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## Role of Zero Valent Iron and Organic Substrates in Chlorinated Solvent Degradation: An Ex-Situ Remediation Case Study

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Graduate Program in Chemical and Biochemical Engineering  
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Engineering Science  
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## Abstract

Field practice suggests that a combination of biotic and abiotic technologies to treat soil impacted by chlorinated solvents positively influences a remediation project's success rate. Two large remediation programs have used a material containing both zero-valent iron (ZVI) and a dry organic substrate to abiotically reduce contaminants and increase anaerobic bioremediation in soil contaminated with tetrachloroethylene and 1,2-dichloroethylene using ex-situ mixing techniques. This research assesses the contributions made by the dry organic substrate and ZVI to the observed changes in chlorinated solvent concentrations by analyzing field samples collected from the sites previously remediated, as well as conducting bench-scale batch reactor experiments designed to test the individual contributions of the ZVI and the organic substrate to dechlorination processes. Laboratory experiments suggest the mixture of ZVI and organic substrate does not lead to the concentration decreases observed in the full-scale remediation projects, and that volatilization may be the most prominent contributing process for contaminant removal from soil. Field samples analyzed for microorganisms show a community shift in the area remediated as well as a decrease in *Dehalococcoides* population size, indicating soil mixing is detrimental to microbial dechlorination activity.

## Keywords

Remediation, zero valent iron, ZVI, bioremediation, biostimulation, *Dehalococcoides*, tetrachloroethylene, 1,2-dichloroethane, chlorinated solvents.

# Co-Authorship Statement

This thesis was written in accordance with regulations and guidelines for integrated-article format by the Faculty of Graduate and Postdoctoral Studies at the University of Western Ontario. Experimental design, data collection, and data analysis for both laboratory and field investigations were conducted by the candidate under the supervision and guidance of Dr. Jose Herrera. The co-authorship of Chapter 3 is as follows:

Chapter 3: Abiotic and Biotic Effects of Zero Valent Iron and Organic Substrates in an Ex-Situ Chlorinated Solvent Contaminated Soil Remediation Project

By Alexander Stevenson and Jose Herrera

Contributions:

- |              |                                                                                                                                             |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| A. Stevenson | Initiated research topic, designed and conducted field and laboratory experiments, performed data interpretation, and wrote chapter drafts. |
| J. Herrera   | Assisted in experiment design and data interpretation, and reviewed/revised draft chapters.                                                 |

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# Table of Contents

Abstract.....	i
Co-Authorship Statement.....	ii
Acknowledgments.....	iii
Table of Contents.....	iv
List of Tables .....	vii
List of Figures .....	viii
List of Appendices .....	xi
Chapter 1.....	1
1 Introduction.....	1
1.1 Background.....	1
1.2 Research Objectives.....	3
1.3 Thesis Outline.....	4
1.4 References.....	5
Chapter 2.....	7
2 Literature Review.....	7
2.1 Introduction.....	7
2.2 Understanding Chlorinated Compound Contamination.....	7
2.2.1 State of the Practice .....	7
2.2.2 Physical and Chemical Properties.....	9
2.2.3 Dechlorination Reactions.....	11
2.3 Zero Valent Iron.....	13
2.3.1 History.....	13
2.3.2 Technical Basis .....	14
2.3.3 Advantages and Limitations of Zero-Valent Iron.....	15

2.4	Bioremediation.....	16
2.4.1	History.....	16
2.4.2	Technical Basis .....	17
2.4.3	Advantages and Limitations of Bioremediation .....	19
2.5	Combining Zero Valent Iron and Bioremediation .....	20
2.6	Summary .....	21
2.7	References.....	22
Chapter 3.....		28
3	Abiotic and Biotic Effects of Zero Valent Iron and Organic Substrates in an Ex-Situ Chlorinated Solvent Contaminated Soil Remediation Project.....	28
3.1	Introduction.....	28
3.1.1	Short-Term Concentration Changes .....	31
3.1.2	Long-Term Concentration Changes.....	31
3.2	Site Description.....	32
3.3	Materials and Methods.....	34
3.3.1	Chemicals.....	34
3.3.2	Experimental Systems.....	34
3.3.3	Analytical Methods.....	37
3.4	Results and Discussion .....	39
3.4.1	Amendment Characterization .....	39
3.4.2	Short-Term Changes .....	41
3.4.3	Long Term Changes.....	52
3.5	Summary .....	60
3.6	References.....	62
Chapter 4.....		66
4	Conclusions and Recommendations .....	66

