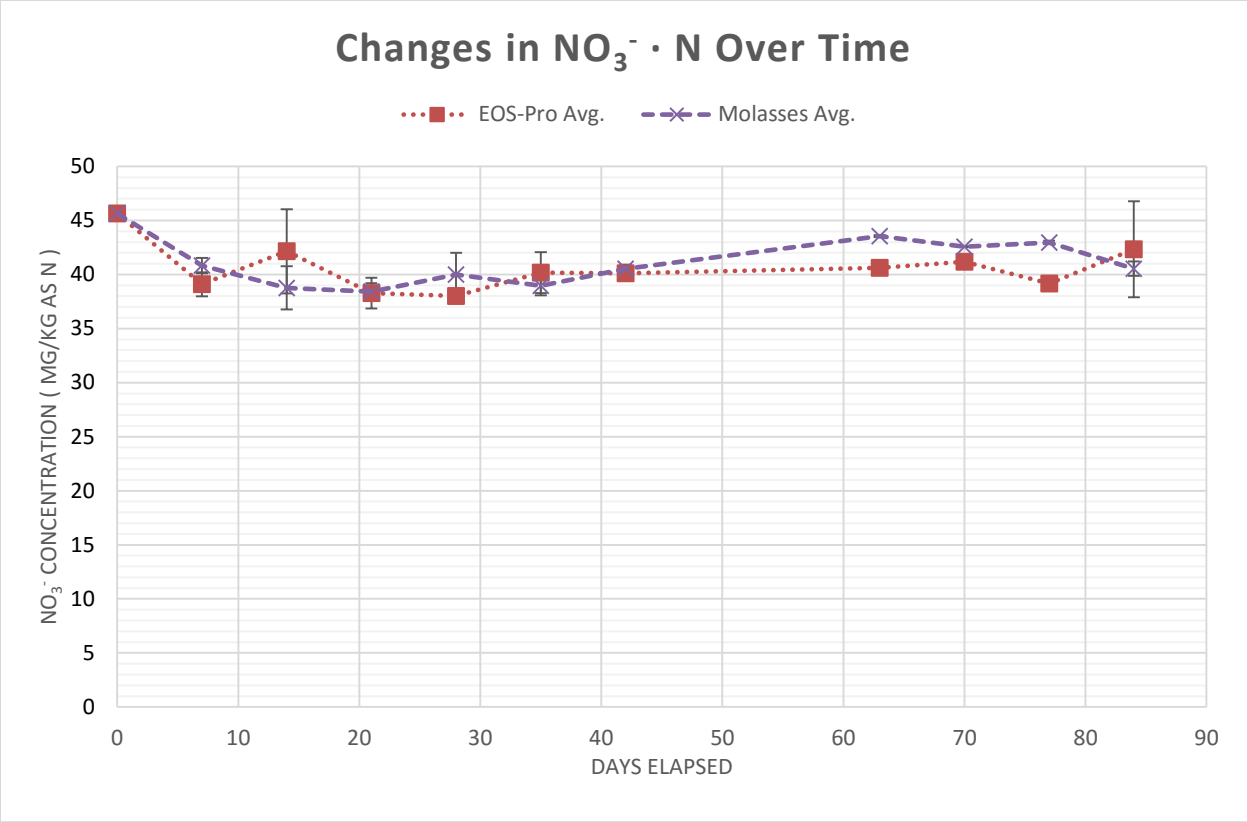

Figure 4-4: Biotic reduction of NO_3^- [in mg NO_3^- · N/kg soil] observed in the anaerobic experiment microcosms treated with nutrients and EOS-Pro and molasses as an electron donor/carbon source. Data points represent the means of duplicate NO_3^- · N analysis ± 1 standard deviation.

This lack of meaningful NO_3^- removal over time suggests that one or more factors inhibited soil denitrification; the main factor being the presence of Cr(VI). Contaminant redox potentials indicate that Cr(VI) is the first to degrade, followed by NO_3^- and ClO_3^-. As 35 — 55% of Cr(VI) still remained after reduction, less degradation of the other contaminants was expected. Furthermore, soil gradation and mineralogy had an effect; as this was a fine-grained clayey soil, the low soil permeability prevented the carbon sources from penetrating the sample, thus limiting carbon source for reduction. Denitrification is known to be enhanced in soils with an adequate carbon supply, much like Cr(VI) reduction (Losi et al., 1994; Jordan et al., 2007). As Cr(VI) reduction was not complete, NO_3^- reduction was inhibited as a result.

Another potential factor affecting NO_3^- reduction is soil moisture content; denitrification is also known to be enhanced in wet soil. In particular, Bouwman (1998) states that soil denitrification largely

occurs at high soil moisture contents [> 60% water-filled pore space]. As the soil samples were prepared assuming arid deep vadose zone conditions and a typical soil moisture of 35%, the relatively dry conditions under which anaerobic bioremediation took place would not have been conducive to NO_3^- reduction, thus resulting in little to no denitrification occurring. Thus, NO_3^- reduction was negatively impacted by the lack of complete Cr(VI) reduction, limited carbon source, and low soil moisture.

4.2.5 – Biotic Reduction of ClO_3^-

Similar to NO_3^-, ClO_3^- degradation was minimal (Table 4-5). Even with the addition of sludge and nutrient mix to stimulate growth, ClO_3^- does not degrade at all regardless of the carbon source used, with contaminant levels remaining relatively constant over time (Figure 4-5).

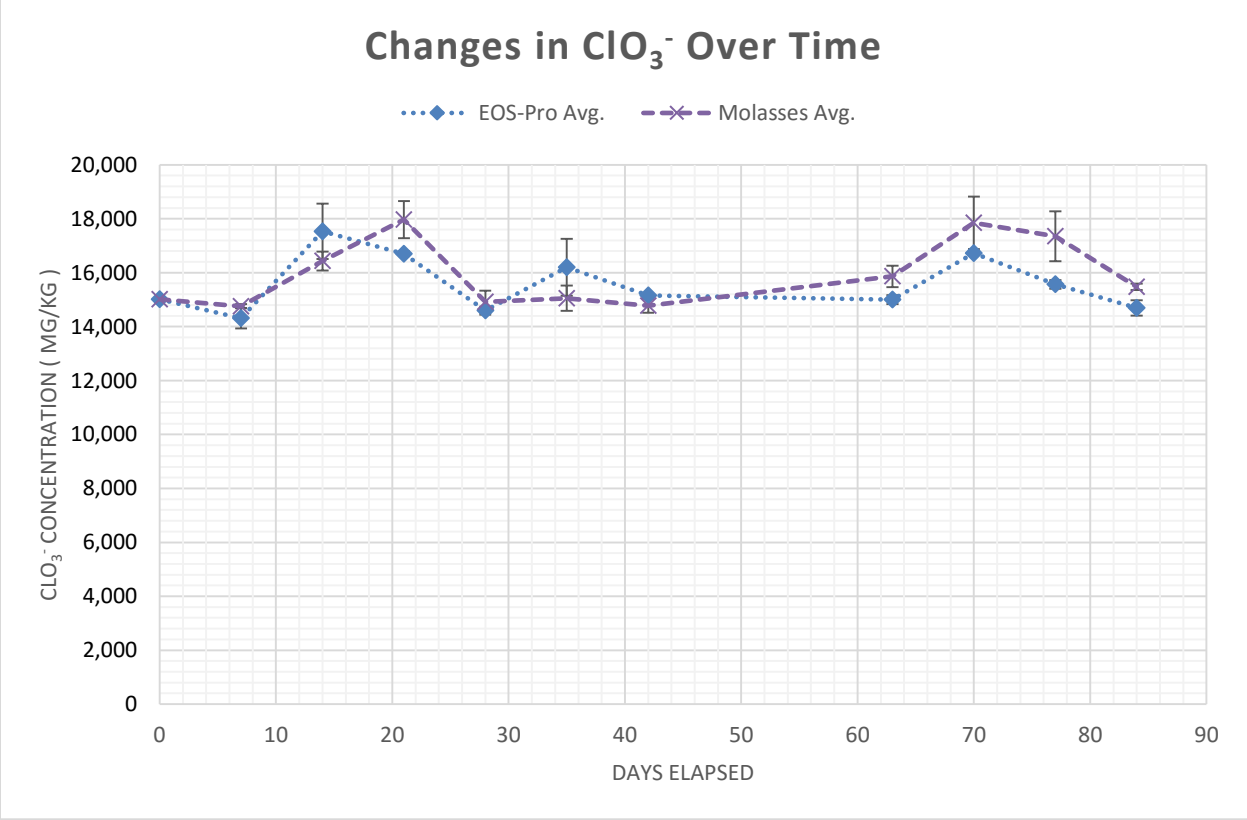

Figure 4-5: Biotic reduction of ClO_3^- [in mg ClO_3^-/kg soil] observed in the anaerobic experiment microcosms treated with nutrients and EOS-Pro and molasses as an electron donor/carbon source. Data points represent the means of duplicate ClO_3^- analysis ± 1 standard deviation.

The lack of ClO_3^- degradation in samples is easily explained in the context of Figure 4-4. ClO_3^- degradation is known to be significantly inhibited in the presence of NO_3^- (van Ginkel, Plugge and Stroo, 1995). As NO_3^- levels were relatively stable after seven days and remained so throughout the experiment timeline, ClO_3^- levels are similarly expected to remain relatively stable. Thus, these findings verify that despite modeling anaerobic conditions, ClO_3^- degradation was inhibited in the presence of NO_3^-, as NO_3^- is a more preferable electron acceptor for microorganisms than ClO_3^- (van Ginkel, Plugge and Stroo, 1995).

4.2.6 – Blank Sample Analysis

Figures 4-6, 4-7 and 4-8 depict the change in Cr(VI), $NO_3^- \cdot N$ and ClO_3^- in the experimental blanks during the experiment. As more individual samples were taken from these blank samples compared to the anaerobic experiment samples, the data points depict the average contaminant levels at the end of the experiment, with the standard deviations again shown on the graph using vertical error bars.

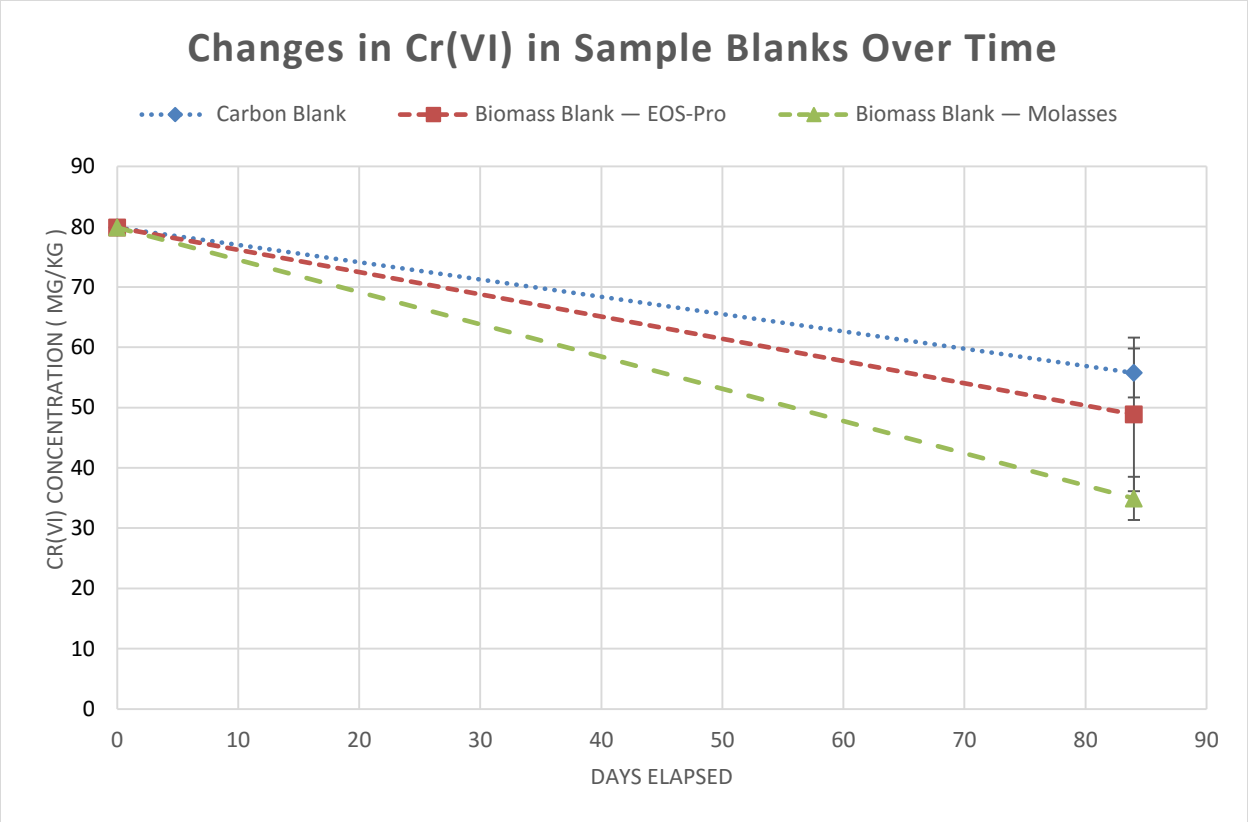

Figure 4-6: Changes in Cr(VI) concentration [in mg Cr(VI)/kg soil] observed in the anaerobic experiment blanks. Data points represent the means of triplicate [biomass blanks] and quadruplicate [carbon blanks] Cr(VI) analysis ± 1 standard deviation.