4.1 Conclusions.....	66
4.2 Recommendations.....	67
Appendices.....	69
Curriculum Vitae .....	72

## List of Tables

Table 2.1 Before and After Treatment Groundwater Concentrations Data from Mcguire, Adamson, Newell, & Kulkarni, 2016. ....	9
Table 2.2 Physical and Chemical Properties of Chlorinated Solvents. Values taken from Cwiertny & Scherer, 2010. ....	10
Table 3.1 Groundwater parameters important to Dhc viability at monitoring wells before and after the ex-situ remediation process. ....	59



# List of Figures

Figure 2.1 Remediation Performance Based on Geometric Mean Concentrations of Total CVOCs. Reprinted from Mcguire, Adamson, Newell, & Kulkarni, 2016..... 8

Figure 2.2 Reductive dechlorination pathways of chlorinated ethenes. Adapted from Arnold and Roberts 2000, and Kocur 2015..... 12

Figure 2.3 Core-shell structure of ZVI depicting various mechanisms for the removal of chlorinated compounds and metals. Adapted from O’Carroll et al., 2013. .... 14

Figure 2.4 Reductive dechlorination pathway leading to detoxification of chlorinated ethenes. Adapted from Loffler et al 2013. .... 18

Figure 3.1 Picture of the amendment used to treat chlorinated solvents ex-situ. .... 28

Figure 3.2 Pictures of the of the remediation process used to treat chlorinated solvent impacted soil ex-situ. The impacted soil is excavated (top left), then the amendment is mixed into the soil (top right and bottom left) before being backfilled to its original location (bottom right)..... 30

Figure 3.3 Flow chart describing the most probable processes impacting CVOC concentrations. .... 31

Figure 3.4 Arial photo of remediated area. The area highlighted in red shows the excavation extent. Arrows point to the locations where soil cores were taken, and to monitoring wells where groundwater samples were collected. .... 33

Figure 3.5 Experimental setup used in ZVI hydrogen production experiments. .... 35

Figure 3.6 Experimental setup used in the batch reactor experiments. .... 36

Figure 3.7 Electron microscope image of ZVI particles from the amendment. .... 39

Figure 3.8 Size distribution of ZVI from the amendment ..... 40

Figure 3.9 XRD results of the ZVI from the amendment..... 41

Figure 3.10 Effect of the amendment components on 1,2-DCA concentrations when combined and separated. Black points represent the control which did not contain amendment. Blue points contained both ZVI and the organic substrate. Orange points contained ZVI, and purple points contained the organic substrate. .... 42

Figure 3.11 Effect of the amendment components on PCE concentrations when combined and separated. Black points represent the control which did not contain amendment. Orange points contained both ZVI and the organic substrate. Red points contained ZVI, and yellow points contained the organic substrate. .... 43

Figure 3.12 Effect of soil addition on CVOC concentrations in an anaerobic environment in the absence of amendment. Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols. .... 45

Figure 3.13 Effect of soil addition on CVOC degradation in an anaerobic environment with amendment present. Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols. .... 46

Figure 3.14 Effect of soil addition on CVOC degradation in an aerobic environment in the absence of amendment. Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols. .... 47

Figure 3.15 Effect of soil addition on CVOC degradation in an aerobic environment with amendment present. Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols. .... 48

Figure 3.16 Effect of previously treated and untreated soil on 1,2-DCA concentrations in an aerobic environment. Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols. .... 49

Figure 3.17 Effect of volatilization on CVOC concentrations. Blue points represent 1,2-DCA concentrations, and orange points represent PCE concentrations. .... 51

Figure 3.18 Change in the proportion of iron in the zero-valent state caused by atmospheric exposure. .... 53

Figure 3.19 XRD patterns of ZVI unexposed to atmosphere (fresh ZVI), and ZVI that was exposed to atmosphere for 3 weeks (aged ZVI). ..... 54

Figure 3.20 Location of the five monitoring wells sampled for DNA analysis (labelled A through E). Burgundy highlighted area represents the area remediated using the ex-situ mixing process. .... 55