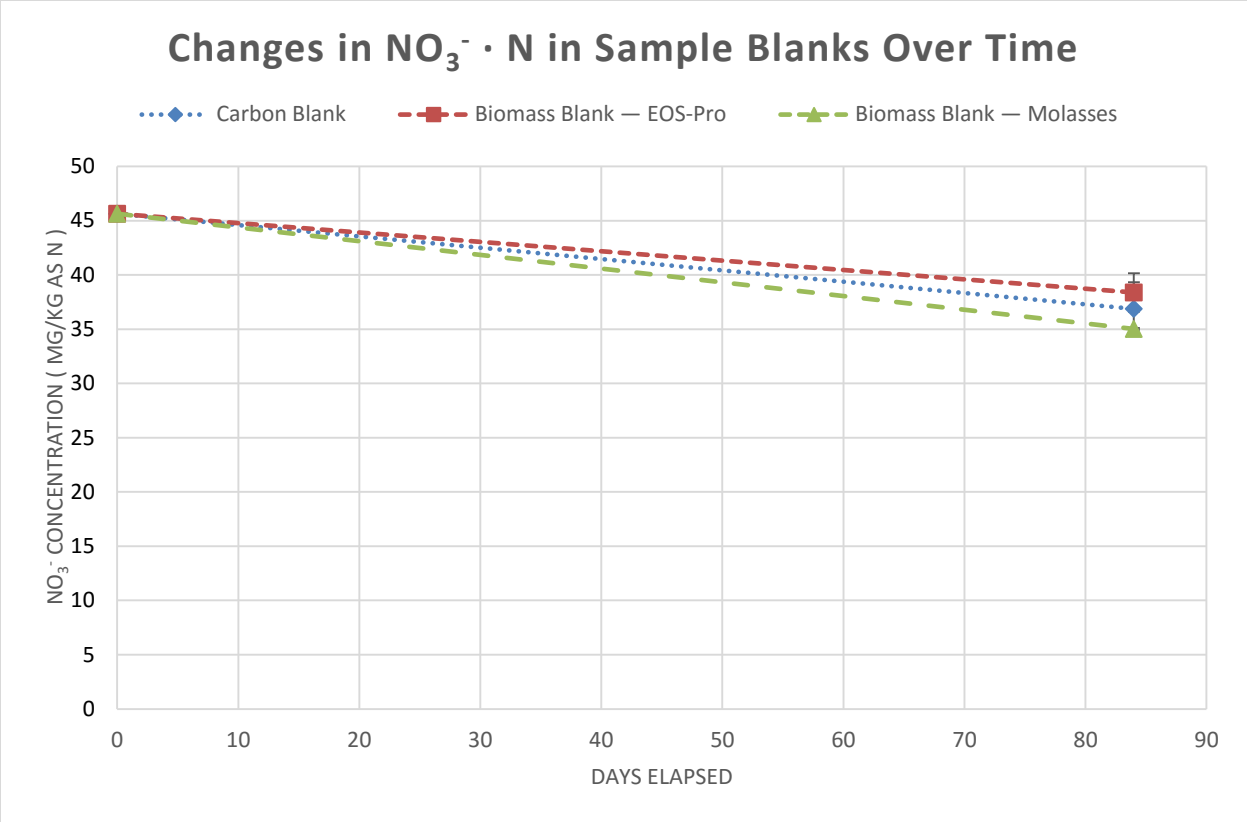

Figure 4-7: Changes in NO_3^- concentration [in mg NO_3^- · N/kg soil] observed in the anaerobic experiment blanks. Data points represent the means of triplicate [biomass blanks] and quadruplicate [carbon blanks] NO_3^- · N analysis ± 1 standard deviation.

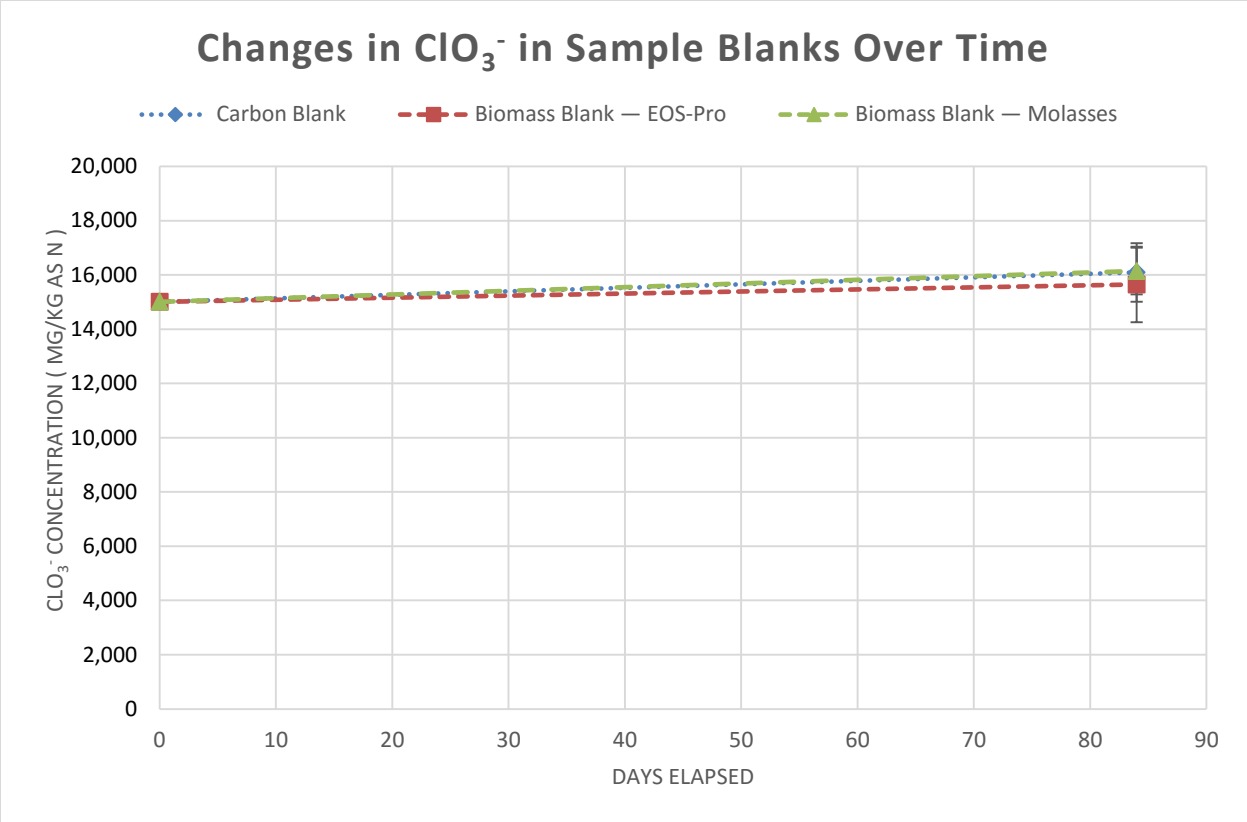

Figure 4-8: Changes in ClO_3^- concentration [in mg ClO_3^-/kg soil] observed in the anaerobic experiment blanks. Data points represent the means of triplicate [biomass blanks] and quadruplicate [carbon blanks] ClO_3^- analysis ± 1 standard deviation.

Like the anaerobic experiment samples, removal rates and % contaminant removal were also calculated. Table 4-8 summarizes average contaminant removal rates per kilogram of contaminated soil, soil moisture, and pH of all three sets of experiment blanks. Positive values for removal rates indicate contaminant reduction.

TABLE 4-8: REMOVAL RATES, PERCENT REMOVAL, SOIL MOISTURE AND PH – ANAEROBIC EXPERIMENT BLANKS

Sample Type	Soil Moisture (%)	pH	Cr(VI)		NO_3^- (as N)		ClO_3^-	
			Removal Rate (mg / d)	% Removal	Removal Rate (mg / d)	% Removal	Removal Rate (mg / d)	% Removal
Blank Samples								
Carbon	33.6%	7.85	0.29	30.2%	0.10	19.2%	-12.92	-7.2%
Biomass EOS-Pro	33.7%	7.89	0.37	38.8%	0.09	15.9%	-7.63	-4.3%
Biomass Molasses	34.8%	8.09	0.53	56.3%	0.13	23.3%	-13.55	-7.6%

Visual inspection of Figures 4-6 through 4-8 and analysis of Table 4-8 all indicate that reductive activity took place in the blank samples, with the most significant reductive activity occurring with Cr(VI) and NO_3^- in the biomass blanks. In contrast, ClO_3^- exhibited little to no degradation (Figure 4-8), with Table 4-7 calculations indicating an apparent increase in ClO_3^- levels. The Cr(VI) results for the carbon blank sample in particular mirror those of Oliver et al. (2003); in the absence of carbon source and in excess amounts of NO_3^-, Cr(VI) % removal ranged from 13 to 66%.

A second one-way ANOVA was performed to determine if there were any statistically significant differences between treatments in the sample blanks. The results of that analysis are shown in Table 4-9.

TABLE 4-9: RESULTS OF THE ONE-WAY ANOVA FOR THE ANAEROBIC EXPERIMENT BLANKS

Parameter	One-Way ANOVA Results - Blanks		
	Cr(VI)	NO_3^-	ClO_3^-
Sample Size (n)	10	10	10
F-value	4.44	1.80	0.13
p-value	0.057	0.23	0.88

Even with the reduction levels observed in the blanks, the results of the Cr(VI) ANOVA are not statistically significant at the 5% level ($p = 0.057$), implying there is no difference between amendments in their reduction capacity, biotic or abiotic. Similar implications are observed for NO_3^- ($p = 0.23$) and ClO_3^- ($p = 0.88$), as there is no statistically significant difference between blank sample treatments for either of them.

It had been assumed during experimental preparation that no bacteria would be present in the vadose zone soil for contaminant reduction. The sample blank results indicated otherwise; while all three samples displayed significant levels of Cr(VI) and NO_3^- reduction, the % removed is more pronounced in the biomass blanks, and in molasses in particular. One possible explanation involves the assumption during experimental preparation that no bacteria would be present in the vadose zone soil for contaminant

reduction. As contaminant reduction was observed in all three sample blanks, it is plausible that the native microbial community utilized the electron donor/carbon sources – such as the residual ethanol in the bacterial seed – and the bacterial seed itself for bacterial growth and contaminant reduction.

As substantial contaminant reduction was observed in the carbon blank, another possible explanation is based off the activated sludge. This seed was taken from a fluidized bed reactor that used ethanol as an electron donor and carbon source for contaminant treatment, which likely ended up in the seed solution and was then transferred into the blank samples, where it was subsequently used for microbial growth and contaminant degradation. In addition, the bacteria of the seed themselves could have been utilized as the carbon source via endogenous respiration; however, the former hypothesis is more plausible.

Similarly substantial contaminant reduction was observed in the biomass samples. Two possible explanations could explain the reduction; one is the aforementioned presence of native microbes that utilized the electron donor/carbon sources for growth. The other explanation is that the electron donor/carbon source itself reduced Cr(VI) and NO_3^- through abiotic reduction. One case study by Chen et al. (2015) reports that it is possible to reduce Cr(VI) chemically using molasses, though the removal rate is dependent on pH; in the presence of reducing agents, Cr(VI) is typically reduced under acidic pH conditions (Duncan et al., 2007; Barrera-Díaz et al., 2012). Removal rates in aqueous solution ranged from 76.8 mg/L · d at pH 2.0 to 2.21 mg/L · d at pH 6.1 for an initial dose of 1 mL molasses/L H_2O. As no experiments currently exist under which molasses is used to chemically reduce Cr(VI) in groundwater, comparisons are limited at best. However, the low reduction rate of 0.53 mg/d at pH 8.09 is in agreement with the expected reduction in contaminant removal at higher pH.

4.2.7 – Concluding Remarks

In both samples and sample blanks, the final % contaminant removal of each follows the expected order of removal based on contaminant E_h (Tables 4-6 and 4-8), with Cr(VI) being reduced first. Incomplete reduction of Cr(VI) resulted in no observable reduction of NO_3^- and ClO_3^- in the experiment

samples, which was also expected. It can be seen that molasses is the more preferable carbon source to use for Cr(VI) reduction, with significantly higher Cr(VI) removal observed in the soils treated with molasses in comparison to the soils treated with EOS-Pro.

If Cr(VI) was the only contaminant of concern, molasses would be the recommended carbon source for biotic reduction under anaerobic conditions. In the presence of other contaminants, however, neither carbon source removed the co-contaminants any differently, if at all. Though the sample blank rate calculations suggest that molasses would have removed more NO_3^- over time compared to EOS-Pro, co-contaminant stabilization in the anaerobic experiment soil samples prevented any further meaningful calculations from being performed. Thus, it was not possible to determine which carbon source, if either of them, would have been more preferable in removing NO_3^- and ClO_3^-.

4.3 – Microcosm Tests: Contaminant Reduction Using Zero-Valent Iron and Organic Electron Donors

Two data values were taken from each soil sample plot; the average of both original and duplicate samples was graphed with time, with standard deviations being graphically displayed as vertical error bars. Every two points on the graph likewise represents one full sample plot; anywhere from three to three and a half plots total were used up in data collection from the ZVI remediation experiments. Again, all sample plots used were mixed and formed using the same soil mix (Table 3-4).

4.3.1 – Soil Moisture and pH Measurements

Soil moisture and pH were monitored during the experiment timeline to determine if reductive activity was taking place. These are depicted in Figures 4-9 and 4-10.

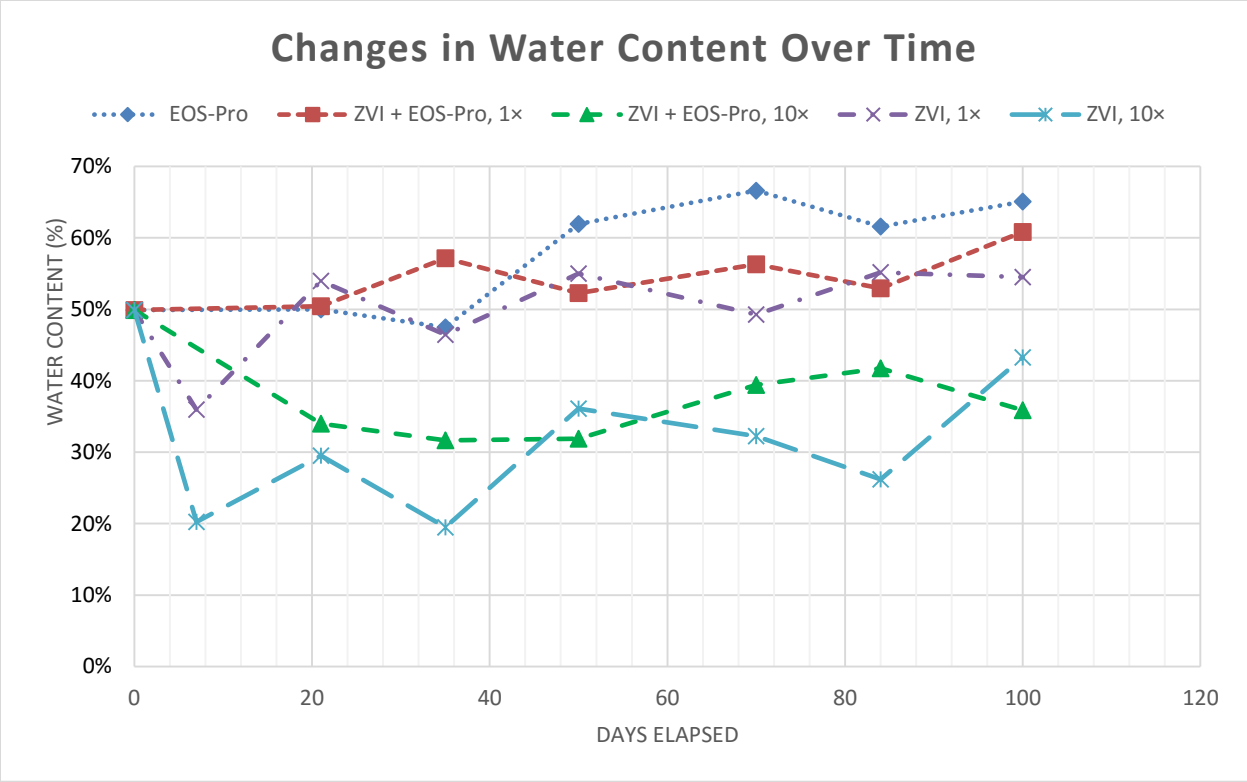

Figure 4-9: Soil moisture readings taken from the ZVI remediation experiment microcosms treated using a combination of biotic reduction with EOS-Pro as electron donor/carbon source, abiotic reduction, and bio-ZVI reduction with EOS-Pro. Data points represent a single measurement of the soil moisture taken at the time of sample collection.

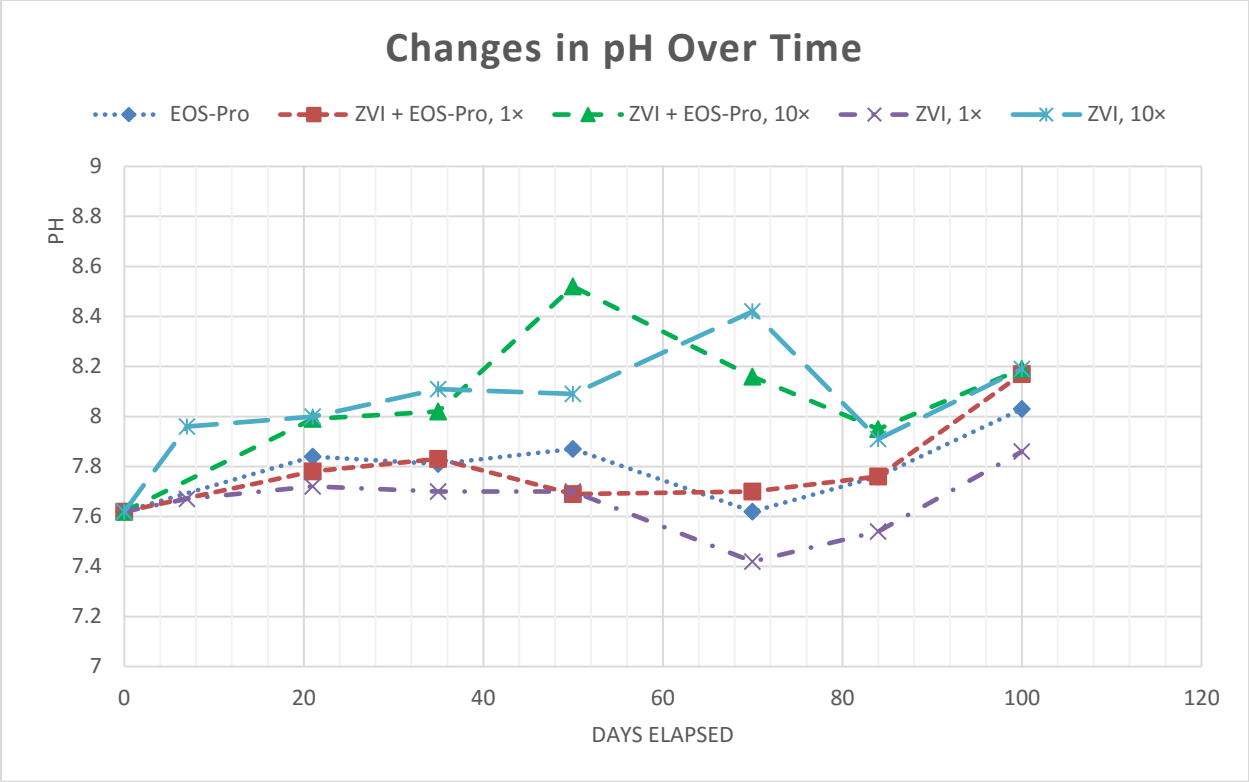

Figure 4-10: pH measurements of the ZVI remediation experiment microcosms treated using a combination of biotic reduction with EOS-Pro as electron donor/carbon source, abiotic reduction, and bio-ZVI reduction with EOS-Pro. Data points represent a single measurement of the pH taken at the time of sample collection.

Like the anaerobic experiments, a one-way ANOVA was performed to determine if there were statistically significant differences between treatments on soil moisture and pH. The results are seen in Table 4-10.

TABLE 4-10: RESULTS OF THE ONE-WAY ANOVA FOR SOIL MOISTURE AND PH EFFECTS ON ZVI REMEDIATION MICROCOSMS

Parameter	One-Way ANOVA Results	
	Soil Moisture	pH
Sample Size (n)	37	37
F-value	15.41	5.98
p-value	4.00E-07	0.001

It can be seen that at the 5% level, there's a statistically significant difference between treatments and their effects on soil moisture and pH. As multiple treatments were employed in contaminant

reduction, the Tukey post-hoc HSD test was performed to determine which pairs were statistically different from each other. The results are seen below in Table 4-11.

TABLE 4-11: TUKEY POST-HOC COMPARISONS OF TREATMENT METHODS AND THEIR EFFECTS ON SOIL MOISTURE AND PH – ZVI REMEDIATION MICROCOSMS

Treatment Comparison	*Soil Moisture*			*pH*		
	q	*p*-Value	Statistically Significant?	q	*p*-Value	Statistically Significant?
EOS-Pro to ZVI + EOS-Pro, 1x	1.134	0.900	NO	0.000	0.900	NO
EOS-Pro to ZVI + EOS-Pro, 10x	6.879	0.001	YES	3.611	0.104	NO
EOS-Pro to ZVI, 1x	2.614	0.332	NO	1.851	0.644	NO
EOS-Pro to ZVI, 10x	8.853	0.001	YES	3.254	0.148	NO
ZVI + EOS-Pro, 1x to ZVI + EOS-Pro, 10x	5.746	0.003	YES	3.611	0.104	NO
ZVI + EOS-Pro, 1x to ZVI, 1x	1.480	0.794	NO	1.851	0.644	NO
ZVI + EOS-Pro, 1x to ZVI, 10x	7.720	0.001	YES	3.254	0.148	NO
ZVI + EOS-Pro, 10x to ZVI, 1x	4.265	0.029	YES	5.461	0.003	YES
ZVI + EOS-Pro, 10x to ZVI, 10x	1.974	0.594	NO	0.356	0.900	NO
ZVI, 1x to ZVI, 10x	6.240	0.001	YES	5.105	0.004	YES

The Tukey post-hoc analysis of Table 4-11 indicates that soil moisture effects are overall the greatest when comparing between biotic reduction and bio-ZVI reduction at a ZVI dosage of 10:1, and in sample plots where the ZVI dosages are different, whether for abiotic or bio-ZVI reduction. Similarly, the pH effects are the most significant when comparing between different ZVI dosages.

As a result of regular soil wetting throughout the experiment, soil moisture varied from 20% at its lowest to 67% at its highest. It was noted throughout the experiment that the soil moisture sensors used did not give exact moisture measurements, only indicating whether the soil was dry or not. The soil moisture levels are noted to be the lowest in the sample plots amended with ZVI at a ratio of 10:1, while the biotic reduction and the sample plots amended with ZVI at a ratio of 1:1 exhibited elevated levels of soil moisture. This can be explained as a result of soil mass; the samples with higher ZVI dosages had

less soil mass — and less soil moisture — to react with the ZVI (Table 3-4). Soil moisture was thus decreased as a result of the reaction with ZVI drying out the soil.

As a result of the soil moisture being consumed in the reaction with ZVI, however, the sample plots amended with ZVI at a 10:1 ratio exhibited higher pH levels in the rinsate compared to the biotic reduction and the sample plots amended with ZVI at a 1:1 ratio. The reactions between ZVI/Cr(VI) and ZVI/NO_3^- (Table 2-5 and Equation 1) are known to consume H^+ and produce OH^- as an end-product, which would result in significant pH increases much like those observed in Figure 4-10. In the biotic reduction and sample plots where the ZVI to contaminants ratio was 1:1, the pH became more acidic; in the latter, it was attributed to soil moisture being in excess of ZVI, resulting in less OH^- being produced. For the former sample type, it was eventually determined to be the result of EOS-Pro degradation. As EOS-Pro is composed largely of vegetable oil, one of its degradation products is acetic acid, which would lower the pH.

4.3.2 – Contaminant Removal Rates, Kinetic Parameters and Percent Removal

Contaminant removal rates were calculated using a combination of linear regression and calculation via the rate law. Like the anaerobic experiment results, removal rates were calculated in terms of the overall rate, the maximum instantaneous rate, and the long-term rate constants. Long-term rates were calculated for data after 7 or 21 days depending on sample type. Furthermore, for long-term conditions, zero, first, and second-order models were fitted to the data to determine the kinetic order of degradation following the initial rapid contaminant reduction. The ZVI remediation experiment removal rates for all three contaminants are summarized in Tables 4-12 through 4-14. Positive values for the overall and maximum instantaneous removal rates indicate contaminant removal, and removal rates are calculated per kilogram of contaminated soil. R-values were again calculated for the long-term data to determine how closely the assumed kinetic model fitted the data.

TABLE 4-12: CHROMIUM(VI) KINETICS AND REMOVAL RATES OVER TIME – ZVI REMEDIATION EXPERIMENTS

Sample Type	Cr(VI)			
	Removal Rate (mg / d)	**Rate Constant *k***	***k* Units**	**R^2**
EOS-Pro				
Overall Rate	0.50	—	—	—
Max. Inst.	2.79	—	—	—
Long-Term				
Zero Order	—	0.11	mg / kg · d	0.792
First Order	—	3.8E-03	d^{-1}	0.836
Second Order	—	-1.0E-04	kg / mg · d	0.831
ZVI + EOS-Pro, 1x				
Overall Rate	0.88	—	—	—
Max. Inst.	4.18	—	—	—
Long-Term				
Zero Order	—	—	—	—
First Order	—	-0.01	d^{-1}	0.056
Second Order	—	0.04	kg / mg · d	0.096
ZVI + EOS-Pro, 10x				
Overall Rate	0.88	—	—	—
Max. Inst.	4.18	—	—	—
Long-Term				
Zero Order	—	—	—	—
First Order	—	-0.03	d^{-1}	0.621
Second Order	—	0.15	kg / mg · d	0.512
ZVI, 1x				
Overall Rate	0.88	—	—	—
Max. Inst.	12.49	—	—	—
Long-Term				
Zero Order	—	—	—	—
First Order	—	-0.01	d^{-1}	0.071
Second Order	—	0.04	kg / mg · d	0.096
ZVI, 10x				
Overall Rate	0.88	—	—	—
Max. Inst.	12.54	—	—	—
Long-Term				
Zero Order	—	—	—	—
First Order	—	6.7E-03	d^{-1}	0.050
Second Order	—	-3.7E-02	kg / mg · d	0.092

TABLE 4-13: NITRATE KINETICS AND REMOVAL RATES OVER TIME – ZVI REMEDIATION EXPERIMENTS

Sample Type	NO_3^- (as N)			
	Removal Rate (mg / d)	**Rate Constant *k***	***k* Units**	**R^2**
EOS-Pro				
Overall Rate	0.28	—	—	—
Max. Inst.	1.33	—	—	—
Long-Term				
Zero Order	—	0.03	mg / kg · d	0.076
First Order	—	1.3E-03	d^{-1}	0.078
Second Order	—	-6.0E-05	kg / mg · d	0.078
ZVI + EOS-Pro, 1x				
Overall Rate	0.42	—	—	—
Max. Inst.	1.53	—	—	—
Long-Term				
Zero Order	—	-0.11	mg / kg · d	0.810
First Order	—	-0.01	d^{-1}	0.811
Second Order	—	1.9E-03	kg / mg · d	0.770
ZVI + EOS-Pro, 10x				
Overall Rate	0.44	—	—	—
Max. Inst.	1.96	—	—	—
Long-Term				
Zero Order	—	-0.03	mg / kg · d	0.364
First Order	—	-0.01	d^{-1}	0.475
Second Order	—	2.6E-03	kg / mg · d	0.563
ZVI, 1x				
Overall Rate	0.42	—	—	—
Max. Inst.	4.90	—	—	—
Long-Term				
Zero Order	—	-0.09	mg / kg · d	0.610
First Order	—	-0.01	d^{-1}	0.651
Second Order	—	1.9E-03	kg / mg · d	0.669
ZVI, 10x				
Overall Rate	0.36	—	—	—
Max. Inst.	6.00	—	—	—
Long-Term				
Zero Order	—	0.03	mg / kg · d	0.194
First Order	—	3.1E-03	d^{-1}	0.073
Second Order	—	-2.0E-04	kg / mg · d	0.005

TABLE 4-14: CHLORATE KINETICS AND REMOVAL RATES OVER TIME – ZVI REMEDIATION EXPERIMENTS

Sample Type	ClO_3^-			
	Removal Rate (mg / d)	**Rate Constant *k***	***k* Units**	**R^2**
EOS-Pro				
Overall Rate	54.97	—	—	—
Max. Inst.	385.70	—	—	—
Long-Term				
Zero Order	—	38.34	mg / kg · d	0.6895
First Order	—	4.0E-03	d^{-1}	0.7112
Second Order	—	-4.0E-07	kg / mg · d	0.7302
ZVI + EOS-Pro, 1x				
Overall Rate	233.65	—	—	—
Max. Inst.	685.39	—	—	—
Long-Term				
Zero Order	—	-16.96	mg / kg · d	0.4058
First Order	—	-0.08	d^{-1}	0.5833
Second Order	—	3.9E-03	kg / mg · d	0.0128
ZVI + EOS-Pro, 10x				
Overall Rate	233.65	—	—	—
Max. Inst.	778.84	—	—	—
Long-Term				
Zero Order	—	—	—	—
First Order	—	0.01	d^{-1}	0.3989
Second Order	—	-0.03	kg / mg · d	0.4364
ZVI, 1x				
Overall Rate	233.64	—	—	—
Max. Inst.	1400.87	—	—	—
Long-Term				
Zero Order	—	-56.23	mg / kg · d	0.6027
First Order	—	-0.11	d^{-1}	0.8266
Second Order	—	2.1E-03	kg / mg · d	0.0363
ZVI, 10x				
Overall Rate	233.65	—	—	—
Max. Inst.	2336.11	—	—	—
Long-Term				
Zero Order	—	—	—	—
First Order	—	-0.01	d^{-1}	0.1089
Second Order	—	-0.01	kg / mg · d	0.0106

The data shows that contaminant degradation is overall enhanced in the presence of ZVI, and that its addition promoted rapid degradation rates. For Cr(VI) alone, degradation occurs more rapidly in the bio-ZVI reduction samples than in the biotic reduction samples; the overall rate is 1.76 times larger, and the maximum instantaneous rate is 1.5 times larger. However, higher ZVI doses did not promote faster degradation rates.