Figure 3.21 Relative abundance of the most common microorganisms capable of dechlorination as well as the Shannon Species Diversity Index at monitoring wells up-gradient, within, and down-gradient of a previously treated site..... 56

Figure 3.22 Dehalococcoides gene copies/mL at five monitoring wells sampled on a previously remediated site. Error bars are the standard deviation of duplicate samples. .... 58

# List of Appendices

Appendix A Photo of batch reactors used in the bench scale experiments. .... 69

Appendix B Results for qPCR duplicate samples. Gene copies/mL was determined by multiplying the extracted DNA concentration by dilution and total extracted volumes. .... 69

Appendix C Standard curve statistics for qPCR analysis. .... 70

Appendix D Percent unclassified, and the top 50 most abundant genera identified at each groundwater well location through Illumina Sequencing. .... 70

## Chapter 1

### 1 Introduction

#### 1.1 Background

*“[a] great deal has been learned, but at the same time the vastness of our ignorance has become even more apparent.”*

Harry Hanson, 1961 Symposium on Ground Water Contamination.

It was at one of the earliest groundwater contamination conferences in the United States where Mr. Hanson, late Director of the Sanitary Engineering Center, made this comment in the opening remarks to approximately 300 attendants. It's unlikely anyone present would have predicted the amount of time it would take the industries and government agencies most responsible, as well as the rest of the environmental community to overcome its own ignorance and acknowledge the pervasiveness of groundwater contamination. It took another 20 years after Mr. Hanson made this statement for the rest of the United States to recognize the seriousness of the issue and begin taking any meaningful action. In 1980 EPA Administrator Costle made clear, while speaking on NBC's *Meet the Press*, that groundwater protection is a principle EPA priority. This point in time is recognized as the catalyst for environmental action in the United States (Pankow et al., 1996). In the same year, U.S Congress passed the *Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)*, better known as “Superfund” to mandate large scale groundwater monitoring at disposal sites. By the time these actions were taken, considerable damage to aquifers had already been done. A 1975 study led by the EPA on 113 different public drinking water supplies in the US revealed that a common metal degreaser, trichloroethene (TCE), was present in nearly 25% of all sites tested (Brass et al., 1977).

Because of the multiple decades of poor disposal practices, as well as lack of sufficient environmental oversight, the financial repercussions associated with remediation are still felt today. In 1985, the US Department of Defense (DoD) estimated it would cost between

\$5 billion and \$10 billion to clean the 400 to 800 US sites requiring environmental remediation. After spending approximately \$12 billion over the next ten years, the cost-to-complete estimates had grown to over \$20 billion. By 2007, after spending over \$20 billion in the just the past decade, the DoD estimated another \$13 billion would still be required (McCarty, 2010).

Within Canada, a 15-year, \$4.33 billion program called the Federal Contaminated Sites Action Plan (FCSAP) started in 2005 to reduce environmental and human health risks from federal contaminated sites. As of 2016, the Government of Canada still holds an estimated \$6.27 billion in environmental liability (FCSAP, 2017). In 2011, the FCSAP created a 5-year plan to reduce the Government's liability by \$576 million; the result after 2016 was a \$1 billion increase. As a part of the same 5-year plan, only 48% of projects were successfully completed of the targeted 368 sites (FCSAP, 2017).

This sluggish progress reflects how the remediation community did not properly assess the seriousness of the problem, believing instead that off-the-shelf technologies would be adequate in cleaning up all types of spills. In a 1994 report titled "Alternative for Ground Water Cleanup", the US National Research Council showed clear evidence that the default pump-and-treat method that had been predominately used over the past 15 years was largely ineffective (Peters, 1995).