In terms of overall order, neither Cr(VI) nor ClO_3^- reduction overall follows a set kinetic order overall, possibly a result of both contaminants being reduced almost completely with the first 7 to 21 days of treatment. As a result, a simple model would not completely fit the reduction data. This was also observed in kinetic calculations; as a result of contaminant levels going down to zero rapidly, multiple errors were encountered in calculating kinetic parameters assuming first and second-order kinetics, resulting in low R^2 values for the Cr(VI) and ClO_3^- data. For example, ClO_3^- reduction by a 1:1 ratio of ZVI to contaminants appears to follow first-order kinetics; however, as concentrations were essentially zero after 50 days, calculation errors occurred that lowered the R^2 value ($R^2 = 0.8266$). It was thus decided that the most effective way of describing the data was to use two sets of data: the maximum instantaneous rate to depict the initial rapid degradation of contaminants, and the kinetic models generated using the flat part of the curve.

NO_3^- was the only contaminant in where a “kinetic” order was observed; however, these results are due to an issue with NO_3^- reduction with ZVI that will be discussed in the appropriate section.

Percent removals were also calculated for contaminants based on each type of treatment. The summarized results are shown in Table 4-15.

TABLE 4-15: PERCENT REMOVAL – ZVI REMEDIATION EXPERIMENTS

Sample Type	Cr(VI)	NO_3^-	ClO_3^-
	Percent Removal		
EOS-Pro	59.0%	61.0%	33.6%
ZVI + EOS-Pro, 1x	99.4%	89.9%	100.0%
ZVI + EOS-Pro, 10x	99.9%	94.7%	100.0%
ZVI, 1x	99.9%	91.7%	100.0%
ZVI, 10x	99.8%	79.2%	100.0%

4.3.3 – Statistical Analysis

The results of the two-way ANOVA with replication are summarized in Table 4-16; F-values and *p*-values are organized by row effects (treatment method), column effects (treatment time) and interaction effects.

TABLE 4-16: RESULTS OF THE TWO-WAY ANOVA FOR THE ZVI REMEDIATION EXPERIMENTS

Parameter	Two-Way ANOVA Results		
	Cr(VI)	NO_3^-	ClO_3^-
Sample Size (n)	70	70	70
Treatment Method Effects			
F-value	11046.02	377.87	1764.01
p-value	9.77E-54	2.92E-28	7.92E-40
Time Effects			
F-value	46829.88	1752.29	2786.61
p-value	8.40E-67	7.39E-42	2.26E-45
Interaction Effects			
F-value	332.52	22.41	61.58
p-value	1.32E-34	8.08E-15	5.08E-22

It can be seen that all results are statistically significant at the 5% level, with treatment method and treatment time both affecting the final contaminant levels. Statistically significant interactions between treatment method and time were also observed for all three contaminants. As the *p*-value for the interactions for all contaminants are small, the additive model assumption was not valid and hypothesis tests for the main effects were not performed.

An additional series of one-way ANOVA tests were performed to determine if there are any statistical differences between ZVI doses on contaminant removal, and if bio-ZVI reduction treats samples any differently than abiotic reduction with ZVI by itself. Tables 4-17 through 4-19 show the results of the individual analyses for Cr(VI), NO_3^-, and ClO_3^-.

TABLE 4-17: RESULTS OF THE ONE-WAY ANOVA FOR CR(VI) REDUCTION IN THE ZVI REMEDIATION EXPERIMENTS

Parameter	Cr(VI) Analysis - ZVI Dose and Treatment		
	ZVI Dosage	*Abiotic vs. Bio-ZVI*	
		ZVI = 1:1	ZVI = 10:1
Sample Size (n)	28	28	28
F-value	7.4E-06	1.8E-05	1.1E-07
p-value	1.00	1.00	1.00

TABLE 4-18: RESULTS OF THE ONE-WAY ANOVA FOR NO_3^- REDUCTION IN THE ZVI REMEDIATION EXPERIMENTS

Parameter	NO_3^- Analysis - ZVI Dose and Treatment		
	ZVI Dosage	*Abiotic vs. Bio-ZVI*	
		ZVI = 1:1	ZVI = 10:1
Sample Size (n)	28	28	28
F-value	1.1E-07	8.5E-03	1.9E-02
p-value	1.00	0.93	0.89

TABLE 4-19: RESULTS OF THE ONE-WAY ANOVA FOR ClO_3^- REDUCTION IN THE ZVI REMEDIATION EXPERIMENTS

Parameter	ClO_3^- Analysis - ZVI Dose and Treatment		
	ZVI Dosage	*Abiotic vs. Bio-ZVI*	
		ZVI = 1:1	ZVI = 10:1
Sample Size (n)	28	28	28
F-value	1.6E-02	6.5E-03	2.3E-07
p-value	0.90	0.94	1.00

Interestingly, in all cases and for all contaminants, there were no statistically significant differences between ZVI dosage or whether bio-ZVI or abiotic reduction was more preferable for contaminant removal, implying that neither ZVI dosage nor treatment method using ZVI had any significant differences from each other with respect to contaminant removal.

4.3.4 – Cr(VI) Reduction

Cr(VI) removal rates were significantly higher in the ZVI-amended sample plots than in the plots treated using biotic reduction alone, with Cr(VI) essentially being reduced completely in the former set of samples in the first 7 to 21 days of treatment (Figure 4-11). As seen in Table 4-12, Cr(VI) was reduced at a maximum rate of 4.18 to 12.5 mg/d. Gheju (2011) reports a range of removal rate constants for ZVI reduction of Cr(VI); two data entries with the same units as the removal rates calculated for these results report ranges of 2.8 to 8.5 mg/L · min for a pH range of 2 — 3, and 0.08 to 1.25 mg/L · h · g Fe for a pH range of 3 — 10. Using the sample design matrix in Table 3-4 and the 4:1 ratio of mL DI water to g soil used for soil contaminant extraction into the rinsate, these become 16.1 to 46 g/kg · d at pH 2 — 3, and 0.11 to 1.7 g/kg · d at pH 3 — 10, much higher than the removal rate reported in the thesis. However, many of these reaction rates in Gheju (2011)'s report were calculated using treatment times of minutes and hours and not days, and were for water instead of soil.

One reason for this discrepancy between the reported and the published Cr(VI) removal rates is the medium in which Cr(VI) is reduced; as noted before, the published rates are for water, not for soil. Gheju (2011) reports that inorganic substances like hardness and carbonate can reduce ZVI removal capacity by as much as 42% as a result of the formation of precipitates like $CaCO_3$. As the soil used in this experiment consists of fine-grained clayey soils with minerals containing those and other ions that readily dissolve into water, it is possible that the H_2 produced by the oxidizing ZVI also oxidized several of these minerals along with reducing Cr(VI), thus decreasing the overall reduction capacity.

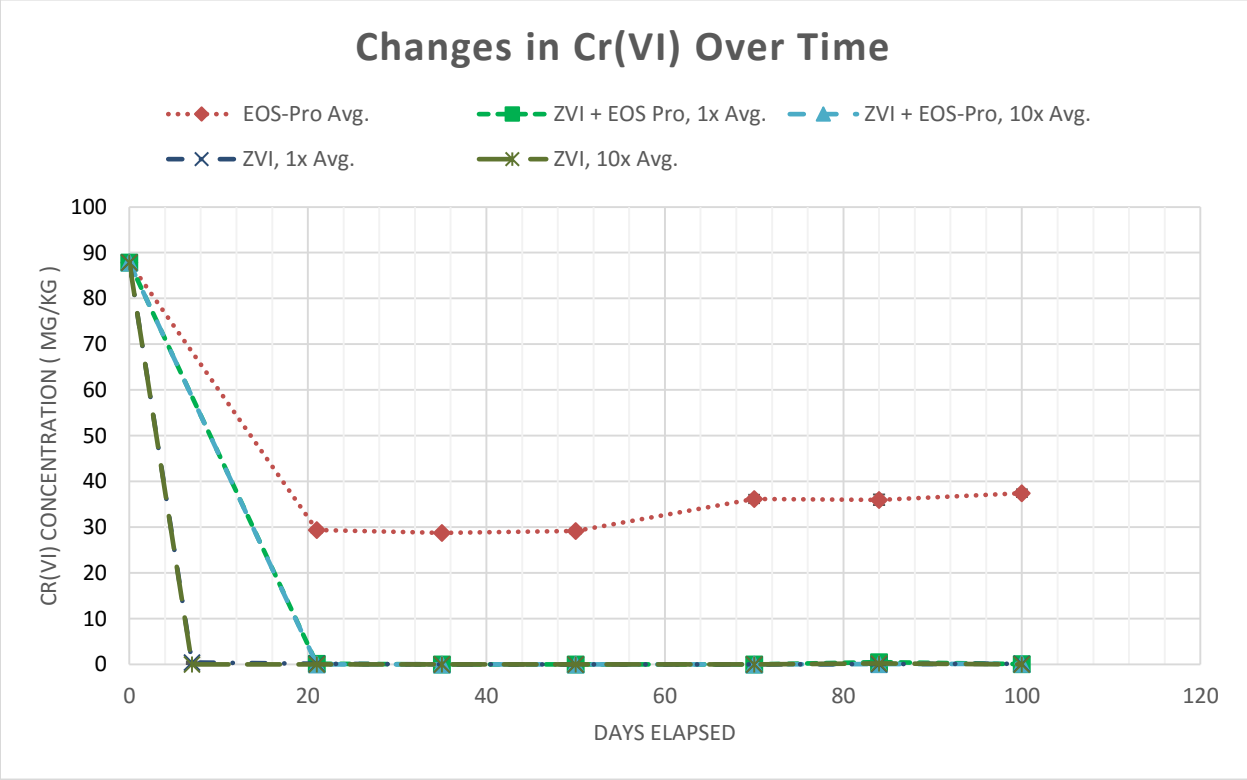

Figure 4-11: Reduction of Cr(VI) [in mg Cr(VI)/kg soil] observed in the ZVI remediation experiment microcosms treated using a combination of biotic reduction with EOS-Pro, abiotic reduction, and bio-ZVI reduction using EOS-Pro. Data points represent the means of duplicate Cr(VI) analysis ± 1 standard deviation.

In the case study by Němeček et al. (2014), removal rates of Cr(VI) with ZVI can be directly calculated using the graphs in figure 3 of the paper. These rates range from 0.1 to 0.11 mg/L · d, which translate to 0.41 to 0.44 mg/kg · d using the 4:1 ratio of mL DI water to g soil used for soil extraction.

Though these removal rates differ by roughly 15 to 20%, they are still in agreement with the average removal rates calculated for Cr(VI) reduction.

Compared to the anaerobic experiments, the biotic reduction samples using EOS-Pro in this experiment set exhibited greater Cr(VI) reduction (compare Tables 4-6 and 4-15), though the initial Cr(VI) removal rates were similar for both experiments (compare Tables 4-3 and 4-12). Cr(VI) stopped reducing in the biotic reduction samples after 21 days, with concentrations remaining relatively constant to the end of the experiment despite the samples being dosed with the same stoichiometric ratio of EOS-Pro to contaminated soil. The reason why becomes apparent upon analyzing the co-contaminant data.

4.3.5 – NO_3^- Reduction

Like Cr(VI), NO_3^- removal rates were significantly higher in the sample plots amended with ZVI, abiotic and bio-ZVI alike, compared to the biotic reduction sample plots (Figure 4-12). Removal rates ranged from 1.3 to 6 mg/d (Table 4-13). Zhang et al. (2019) reports that for their experiments, 25 mg/L of $NO_3^- \cdot N$ was reduced completely in the presence of ZVI and microorganisms within 3 days, which translates to a maximum average removal rate of 8.3 mg/L · d for bio-ZVI reduction. Similarly, 82% of $NO_3^- \cdot N$ and 22% of $NO_3^- \cdot N$ was reduced by microorganisms by themselves and ZVI by itself after 80 hours, which can be calculated to an average removal rate of 6.15 mg/L · d for biotic reduction and 1.1 mg/L · d for abiotic reduction.

Using the same 4:1 ratio of mL DI water to g soil for contaminant extraction previously mentioned, NO_3^- removal rates become 4.4, 24.6 and 33.2 mg/kg · d for abiotic, biotic and bio-ZVI reduction, respectively. Of these, removal rates are in agreement for the abiotic reduction of NO_3^-, while biotic and bio-ZVI reduction rates are much higher in the literature than those in the thesis. However, these were calculated within five days instead of the 7 to 21 days used for the sampling rate; thus, much like Cr(VI), it is possible that actual reduction rates were higher than those calculated for the thesis for much the same reasons outlined previously.

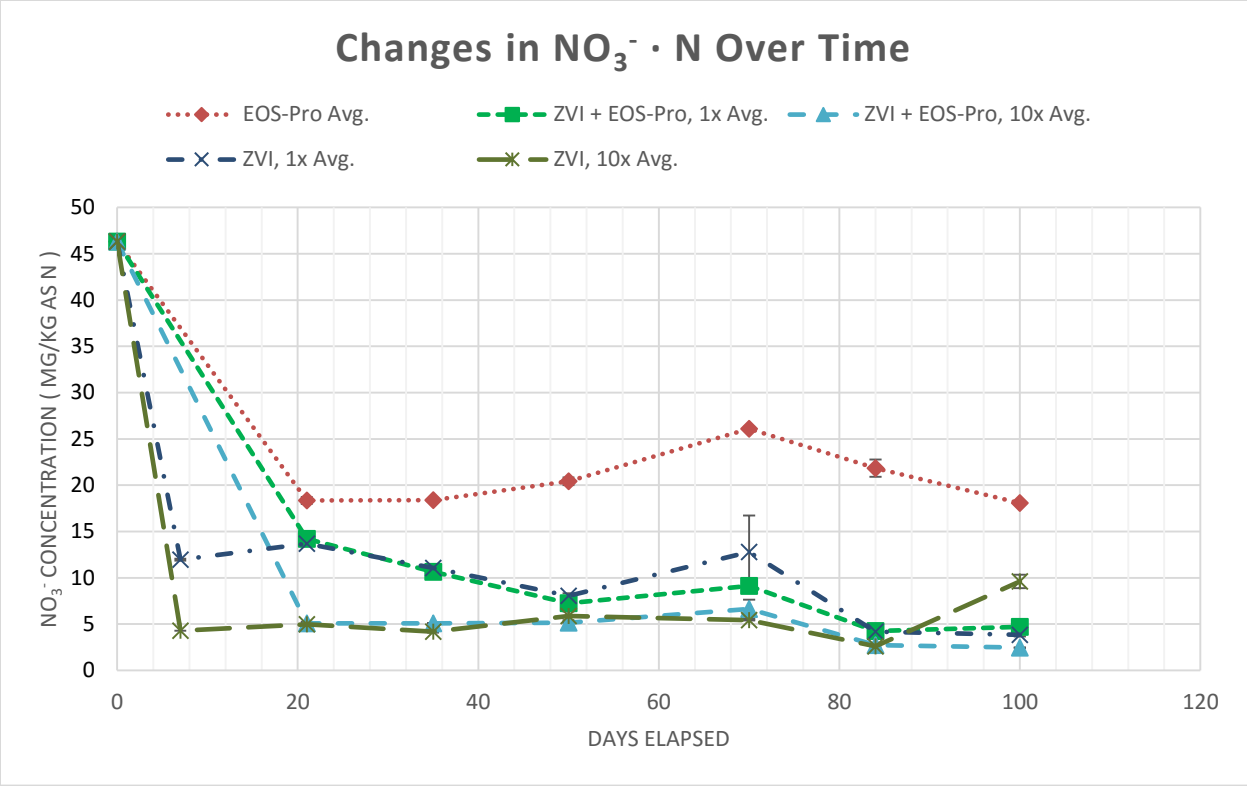

Figure 4-12: Reduction of NO_3^- [in mg NO_3^- · N/kg soil] observed in the ZVI remediation experiment microcosms treated using a combination of biotic reduction with EOS-Pro, abiotic reduction, and bio-ZVI reduction using EOS-Pro. Data points represent the means of duplicate NO_3^- · N analysis ± 1 standard deviation.

It is observed in Figure 4-12 that not all NO_3^- · N was reduced and removed from the ZVI-amended soil sample plots, with % removals ranging from 79% to 95% (Table 4-15). NO_3^- does not adsorb strongly onto soil particles due to its negative charge (Follett, 1995; Bhatnagar and Sillanpää, 2011), and based on redox potential NO_3^- should have been completely removed since ClO_3^- was completely degraded in the ZVI-amended samples.

One potential explanation could be in the measurement of NO_3^- · N. As the soil had high TDS concentrations, the samples had to be sent off to an outside laboratory for analysis; their method detection limit is 0.1 mg/L. If it was not possible to read levels of NO_3^- · N lower than 0.1 mg/L, this would result in up to 2 mg/kg of NO_3^- · N that was apparently left untreated in the rinsate. Another potential explanation is that the NO_3^- · N observed in the rinsate isn't actually NO_3^-, but nitrite [NO_2^-]. Su and Puls (2004) and Suzuki et al. (2012) report that trace amounts of NO_2^- are produced as an intermediate product

of nitrate reduction by ZVI. However, the USUAL analysis confirmed that the N in the rinse was from NO_3^- and not NO_2^-.

The most plausible explanation is the passivation of ZVI by NO_3^-; Luo et al. (2010) and Chen et al. (2013) both report that ZVI passivation by NO_3^- has hindered its reduction. These results appear to contradict the ClO_3^- results; however, the reason for why ClO_3^- was completely reduced by ZVI will be explained in the following section.

Like Cr(VI), $NO_3^- \cdot N$ concentrations in the biotic reduction sample plots remained relatively constant after 21 days of reduction (Table 4-15). Comparing these results with the anaerobic experiment results, where NO_3^- and ClO_3^- remained essentially untouched (Table 4-6), the lack of observable reduction beyond the first 21 days is suggested to be attributed to the microbial population consuming all available carbon source, instead of soil moisture levels inhibiting denitrification and ClO_3^- biodegradation.

4.3.6 – ClO_3^- Reduction

ClO_3^- removal rates in the ZVI-amended sample plots ranged from 685 mg/d to 2336 mg/d, compared to 385.7 mg/d in the biotic reduction sample plots (Table 4-14). Unlike Cr(VI) and NO_3^-, data for ClO_3^- reduction by ZVI is scarce, with Westerhoff (2003) being the only published paper this author could find where data was available. Westerhoff reports that 10 mM ClO_3^- was reduced with ZVI at neutral conditions within 8 hours, which calculates to a removal rate of 2500 mg/L · d or 10000 mg/kg · d for abiotic reduction of ClO_3^-. As abiotic reduction rates of ClO_3^- were in the order of thousands of milligrams reduced over several days, the thesis results are in agreement with Westerhoff (2003)'s work, though again, it is possible that reduction occurred faster than the sampling rate used for the experiments.

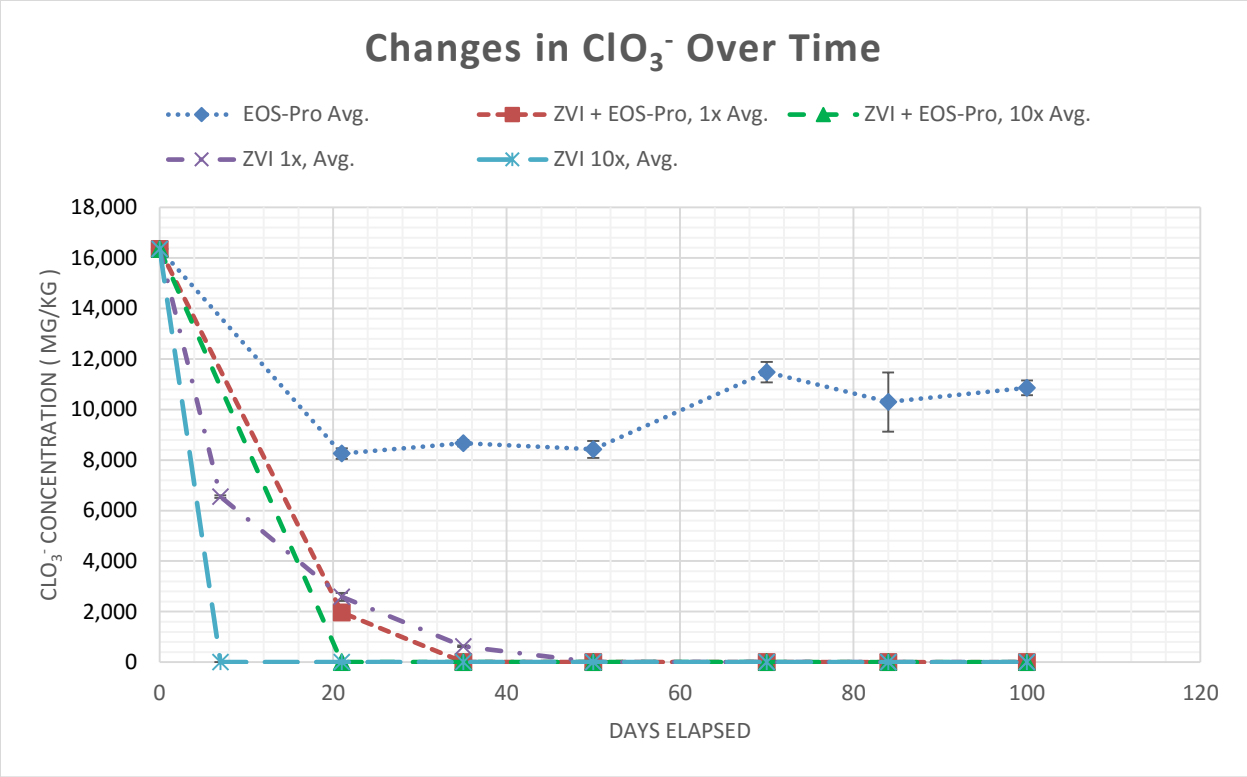

Figure 4-13: Reduction of ClO_3^- [in mg ClO_3^-/kg soil] observed in the ZVI remediation experiment microcosms treated using a combination of biotic reduction with EOS-Pro, abiotic reduction, and bio-ZVI reduction using EOS-Pro. Data points represent the means of duplicate ClO_3^- analysis ± 1 standard deviation.

It can be seen in Figure 4-13 that ClO_3^- is completely reduced in the ZVI-amended sample plots after 50 days of treatment, compared to the biotic reduction sample plots (Table 4-15), where contaminant levels remain stable after 21 days of reduction.

In his research, Westerhoff (2003) reports that ClO_3^- has a higher affinity for removal by ZVI (E_h = 1.89 V) than NO_3^- (E_h = 1.32 V). Comparing the ClO_3^- removal with the removal of NO_3^- from the sample plots thus makes it clear that following Cr(VI) reduction, ClO_3^- was reduced completely in the presence of ZVI, and that following its removal NO_3^- was reduced. However, as a result of passivation of ZVI, NO_3^- was not completely removed from the soil. This explanation not only follows the sequence of events suggested in the literature (Westerhoff, 2003; Luo et al., 2010; Chen et al., 2013), but also raises important implications about the contaminant reduction observed in the ZVI remediation samples.

4.3.7 – ZVI Remediation Byproducts

As Fe and NH_3 were major byproducts of concern from treatment using ZVI, additional graphs were constructed depicting the changes in dissolved Fe and aqueous NH_3 levels over the life of the experiment. Figures 4-14 and 4-15 depict Fe and $NH_3 \cdot N$ levels, respectively, over time in the abiotic and bio-ZVI reduction samples, where ZVI was used for treatment. Standard deviations in sample data are graphically displayed as vertical error bars.

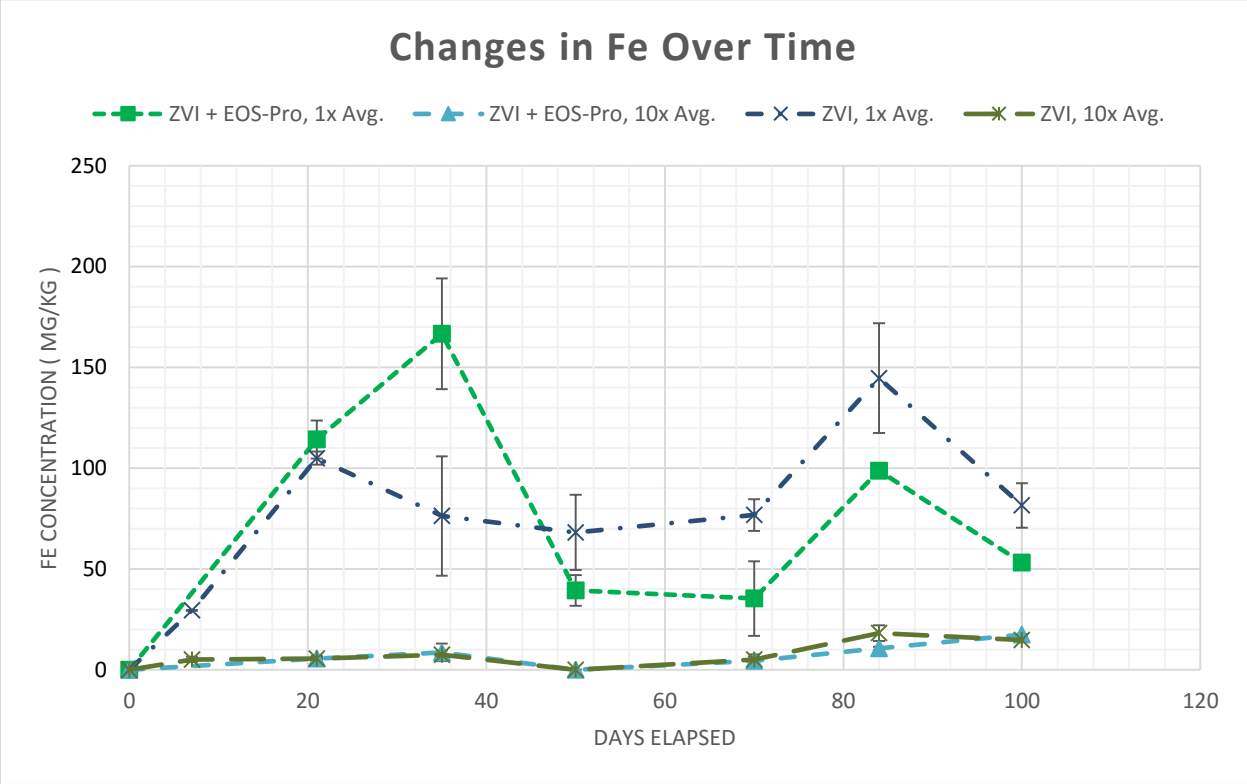

Figure 4-14: Fe concentrations [in mg Fe/kg soil] observed in the ZVI remediation experiment microcosms, in the abiotic reduction and bio-ZVI reduction samples where ZVI was among the amendments added to soil. Data points represent the means of duplicate Fe analysis ± 1 standard deviation.