One reason why the remediation efforts between 1980 and 1990 had been so ineffective was a poor understanding of the physical and chemical properties of the most predominant contaminants; specifically, a class of chemicals known as chlorinated solvents. Chlorinated solvents have proven to be one of the most pervasive groups of groundwater contaminants, and have been found in approximately 80% of all U.S. Superfund Sites (Westrick et al., 1984). The solvents most commonly encountered include tetrachloroethene (PCE), trichloroethene (TCE), carbon tetrachloride (CT), and 1,2-dichloroethane (1,2-DCA). These chemicals typically enter the subsurface as a dense non-aqueous phase liquid (DNAPL). A DNAPL exists in its own phase and is denser than water, giving it the ability to migrate through the subsurface and below the water table. This, along with characteristically low viscosities and solubility, combine to make these types of

contaminants very recalcitrant to treatment once in the subsurface (Kueper et al., 2014b). As one of the most prevalent groundwater contaminants, technologies are continually being developed and improved to remediate soil and groundwater impacted by chlorinated solvents. Of these technologies, the use of zero valent iron (ZVI) as a reductant, and bioremediation (naturally occurring, or introduced microorganisms which can biodegrade contaminants) have received significant attention since the mid-1990s (O'Carroll et al., 2013) (Dzionic et al., 2016).

The purpose of this study is to examine a full-scale remediation project based on the use of a ZVI/organic substrate mixture used to treat soil predominantly contaminated with PCE and 1,2-DCA. Since these two chlorinated compounds are relatively common soil and groundwater contaminants, there have already been many successful full-scale remediation projects targeting these compounds. The full-scale project being examined in this study was unique due to the novel ex-situ amendment application process, although the relative contribution made by the most prominent mechanisms contributing to contaminant destruction is not well understood.

## 1.2 Research Objectives

The main objective of this work is to identify the main mechanism of remediation through which the amendment effectively removes chlorinated solvents from soil in large-scale field applications. The amendment used in this project contains two primary constituents: ZVI, and proprietary organic material, designed to destroy contaminants either abiotically or biotically respectively. Abiotic degradation describes the reduction of chlorinated solvents by micro-sized ZVI. The biotic degradation is caused by supplying a microbial food source to increase the population of naturally occurring microorganisms in the soil; some of which can break down chlorinated solvents through their metabolic process.

Data collected during the field scale project suggest that the amendment and application strategy was successful in decreasing the contaminant concentrations to the project's target criteria, although the relative role ZVI and bioremediation had on the observed decreases was not determined. In addition, the effect the ex-situ amendment application process had on contaminant concentrations is also not well understood. It has been hypothesized that

volatilization may be significantly contributing to the observed losses in contaminant concentrations. If so, the contaminant decreases being attributed to the amendment may be overestimated.

### 1.3 Thesis Outline

This thesis is written as an “Integrated Article”. A summary of the chapters is given below:

Chapter 1: Introduces the topic and presents the main research objective.

Chapter 2: Provides a review to the relevant literature on the use of ZVI and biostimulation as remediation techniques both separately and combined.

Chapter 3: Describes the materials used, as well as procedures used for laboratory and field work. Experimental results are also presented and discussed.

Chapter 4: Summarizes the findings of this study and provides recommendations for future work.

Appendices: Contain supplementary material for Chapter 3.



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## Chapter 2

### 2 Literature Review

#### 2.1 Introduction

Through the mid to late 1900s, lax regulation and oversight led to liquid halogenated organic waste being directly released into the ground (O'Carroll et al., 2013). Much of the contaminated sites being handled today are a result of these practices before the implementation of modern legislation. Today's financial penalties for uncontrolled releases, as well as the amount of liability required for contaminated sites has led government and industry to minimize their environmental footprint. Penalty avoidance, and liability reduction are also the main motivators for the remediation of contaminated sites by owners (Nielsen, 2006). Although the financial consequences of modern environmental legislation are the main driver for today's remediation projects, they can still be cost inhibitive. Reducing this cost, and making more efficient technologies are main objectives in soil and groundwater remediation research.

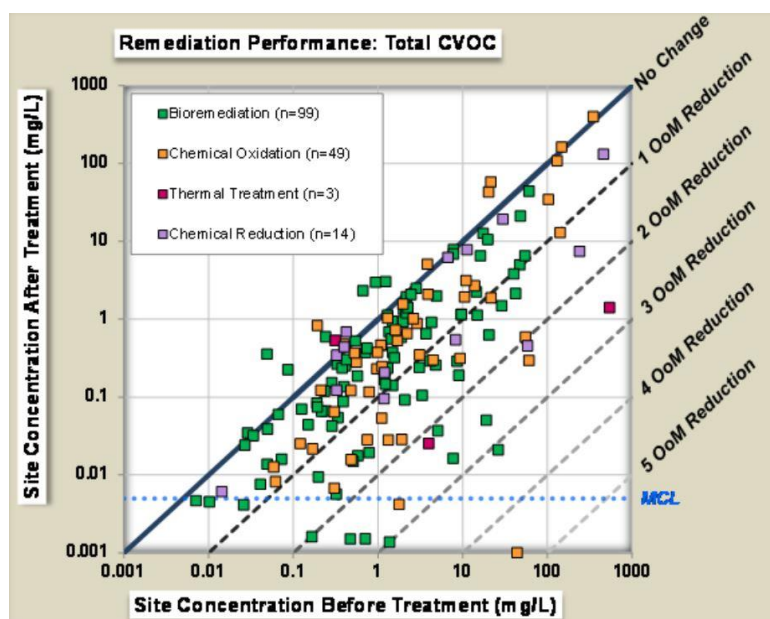
While zero valent iron (ZVI) and bioremediation are proven remediation technologies, the implementation of their use to treat highly recalcitrant chlorinated solvents ex-situ is an area where several research efforts converge. Therefore, the efficacy of this novel remediation technique is the basis of this work. It is hypothesized that there are three processes contributing to the chlorinated solvent decreases observed in the remediation project being investigated: ZVI mediated reduction, bioremediation, and partitioning between soil, water, and air. As such, each will be introduced, along with a summary of how these compounds act in the subsurface.

#### 2.2 Understanding Chlorinated Compound Contamination

##### 2.2.1 State of the Practice

It is important to note that the most commonly used remediation technologies do not often succeed at reducing chlorinated volatile organic compound (CVOC) concentrations to the maximum allowed contaminant level in drinking water. Because drinking water criteria for

CVOCs are so low (0.1 mg/L for PCE and 0.005 mg/L for 1,2-DCA in Ontario), it is not uncommon for a remediation technology to have to reduce contaminant concentrations 3 orders of magnitude (99.9%) or more to meet regulations for drinking water. For this reason, in some cases, returning a site to drinking water guidelines is not financially feasible, and other strategies such as containment, or risk analyses are chosen instead of remediation (Mcguire et al., 2016). In 2016, a meta-analysis of 235 remediation projects was conducted for the Department of Defense Environmental Security Technology Certification Program (ESTCP). This analysis indicated that while most projects were able to decrease CVOC concentrations 90-99%, only 7% of sites actually achieved drinking water standards (Mcguire et al., 2016). Figure 2.1 depicts the main findings of this study comparing before and after treatment contaminant concentrations, and whether they successfully reached the maximum contaminant level (MCL).



**Figure 2.1 Remediation Performance Based on Geometric Mean Concentrations of Total CVOCs.** Reprinted from Mcguire, Adamson, Newell, & Kulkarni, 2016.

When each remediation technology is grouped, bioremediation and chemical reduction (such as ZVI) reduced CVOC concentrations on average 96% and 93% respectively, i.e. slightly above a one order of magnitude decrease in concentration (Table 2.1).

**Table 2.1 Before and After Treatment Groundwater Concentrations** Data from McGuire, Adamson, Newell, & Kulkarni, 2016.

	<b>Median Geomean Before (mg/L)</b>	<b>Median Geomean After (mg/L)</b>	<b>% Reduction in Concentration</b>	<b>Order of Magnitude Reduction in Concentration</b>
Bioremediation	0.74	0.027	96%	1.4
Chemical Reduction	1.8	0.13	93%	1.1

## 2.2.2 Physical and Chemical Properties

The mobility and fate of different contaminants can vary greatly depending on their physical and chemical properties, their biological interactions, as well as the hydrogeological characteristics of the surrounding area. Compared to many other types of contaminants, the properties of chlorinated solvents make them especially recalcitrant (McCarty, 2010). In the context of groundwater contamination, these compounds are commonly referred to as dense non-aqueous phase liquids (DNAPLs) when they are in their own phase in the subsurface. Their greater density than water allows chlorinated solvents to penetrate the water table and sink through the saturated zone becoming more difficult and costly to remediate. Their characteristically low viscosity allows for this downward movement to be relatively rapid. DNAPLs are also sparingly soluble in water, allowing them to travel through aquifers as a separate phase and spread out vertically and horizontally as the DNAPL preferentially travels through the path of least resistance, or pools on top of lenses of lower permeability soil where it can then slowly dissolve into the groundwater. Because the drinking water standards for chlorinated solvents are so low, even this slow dissolution can result in dissolved phase plumes with CVOC concentrations orders of magnitude greater than regulatory guidelines (Pankow et al., 1989).