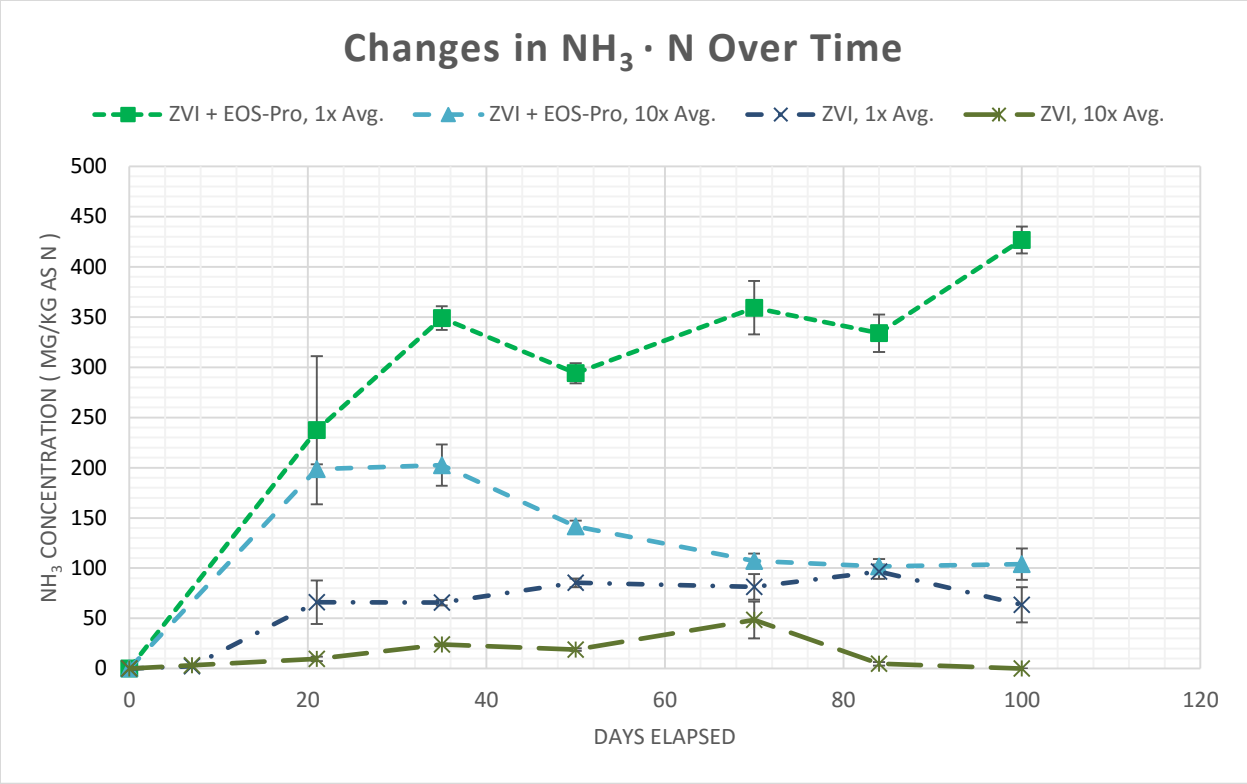

Figure 4-15: NH_3 concentrations [in mg NH_3 · N/kg soil] observed in the ZVI remediation experiment microcosms, in the abiotic reduction and bio-ZVI reduction samples where ZVI was among the amendments added to soil. Data points represent the means of duplicate NH_3 · N analysis ± 1 standard deviation.

Fe levels were observed to be overall higher in the sample plots with a 1:1 ZVI to contaminants ratio (Figure 4-14), while NH_3 · N levels were higher in the bio-ZVI reduction sample plots (Figure 4-15). With respect to Fe, the fluctuation in dissolved Fe concentrations is directly linked to soil moisture (Figure 4-9). As a result of lower soil moisture levels observed in the sample plots with a 10:1 ratio of ZVI to contaminants, less Fe was dissolved into the rinsate following reduction. In contrast, as there was excess soil moisture in the sample plots with a 1:1 ratio of ZVI to contaminants, the ZVI in those was completely dissolved into the rinsate, resulting in elevated dissolved Fe levels due to reduction of Cr(VI), NO_3^- and ClO_3^- releasing Fe into the rinse water.

NH_3 · N levels generated were expected to be directly proportional to the initial soil NO_3^- · N levels based on reaction stoichiometry (Equation 1). Based on Table 4-15, it was expected that 80 to 95% of the NO_3^- · N in the ZVI-amended samples [37 — 44 mg N/L] would be converted to NH_3 · N.

However, the bio-ZVI reduction samples consistently exhibited elevated levels of $NH_3 \cdot N$ in the rinse solution (Figure 4-15), raising the possibility that something else in the bio-ZVI reduction samples, like the nutrient amendments, were contributing to final $NH_3 \cdot N$ levels. The results of this analysis are shown in Table 4-20.

TABLE 4-20: AMMONIA NITROGEN ANALYSIS FOR BIO-ZVI REDUCTION NUTRIENT AMENDMENTS

Measured $NH_3 \cdot N$ Levels	
Component	Concentration (mg N/L)
Urea	12,500
EOS-Pro	180
Bacteria	400
Vitamin B12	0

The diluted EOS-Pro solution, the activated sludge bacteria, and the urea solution all contributed to the final $NH_3 \cdot N$ concentration, with the urea solution contributing the most N. This raises an important implication regarding nutrient and microbial amendments; depending on which amendments are added to vadose zone soils, further treatment of undesirable bioremediation byproducts may be required post-treatment.

Based on these results, ZVI dosage can affect the final soil and rinse quality. While higher doses of ZVI result in less Fe present in both soil and rinse, it also raises the implication that not all of the ZVI was used in reduction due to being present in excess; it was observed during the experiment that large pieces of unoxidized ZVI were among the particles that sank to the bottom during centrifugation. Soil moisture measurements of the samples with a 10:1 ratio of ZVI to contaminants confirm this implication (Figure 4-9), as soil moisture was consistently less than the initial measurement of 49.9% due to more ZVI being present in the sample than contaminated soil. Conversely, low doses of ZVI increase the Fe concentration in the final rinse, which in turn can affect soil and water quality if Fe is provided a means to mobilize into the underlying water table. ZVI doses can in turn affect $NH_3 \cdot N$ levels if it is combined with bioremediation methods.

4.3.8 – Blank Sample Analysis

Figures 4-16 through 4-18 illustrate the changes in the levels of Cr(V), $NO_3^- \cdot N$, and ClO_3^- in the carbon and biomass blank samples. The data shown is the average concentrations of contaminant in the soil, with the standard deviations shown on the graphs using vertical error bars.

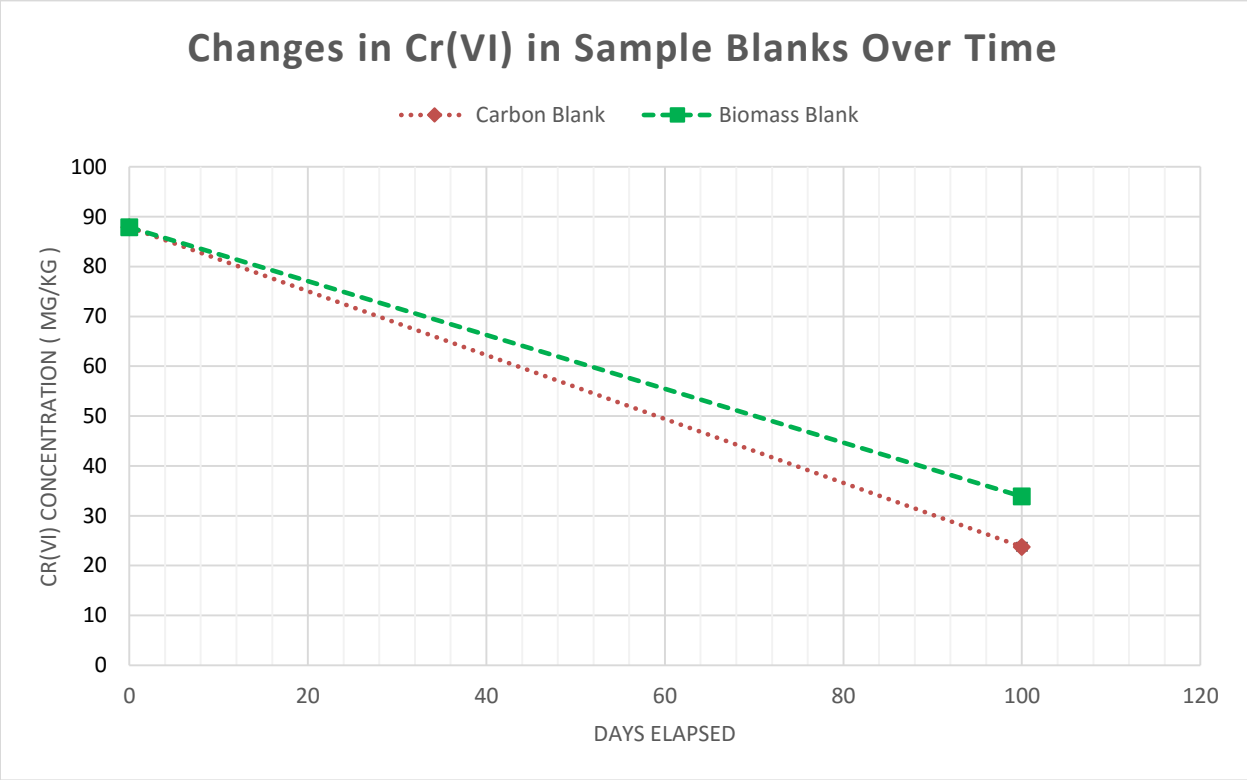

Figure 4-16: Changes in Cr(VI) concentrations [in mg Cr(VI)/kg soil] observed in the ZVI remediation experiment blanks. Data points represent the means of duplicate Cr(VI) analysis ± 1 standard deviation.

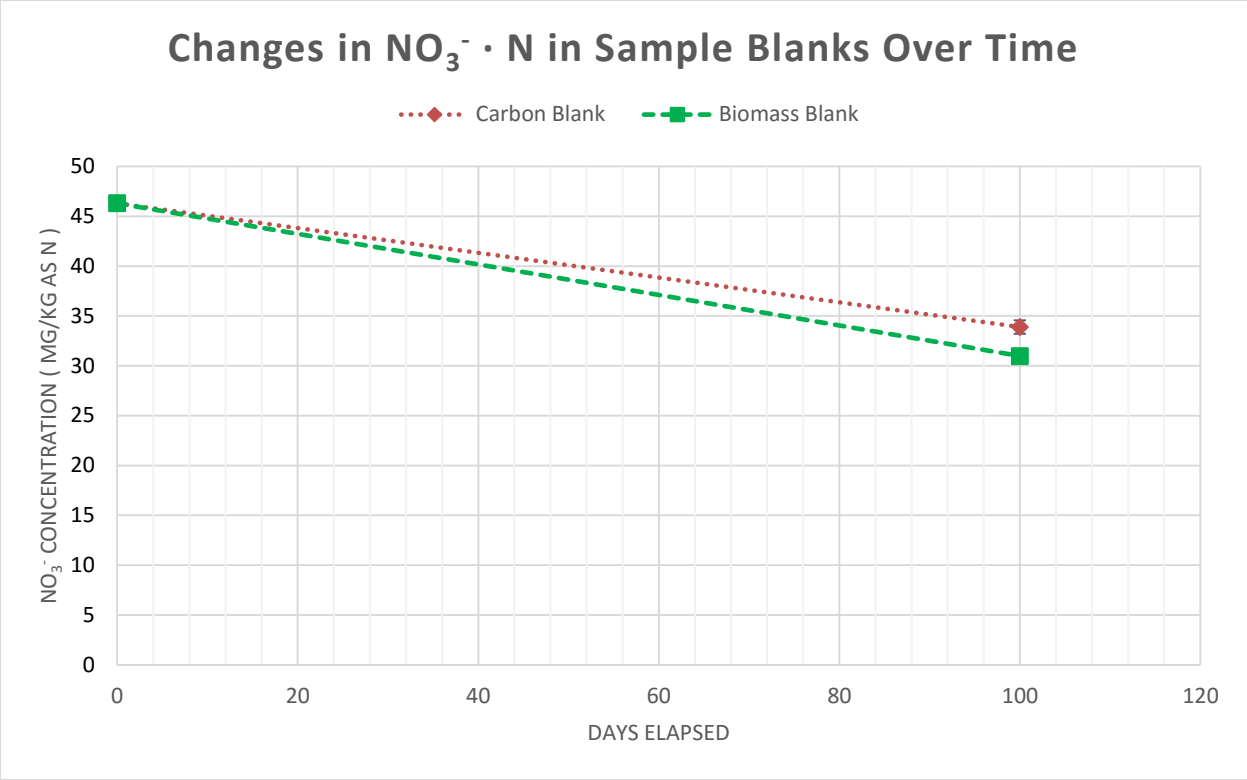

Figure 4-17: Changes in NO_3^- concentrations [in mg NO_3^- · N/kg soil] observed in the ZVI remediation experiment blanks. Data points represent the means of duplicate NO_3^- · N analysis ± 1 standard deviation.

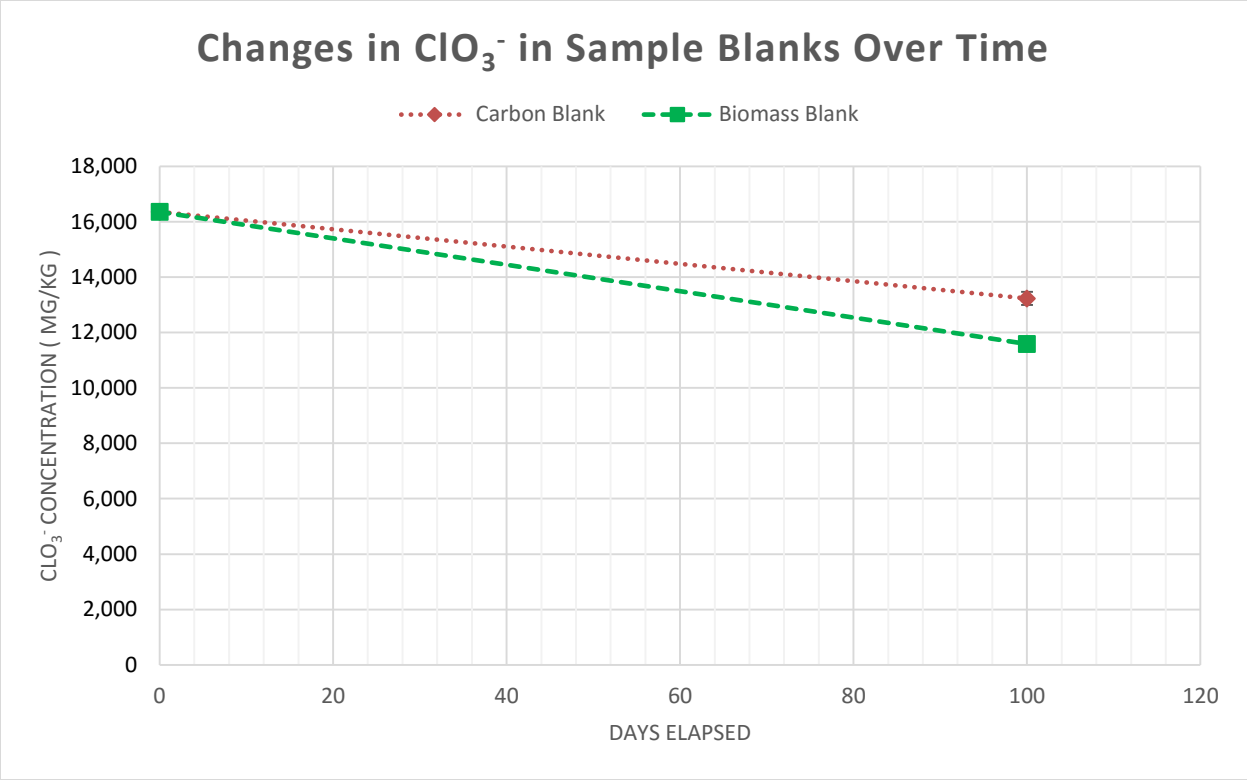

Figure 4-18: Changes in ClO_3^- concentrations [in mg ClO_3^-/kg soil] observed in the ZVI remediation experiment blanks. Data points represent the means of duplicate ClO_3^- analysis ± 1 standard deviation.