The physical and chemical properties of chlorinated solvents also control their partitioning between the phases present in the subsurface. The properties that govern the partitioning between air, water, and aquifer solids are summarized in Table 2.2.

**Table 2.2 Physical and Chemical Properties of Chlorinated Solvents.** Values taken from Cwiertny & Scherer, 2010.

Chlorinated Solvent	Ontario Drinking Water Standards (mg/L)	Density (g/cm <sup>3</sup> )	Solubility in Water (mg/L)	Henry's Law Constant, K <sub>H</sub> (atm/M)	Absolute Viscosity (cP)	Octanol/Water Partition Coefficient (log K <sub>ow</sub> )	Vapour Pressure (torr)
Water	N/A	0.997	N/A	N/A	0.894	N/A	N/A
Tetrachloroethylene (PCE)	0.01	1.63	150	26.3	0.9	2.88	18.1
Trichloroethylene (TCE)	0.005	1.46	1,100	11.7	0.57	2.53	74.2
1,2-Dichloroethane (1,2-DCA)	0.005	1.25	8,606	1.2	0.84	1.48	79
Dichloromethane	0.05	1.33	13,200	1.7	0.44	1.25	415
Carbon Tetrachloride	0.002	1.59	800	28.9	0.97	2.64	153.8

### 2.2.2.1 Solid-Water Partitioning

As a class, chlorinated solvents are considered moderately hydrophobic. Their affinity for aquifer solids is less than other organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenols (PCBs). The most practical measure of a compound's hydrophobicity is the octanol-water partitioning coefficient (K<sub>ow</sub>) and is defined as the ratio of their concentration in the octanol and in water after the partition equilibrium has been reached (Cwiertny & Scherer, 2010).

$$K_{ow} = \frac{C_{octanol}}{C_{water}} \quad \text{Equation 2.1}$$

In this calculation, the concentration of octanol is used as a proxy for the concentration of organic carbon in the subsurface. Large values of K<sub>ow</sub> correspond to compounds that are expected to sorb to soils and organics more readily.

### 2.2.2.2 Air-Water Partitioning

The partitioning between air and water is commonly described by Henry's Law, which applies to low concentrations of solvents in water. This law relates the concentration of the solvent in air to its concentration in water at equilibrium (Cwiertny & Scherer, 2010).

$$K_H = \frac{c_{air}}{c_{water}} \quad \text{Equation 2.2}$$

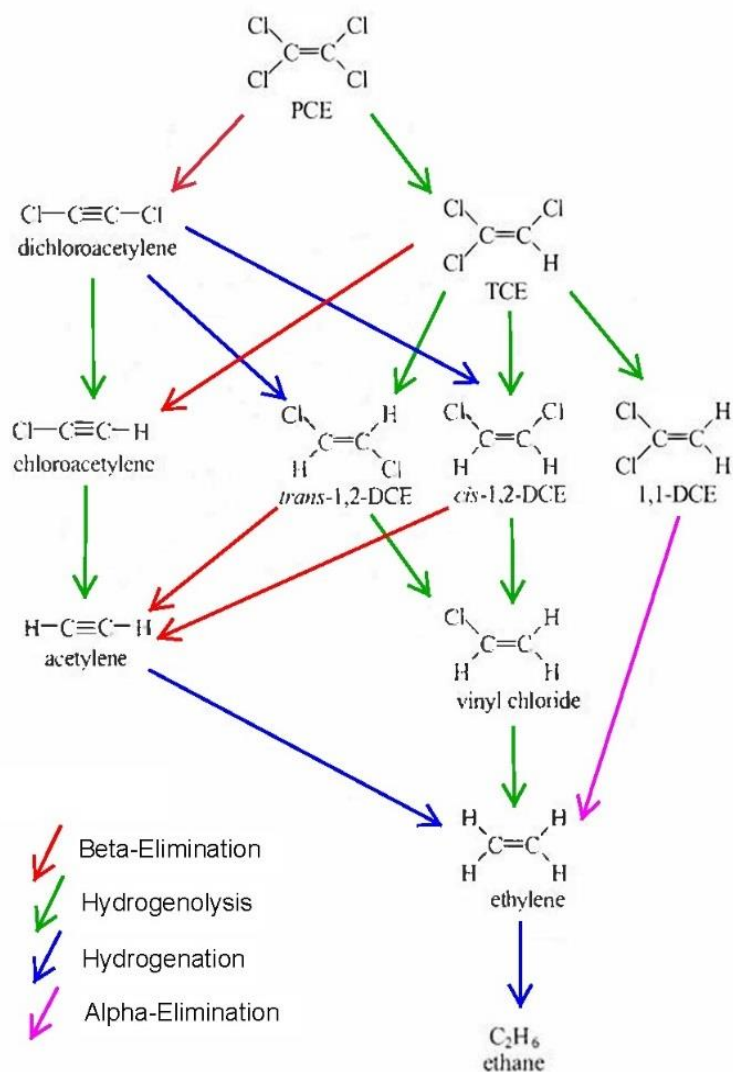
Large values of  $K_H$  describe chemicals that readily partition from water to air. It should be noted that the Henry's constant for a given compound can be influenced by several environmental factors.