Contaminant removal rates and % removal were also calculated for the ZVI remediation sample blanks; these along with the final soil moisture and pH are summarized in Table 4-21. Positive values for removal rates indicate contaminant reduction, and removal rates are calculated per kilogram of contaminated soil.

TABLE 4-21: REMOVAL RATES, PERCENT REMOVAL, SOIL MOISTURE AND PH – ZVI REMEDIATION EXPERIMENT BLANKS

Sample Type	Soil Moisture (%)	pH	Cr(VI)		NO_3^- (as N)		ClO_3^-	
			Removal Rate (mg / d)	% Removal	Removal Rate (mg / d)	% Removal	Removal Rate (mg / d)	% Removal
Blank Samples								
Carbon	53.1%	8.03	0.64	73.0%	0.12	26.8%	31.25	19.1%
Biomass	56.3%	7.98	0.54	61.5%	0.15	33.1%	47.69	29.2%

Again, visual inspection of Figures 4-16 through 4-18 and an analysis of Table 4-21 confirm that reductive activity took place in the sample blanks, just like with the anaerobic experiments. Cr(VI) alone was reduced by up to 73%. Furthermore, significant NO_3^- reduction (26.8 — 33.1% removal) was also observed, more than double the % removals observed in the anaerobic sample blanks. ClO_3^- was also significantly reduced by 19 — 29%, compared to the anaerobic experiment blanks where no reduction took place (Table 4-21; compare to Table 4-8).

A one-way ANOVA was performed to determine if there were any statistically significant differences between blank sample treatments. The results are shown in Table 4-22.

TABLE 4-22: RESULTS OF THE ONE-WAY ANOVA FOR THE ZVI REMEDIATION EXPERIMENT BLANKS

Parameter	One-Way ANOVA Results - Blanks		
	Cr(VI)	NO_3^-	ClO_3^-
Sample Size (n)	4	4	4
F-value	100.53	9.08	47.06
p-value	0.01	0.09	0.02

At the 5% level, statistically significant reduction is observed for Cr(VI) and ClO_3^- ($p < 0.05$), implying that under these specific conditions, different amendments will reduce both contaminants differently. NO_3^- ($p = 0.09$) is the only contaminant for which no statistically significant differences are observed.

Interestingly, the carbon blank exhibited higher levels of Cr(VI) reduction compared to the biomass blank, while more NO_3^- and ClO_3^- reduction was observed in the biomass blank (Table 4-18). The explanation why is the same for those in the anaerobic experimental blanks; as the bacterial seed used came from a fluidized bed reactor treated with ethanol, some residual ethanol could have been taken with the seed solution and thus been utilized in contaminant removal.

It is also likely that the bacterial seed in the carbon blank and the EOS-Pro solution in the biomass blank were both utilized by the native microbes for contaminant reduction. This raises an

important observation for both the anaerobic and ZVI remediation blanks; it had been assumed that there were no native microbes in the soil to treat the contaminants. However, it was discovered that the addition of either carbon source or bacterial seed was enough to stimulate microbial reduction. In particular, the fluidized bed seed contained more than adequate amounts of carbon for the native microbes to utilize as a carbon source, thus resulting in the elevated rates of contaminant removal observed in the blank samples.

4.3.9 – Concluding Remarks

Compared to the anaerobic remediation results, the addition of ZVI to the soil produced a marked difference in contaminant reduction and removal. In general, the abiotic reduction and bio-ZVI reduction sample plots saw more significant contaminant removal compared to the biotic reduction sample plots. Cr(VI) and ClO_3^- were completely reduced within 21 and 50 days, respectively, while NO_3^- was reduced by ≥ 79% in all of the mixed ZVI samples. The % removal in the sample blanks and the biotic reduction samples followed the expected order of contaminant removal from soils based on redox potentials, with Cr(VI) being significantly reduced first, followed by NO_3^- and ClO_3^- immediately after. However, in the samples amended with ZVI, following Cr(VI) reduction ClO_3^- was completely reduced first before NO_3^- began reducing, and NO_3^- reduction was inhibited. A potential explanation for why NO_3^- was not completely reduced in these microcosms is related to abiotic reduction with ZVI more than biotic reduction with EOS-Pro. The reduction of Cr(VI) and ClO_3^- observed were likely caused via abiotic reduction; NO_3^- is known for its inability to reduce in the presence of ZVI without passivating the ZVI surface (Luo et al., 2010; Chen et al., 2013).

Treatment in the biotic reduction sample plots resulted in % removals of 34 to 61 percent. Compared to the anaerobic experiments, the % removals are higher and more significant (Tables 4-6 and 4-15). This is largely attributed to the fact that, despite anaerobic conditions being observed at the bottom of the sample plots, the samples themselves weren't entirely anaerobic. Though aluminum foil was used to cover all samples, it was meant to prevent soil desiccation rather than free oxygen flow along the top of the sample plots. In actual applications, the ZVI and organic electron donor/carbon sources would be

mixed into the vadose zone soils and be exposed to the atmosphere. Soil moisture levels were also higher than those in the anaerobic experiment as a result of regular soil wetting, which encouraged higher contaminant degradation especially with NO_3^-.

4.4 – Applications to Future Remediation Studies

In light of the results of both experiments, several important observations can be made. While both biotic reduction and bio-ZVI reduction are appealing alternatives for treatment of highly contaminated vadose zone soils, both of them come with their own disadvantages. For both types of treatment, ambient soil conditions will be a major factor in determining treatment strategies; in particular, soil moisture will be a major factor in treatment effectiveness, especially if NO_3^- is among the contaminants at a given site (Bouwman, 1998). For soils without adequate moisture, drip irrigation techniques could be utilized for water supply.

Another factor is the initial soil contaminant concentration; contaminant removal generally decreases with a corresponding increase in the initial concentration (Narayani and Shetty, 2013; Zhang et al., 2019). In particular, for sites where NO_3^- and ClO_3^- are co-contaminants, the presence of NO_3^- will directly impact the efficiency of biological degradation of ClO_3^-, as bacteria will remove NO_3^- first prior to reducing ClO_3^- (van Ginkel, Plugge and Stroo, 1995).

Soil type is also a major consideration; for fine-grained expansive clay soils with high mineral content like the one used in this experiment, soil permeability may be low enough that in-situ treatment methods may prove ineffective or inefficient at contaminant removal (Fruchter, 2002). Furthermore, due to their higher water-holding capacity, fine-grained soils may be contaminant sinks, holding greater contaminant concentrations than coarse-grained soils and thus be more difficult to treat (Dresel et al., 2011). As these are the main factors that largely determine extent and duration of contaminant removal, initial studies should be performed to determine site conditions and soil characterization prior to treatment implementation.

Biotic reduction of contaminants by itself without ZVI is not recommended as a treatment strategy for arid vadose zone soils, as anaerobic soil conditions are generally associated with wetland environments and poorly draining soils, not arid and semi-arid regions (van Keulen, 1977; Inglett, Reddy and Corstanje, 2005). This strategy may be viable for deep vadose zone soils close to the water table, where soil moisture levels are sufficient enough to sustain prolonged conditions for microbial growth.

Another issue to be addressed is flow conditions at the site. Typical vadose zone flow is governed by preferential gravity flow, capillary forces and ephemeral surface infiltration (Dresel et al., 2011); these flow conditions can potentially inhibit any treatment strategy that employs biological degradation, anaerobic or otherwise. Furthermore, any water discharged into the vadose zone to stimulate this process runs the potential risk of mobilizing the contaminants and discharge them into the underlying water table (Hanson et al., 1993; Dresel et al., 2011).

As mentioned before, soil flushing has been used effectively in the past to remove contaminants from vadose zone soils (National Risk Management Research Laboratory, 2000; Jacobs and Rouse, 2005). Soil flushing transports contaminants from the vadose zone to the saturated zone, where it can be pumped and treated either ex-situ or in-situ. However, one of the major drawbacks of soil flushing is the large volume of water typically utilized to flush contaminants from soil. At the United Chrome Products site, up to 4 million gallons of water was utilized to remove 90% of the Cr(VI) from the soil (National Risk Management Research Laboratory, 2000). Flushing techniques also have the potential to instead mobilize the contaminant, producing a lateral and vertical front that can potentially spread it into the underlying groundwater (Hanson et al., 1993; National Risk Management Research Laboratory et al., 2000). In this research study, enhanced soil flushing is proposed as an alternative, where the contaminants are degraded as water travels through the vadose zone. The treatment method is similar to soil flushing in that a flushing solution is added to soil to remove contaminants; however, these techniques are meant to immobilize chromium as Cr(III) and also reduce its co-contaminants to innocuous compounds in place. By using the flushing solution to reduce the contaminant, less water is likely to be used and the

contaminant loading transported to the saturated zone is likely to be significantly reduced. An example of a similar technique is the use of foam to deliver CaS_5 to and immobilize Cr(VI) in soils (Zhong et al., 2009). Enhanced soil flushing thus has the potential to reduce the amount of water used to flush the contaminant — and thus decrease treatment costs and increase sustainability.

Though ZVI has been shown to effectively reduce Cr(VI), NO_3^- and ClO_3^- in a number of studies (Westerhoff, 2003; Gheju, 2011; Mitra et al., 2011; Zhang et al., 2019), the use of ZVI in contaminated vadose zone treatment has drawbacks and potential issues that must be addressed prior to full-scale implementation. One major drawback is the generation of byproducts such as Fe and NH_3. As seen in Figures 4-14 and 4-15, excessive amounts of both byproducts were detected in the rinse water following treatment in the ZVI remediation experiments. The U.S. EPA has set a secondary MCL for Fe at 0.3 mg/L and a lifetime exposure advisory for NH_3 at 30 mg/L (U.S. EPA, 2018); at the water to soil ratio of 4:1 used in the ZVI remediation experiments, Fe and NH_3 concentrations in the rinsate were well in excess of the allowable standards. As these could potentially negatively impact the environment downgradient of the discharge point, additional treatment may be required to remove these byproducts from vadose zone soils post-treatment — which could prove inefficient from an economic standpoint. Another potential drawback is ZVI passivation, especially at sites where NO_3^- is a contaminant; severe passivation of ZVI can impact both the reduction capacity and the long-term performance of ZVI systems and thus have an impact on treatment costs if a strategy for rejuvenating or replacing passivated ZVI isn't developed prior to implementation (Luo et al., 2010).

ZVI is considered a competitive alternative to other reducing agents for the removal of Cr(VI) and its co-contaminants from soil due to its low cost and ease of operations as part of a permeable reactive barrier system (Gheju, 2011; Mitra et al., 2011; Němeček et al., 2014). However, the lack of information about potential environmental risks has prevented it from being used in full-scale applications. Němeček et al. (2014) and Zhang et al. (2019) have published research showing that bacterial communities are not adversely affected by ZVI; the presence of ZVI has stimulated bacterial

growth due to its production of H_2 as an electron donor. However, Gheju (2011) reports that nanoscale ZVI in particle form can be cytotoxic to microbes. The environmental fate of ZVI and its potential risk to the environment, as well as the concern over the mechanism by which ZVI is potentially toxic to microbes and the environment, are both issues that need to be addressed prior to full-scale implementation.

This potential for environmental risk directly affects the ZVI dosage used for treatment. As discussed in section 4.3.7, while a 1:1 ratio of ZVI to contaminant is enough to significantly reduce contaminant levels in the vadose zone, if not completely remove them, the large concentrations of byproducts produced could incur additional costs for removal. However, high ZVI to contaminant ratios such as the 10:1 ratio used in the ZVI remediation experiments could prove to be costly and economically impractical, as not all ZVI would be utilized in contaminant biodegradation. There is also the potential for minerals to precipitate out and reduce ZVI redox capacity as a result of the increase in pH associated with reductive activity (Fruchter, 2002). There are potential advantages to using higher ZVI to contaminant ratios, however, especially if the treatment strategy involves maintaining reductive conditions long enough to ensure complete biodegradation of Cr(VI) and its co-contaminants.

CHAPTER 5

CONCLUSIONS

5.1 – Thesis Significance

Much of the research on the removal of Cr(VI), NO_3^- and ClO_3^- from soils largely involve saturated zone soils and the groundwater table. With respect to Cr(VI) removal from water in general, Narayani and Shetty (2013) and Gheju (2011) both published exhaustive review articles discussing microbial degradation and chemical reduction with ZVI in great detail. Research studies also discuss the available technologies and remediation strategies for removing NO_3^- and ClO_3^- from soil (Bhatnagar and Sillanpää, 2011; Ebrahimi and Roberts, 2013), with several published works focusing on bioremediation (Logan and Lapoint, 2002; Rao et al., 2010; Mastrocicco et al., 2017; Ucar et al., 2017) and reduction using ZVI (Westerhoff, 2003; Su and Puls, 2004; Suzuki et al., 2012).

However, very few studies have been published with respect to Cr(VI) removal from soils under unsaturated flow / vadose zone conditions, with Oliver et al. (2003) being the only published study that the author could find referencing microbial degradation of Cr(VI) in vadose zone soils. Zhong et al. (2009) is similarly the only published work that this author has found that discusses chemical reduction of Cr(VI) in vadose zone soils using reducing agents – in this case, foam laced with CaS_5. In both studies, Cr(VI) was the only contaminant studied; neither the presence of co-contaminants nor the reduction conditions were discussed in detail. Another recently-published study by Zhang et al. (2019) discusses the use of advanced bioremediation techniques with ZVI in the treatment of NO_3^-; however, these were performed under anoxic conditions and in batch microcosm tests in aqueous solution, and not in soil.

The results of the research performed for this thesis thus contribute new knowledge to the removal of Cr(VI) in the presence of competing co-contaminants [NO_3^- and ClO_3^-], vadose zone contaminant removal under anaerobic conditions, and vadose zone treatment using ZVI. This thesis also

discusses the potential for future vadose zone treatment using a combination of bioremediation and geochemical fixation with ZVI.