### 2.2.2.3 Solid-Air Partitioning

A compound's vapour pressure ( $p^\circ$ ) is a measure of the maximum attainable concentration of a chlorinated solvent in air, and is considered a noteworthy variable in solid-air partitioning. A compound with a high vapour pressure will partition more readily between air and soil (Cwiertny & Scherer, 2010).

### 2.2.3 Dechlorination Reactions

The dechlorination pathways of chlorinated compounds have been well studied (Arnold & Lynn Roberts, 1998; Li & Farrell, 2000) and include four main reaction mechanisms depicted and described in Figure 2.2.



**Figure 2.2 Reductive dechlorination pathways of chlorinated ethenes.** Adapted from Arnold and Roberts 2000, and Kocur 2015.

The main mechanisms in dechlorination are hydrogenolysis, which involves the replacement of one chloride atom with a hydrogen atom, and beta and alpha eliminations, where chlorine atoms are released by the chlorinated compounds resulting in the formation of additional unsaturated carbon-carbon bonds. The third possible mechanism is hydrogenation, which involves the addition of hydrogen across a double or triple carbon-carbon bond.



These reductive dehalogenation reactions have been reported to preferentially degrade highly chlorinated compounds (Gillham & O'Hannesin, 1994). This phenomenon can sometimes lead to a temporary, or prolonged accumulation of partially dechlorinated compounds, which has important implications since some daughter products of chlorinated compounds such as vinyl chloride have a greater toxicity (Lien & Zhang, 2005). This is of great concern for remediation projects. If the technology selected results in the accumulation of incomplete dechlorination products such as vinyl chloride, a site may be worse off than it was before the project took place.

## 2.3 Zero Valent Iron

Zero valent iron is a powerful reducing agent capable of donating electrons to a variety of contaminants. While the basis of this study is the use of ZVI in degrading chlorinated solvents, various studies have shown it to be able to react with metalloids (such as arsenic bearing anions), polychlorinated biphenyls (PCBs), chlorinated pesticides, nitro aromatic compounds, and nitrates (O'Carroll et al., 2013). The following section provides a history of ZVI as a soil and groundwater remediation tool, a brief introduction into its mechanism for chemical reduction and the most relevant reactions, as well as the advantages and limitations of its use.