5.2 – Thesis Conclusions

This thesis investigated and analyzed two different types of bioremediation techniques for vadose zone soils: biotic reduction by itself under anaerobic conditions, and contaminant removal using ZVI, both by itself in abiotic reduction and in combination with organic electron donor/carbon sources in bio-ZVI reduction. Microcosm tests were performed using both methods to assess Cr(VI) removal in the presence of two co-contaminants: NO_3^- and ClO_3^-. Anaerobic experiments were performed to compare EOS-Pro and molasses as carbon sources for biological contaminant removal, while ZVI remediation experiments assessed different combinations of ZVI and organic electron donors for contaminant removal. The findings of this research are summarized thus:

1. Molasses was determined to be more efficient than EOS-Pro at reducing Cr(VI) biotically under anaerobic conditions. However, due to lack of complete Cr(VI) reduction in the sample, NO_3^- and ClO_3^- reduction was not observed, as redox potentials favor the complete removal of Cr(VI) prior to reduction of NO_3^- and ClO_3^-. The incomplete removal of Cr(VI) is speculated to be the result of insufficient soil moisture.

2. Soil moisture is an important factor in determining the degree of biological contaminant removal, especially in soils where NO_3^- is a known contaminant. Biotic reduction of contaminants is more likely to be enhanced in soils with higher moisture contents. Furthermore, anaerobic bioremediation of vadose zone soils, especially in arid and semi-arid regions like those modeled in the experiment, is not considered a viable treatment strategy as anaerobic conditions do not occur in and are generally not characteristic of vadose zone soils (van Keulen, 1977; Inglett, Reddy and Corstanje, 2005). In dry vadose zone conditions, additional moisture is needed if bioremediation is to be accomplished.

3. Soils treated using either ZVI by itself or in combination with biotic reduction with organic electron donor/carbon source resulted in more complete contaminant removal compared to bioremediation alone. Cr(VI), NO_3^- and ClO_3^- were all significantly or completely reduced from vadose zone soil within 50 days of treatment. As ZVI requires water to oxidize and produce H_2, enough soil moisture must also be present for this technique to be utilized.

4. Soil amendments added during treatment using ZVI, both for abiotic and bio-ZVI reduction, have an impact on the potential end products and byproducts produced, both directly and indirectly. The carbon source, urea solution, and activated sludge added as soil amendments in the bio-ZVI reduction samples were all detected as excess $NH_3 \cdot N$ in the final rinse.

5. ZVI dosage impacts the final level of Fe detected in the rinse solution. At a 1:1 ratio of ZVI to contaminants in soil, the observed ZVI levels were elevated as a result of the reactions with Cr(VI), NO_3^- and ClO_3^- reducing all present contaminants completely. At a 10:1 ratio, the same level of contaminant removal was observed, but lower Fe concentrations were detected as a result of excess ZVI in the samples not being consumed in the reduction reactions.

6. The sample blanks in both sets of experiments were observed to have significant reduction of Cr(VI), NO_3^- and ClO_3^- following the experiment as a result of providing rich carbon sources to the native microbes in the form of fluidized bed reactor bacterial seed, EOS-Pro and molasses — the last of which can also be used to reduce Cr(VI) abiotically. These observed reduction levels were low compared to the experiment samples and were expected as the soil itself contained native bacteria, which utilized the added activated sludge / electron donor to the controls as a carbon source.

7. Of the two treatment methods, ZVI — either by itself or combined with biotic degradation using organic electron donor/carbon source — is the more effective of the two treatment strategies at removing Cr(VI) and its co-contaminants, NO_3^- and ClO_3^-, from vadose zone soils. However, the

production of undesirable byproducts like Fe and NH_3, the continued controversy over its toxicity to microbes, the potential for ZVI passivation in the presence of NO_3^-, and the overall lack of information about its environmental fate and direct and indirect effects are all issues that need to be addressed prior to using ZVI in full-scale treatment operations.

5.3 – Further Research

Because there is still plenty of work left to be done to further investigate vadose zone bioremediation techniques, the following subjects of research have been suggested for future work. As far as the author is aware, research is either ongoing in or not started on the following topics:

1. Further experiments are needed to determine the degree of which vadose zone soil moisture affects biological treatment. It was observed during the anaerobic experiments that NO_3^- and ClO_3^- degradation was inhibited due to low soil moisture, despite Cr(VI) reduction being observed. A range suggested for future investigations is from 10% to 50% soil moisture, which is within the range of soil moisture levels observed in the highly contaminated vadose zone soils at the Hanford River site in southeast Washington state and the Savannah River Site in South Carolina (Subramanian, 2007; U.S. Department of Energy, 2011).
2. ClO_4^-, a common co-contaminant along with Cr(VI), was not studied in this thesis due to both the competing co-contaminants [NO_3^- and ClO_3^-] that would have inhibited degradation and the high TDS interfering with analysis. As all contaminants were reduced completely with bio-ZVI reduction, there is potential for similar techniques to significantly reduce, if not remove entirely, ClO_4^- from vadose zone soils, especially in the presence of multiple co-contaminants.
3. The interactions between the reducing bacteria and the ZVI are not well-understood. In some applications, the presence of ZVI had no effect on and stimulated the growth of microbes (Němeček et al., 2014; Zhang et al., 2019). However, actual analysis into this interaction was not performed. Studies have reported that ZVI can be potentially toxic to microbes (Gheju, 2011), but

the exact mechanism into how has not yet been fully determined. Future research could further investigate the mechanisms through which bacteria and ZVI interact.

4. Other potential byproducts of contaminant reduction using ZVI need to be studied. For example, Westerhoff (2003) reports that the principal byproduct of ClO_3^- reduction by ZVI is Cl^-; however, Fe and NH_3 were the only two reaction byproducts addressed in this study. Furthermore, very little information about the interactions between ClO_3^- and ZVI has been published, with Westerhoff (2003) being the only study this author could find discussing ClO_3^- degradation by ZVI.
5. Very few remediation studies about vadose zone / unsaturated zone contamination in general have been performed as a result of capillary and pressure gradient flow. As contaminant transport models under vadose zone conditions already exist, potential topics of study could focus on the development of dynamic models for specifically predicting contaminant removal over time; studies can also examine the effect of particle size (coarse vs. fine-grained soil) on vadose zone contaminant removal.

APPENDIX A: NUTRIENT CALCULATIONS FOR BIOTIC CONTAMINANT REDUCTION EXPERIMENTS

A.1 – Organic Electron Donor Requirements

The calculations in this appendix are to supplement the experimental procedures described in section 3.3: "Microcosm Tests: Biotic Contaminant Reduction". Problems in measuring NO_3^- concentrations in the original soil were encountered prior to experimental implementation as a result of high TDS concentrations in the soil, facilitating additional analysis from the USUAL facility to obtain accurate NO_3^- values. No interference was encountered in measuring Cr(VI) or ClO_3^-.

As soil samples were formed prior to receiving corrected data from USUAL, all contaminant values used in this analysis do not account for NO_3^- TDS interference. Using the Hach analytical method for NO_3^-, the aforementioned interference resulted in reported NO_3^- values being higher than the actual NO_3^- values. Furthermore, the calculations were specifically performed for wet soil and not corrected for soil moisture like the contaminant analysis results shown in Table 3-1.

As this calculator was originally developed for contaminant treatment using EOS-Pro as the carbon source, Table A-1 was used to calculate how many milligrams of the contaminants of interest — Cr(VI), NO_3^-, and ClO_3^- — were present in the 125-gram soil cylinders to be incubated. Once the total milligrams of contaminant were calculated, the total EOS-Pro in its undiluted form was calculated using stoichiometry. COD equivalents were used to determine how much molasses was required for treatment, using EOS-Pro and molasses COD to convert between both (Table A-2). The final amount of diluted solution required for each 125-gram individual soil cylinder was then calculated (Table A-3). As the soil samples were made in batches weighing 750 grams total, the total amount of diluted carbon source was multiplied by a factor of six to account for the increased soil mix weight.

TABLE A-1: CONTAMINANT LEVELS IN SOIL A PER 125-GRAM SOIL CYLINDER

contaminants in wet soil			
	mg/kg	mg in 125 g wet soil	lb in 125 g wet soil
Perchlorate (ClO_4^-)	1286	160.75	0.00035365
Nitrate (NO_3^-)	346.6	43.325	0.000095315
Chromate (Cr^{+6})	45.7	5.7125	1.25675E-05
Oxygen (O_2)	132	16.5	0.0000363
Chlorate (ClO_3^-)	8450.38	1056.2975	0.002323855
	total	1282.585	0.002821687
Volume of groundwater to be treated (for soil with porosity of 30%) L	0.10	L	

TABLE A-2: CARBON SOURCE PROPERTIES

	Molasses	EOS-PRO
Density (kg/L)	1.4	0.98
COD (mg/L)	1,000,000.00	2,000,000.00

TABLE A-3: DILUTED EOS-PRO AND MOLASSES SOLUTION REQUIREMENTS FOR BIOTIC REDUCTION EXPERIMENTS

EOS-PRO and Molasses Calculations		
1 lb EOS PRO provides H_2 for	13	lb contaminant
Total volume of groundwater to be treated (L)	0.10	
contaminants in lb	0.002821687	lb
lb EOS-PRO needed to remove the contaminant	0.000217053	lb
EOS-PRO needed to remove the contaminant	**0.098660385**	g EOS-PRO
	0.100673862	mL EOS-PRO
Molasses needed to remove the contaminant	**0.201347724**	mL Molasses
Dilution Factor	10	

	EOS-Pro	**Molasses**
Volume of diluted solution needed (mL)	1.0067	2.0135
Rounded volume of solution (mL)	**1.00**	**2.00**

APPENDIX B: ZVI DOSAGE AND NUTRIENT CALCULATIONS FOR CONTAMINANT REDUCTION USING ZERO-VALENT IRON AND ORGANIC ELECTRON DONORS EXPERIMENTS

B.1 – ZVI Dosage Requirements

The calculations in this appendix are to supplement the experimental procedures described in section 3.4: "Microcosm Tests: Contaminant Reduction Using Zero-Valent Iron and Organic Electron Donors". Like the calculations performed in Appendix A, the contaminant values here do not account for NO_3^- TDS interference, and are performed using saturated soil. Thus, they are also not corrected for soil moisture like the contaminant analysis results seen in Table 3-1.

Tables B-1 through B-3 are part of a pre-existing ZVI calculator designed to calculate the ZVI dose required to reduce contaminants in water; the amount required is based on the stoichiometric amount of ZVI required to react with one mole of contaminant. These molar ratios were previously compiled in another related project. Once the stoichiometric amount of ZVI required for removal of all contaminants present in the soil were calculated, the dosage was then adjusted to calculate for contaminants present in 65 grams of soil, the amount of soil sample placed per seedling pot in the ZVI remediation experiments. These dosage calculations are seen in Tables B-1 through B-3.

TABLE B-1: CONTAMINANT LEVELS IN SOIL A

Compound	**Molecular Weight** *grams/mole*	**Concentration** *mg/kg, avg*	*mmoles/kg*
Oxygen	32	**2.00**	0.063
Chromate (i.e. Cr^{6+})	116	**45.70**	0.394
Nitrate	62	**346.6**	5.591
Chlorate	83.5	**8,450.38**	101.202
Perchlorate	99.5	**1,286.0**	12.924

TABLE B-2: ZVI REQUIRED FOR TREATMENT AT DIFFERENT ZVI : CONTAMINANT RATIOS

Compound	Molar Ratio	Stoichiometric		ZVI needed 1	5	10
	ZVI : Contaminant	*mmoles/kg*	*mg / kg*	*g / kg*	*g / kg*	*g / kg*
Oxygen	2	0.13	7.0	0.007	0.04	0.1
Chromate (i.e. Cr^{6+})	1.5	0.59	33.09	0.033	0.17	0.33
Nitrate	4	22.36	1,252.3	1.252	6.3	12.5
Chlorate	3	303.61	17,002.0	17.002	85.01	170.0
Perchlorate	4	51.70	2,895.0	2.895	14.5	29.0

TABLE B-3: ZVI REQUIRED AT DIFFERENT ZVI : CONTAMINANT RATIOS PER 65-GRAM SOIL SAMPLE PLOT

		ZVI to Contaminant Ratios 1	5	10
Total grams ZVI needed per kilogram of contaminated soil	grams	21.19	106	212
ZVI needed to remove O2, Cr, NO3, ClO3 (co-contaminants)	grams	18.29	91.47	182.94
Ratio ZVI Co-contaminants/ZVI perchlorate	grams	6.32	6.32	6.32
% Total ZVI needed for perchlorate reduction	%	15.82%	15.82%	15.82%

Soil mass	grams	65	65	65
	kilograms	0.065	0.065	0.065
Total grams ZVI needed per mass of soil	grams	**1.377**	**6.887**	**13.773**

B.2 – Carbon Source Requirements

For this set of experiments, only one organic electron donor / carbon source was used for the biotic and bio-ZVI reduction sample plots – diluted EOS-Pro. Like the calculations in Table A-1, the amount of EOS-Pro needed was calculated for the total milligrams of contaminant to be expected in 65 grams of soil sample, and the dose was adjusted accordingly based on dilution factor and the number of samples made. The calculations for the amount of diluted EOS-Pro per individual sample are seen in Tables B-4 and B-5.

TABLE B-4: CONTAMINANT LEVELS IN SOIL A PER 65-GRAM SOIL SAMPLE PLOT

contaminants in wet soil			
	mg/kg	mg in 65 g wet soil	lb in 65 g wet soil
Perchlorate (ClO_4^-)	1286	83.59	0.0001839
Nitrate (NO_3^-)	346.6	22.529	4.956E-05
Chromate (Cr^{+6})	45.7	2.9705	6.535E-06
Oxygen (O_2)	132	8.58	1.888E-05
Chlorate (ClO_3^-)	8450.38	549.2747	0.0012084
		666.9442	0.0014673
Volume of groundwater to be treated (for soil with porosity of 30%) L	0.10	L	

TABLE B-5: DILUTED EOS-PRO SOLUTION REQUIREMENTS FOR ZVI REMEDIATION EXPERIMENTS

EOS-PRO calculations		
1 lb EOS PRO provides H_2 for	13	lb contaminant
Total volume of groundwater to be treated (L)	0.10	
contaminants in lb	0.00146728	lb
lb EOS-PRO needed to remove the contaminant	0.00011287	lb
EOS-PRO needed to remove the contaminant	**0.051303400**	g EOS-PRO
	0.052350408	mL EOS-PRO
Dilution Factor	10	
Volume of diluted solution needed	0.523504082	mL diluted EOS-Pro
Rounded volume of solution	**0.52**	mL diluted EOS-Pro

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THESIS TITLE

Chromium(VI) and Oxyanion Remediation of Vadose Zone Soils with Zero Valent Iron (ZVI) and Biological Reduction

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