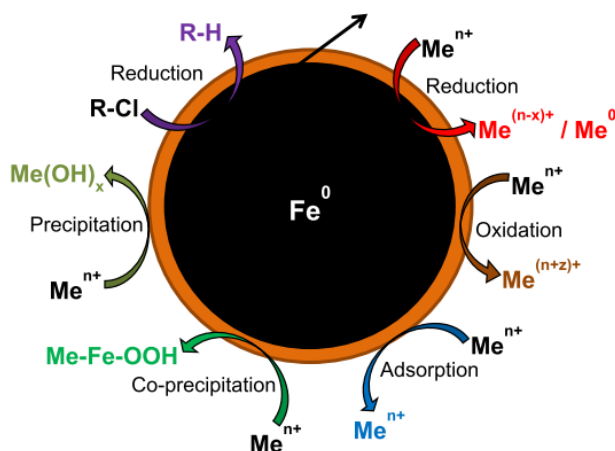
### 2.3.1 History

The first publication documenting the degradation of halogenated compounds by iron was largely an accidental finding by Reynolds and collaborators in 1990, who were evaluating the possible sampling bias that different groundwater monitoring construction materials could impart (Reynolds et al., 1990). One of the first papers actually studying ZVI as a possible remediation tool was conducted by Gillham and O'Hannesin in 1994, who concluded that ZVI is highly effective at enhancing the rate of degradation of a wide range of chlorinated compounds (Gillham & O'Hannesin, 1994). The research team of O'Hannesin and Gillham followed up this work with the first field trial using ZVI by placing the reductant in a trench to act as a permeable reactive barrier (PRB) (O'Hannesin & Gillham, 1998). Since the acceptance of ZVI as an effective remediation tool and the success of PRBs, many other advancements have been made. These include the use of

nano-scale ZVI, which increases the iron surface area and consequently the reaction rate (Zhang & Wang, 1997), or encapsulating ZVI in an emulsion causing DNAPL to solubilize into it to preferentially react with the ZVI within (ITRC, 2011). Advancements such as these have allowed practitioners to tailor the ZVI to better treat site specific contaminant sources and plumes in varied circumstances.

### 2.3.2 Technical Basis

ZVI is best described as having a core-shell structure (Zhang & Wang, 1997) (Figure 2.3). Through reactions with oxygen and water, the surface of the particle passivates and forms an iron oxy/hydroxide layer, which limits the transfer rate of electrons between the zero-valent iron core, and outer oxidants (Nurmi et al., 2005).

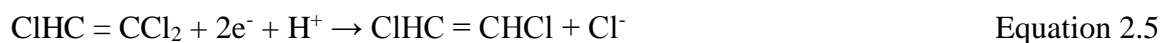


**Figure 2.3 Core-shell structure of ZVI depicting various mechanisms for the removal of chlorinated compounds and metals.** Adapted from O'Carroll et al., 2013.

Reactions with contaminants take place on the surface of the ZVI particle where the strong reduction potential of the ZVI (-0.44V) (Eq. 2.3) allows for the breakage of carbon chlorine bonds, releasing chloride ions (Eq. 2.4) (Lien & Zhang, 2005).



The most common reduction reactions responsible in the dehalogenation of chlorinated compounds are hydrogenolysis (Eq. 2.5), and reductive elimination (Eq. 2.6). Polychlorinated compounds can undergo sequential hydrogenolysis, resulting in a characteristic sequence of partially dechlorinated products. As the chlorine ions are removed, further dechlorination becomes both thermodynamically and kinetically less favourable, possibly increasing the concentration of persistent, and sometimes more toxic, partially dechlorinated daughter products. The other major reduction pathway involves eliminating two chlorine ions at the same time, resulting in the formation of a carbon-carbon double or triple bond. When the two chlorines are cleaved from the same carbon, the process is named  $\alpha$ -elimination, and when the chlorines are on adjacent carbons the process is a  $\beta$ -elimination (Eq. 2.6). The reaction step that commonly follows elimination reactions is hydrogenation. This involves hydrogen being added to a double or triple carbon-carbon bond (Arnold & Lynn Roberts, 1998).



The significance of each reaction pathway has been shown to depend on a variety of factors including contaminant structure, properties of the reductant, as well as environmental conditions (Kim et al., 2008). Hydrogenolysis is more prevalent when higher chlorinated compounds are reduced using less reactive species. Reductive elimination tends to be more important when there are fewer chlorines per carbon, or when stronger reductants are used (Tratnyek et al., 2003).

### 2.3.3 Advantages and Limitations of Zero-Valent Iron

Advancements in ZVI technology have allowed practitioners to tailor its physical and chemical properties to work best for a specific application. These properties can be both an advantage when exploited to increase the technology's remedial potential, or they can act to limit ZVI's effectiveness when not controlled for or properly considered. Factors include variation in surface area, effects of pH, as well as contaminant identity and other ion concentrations in the groundwater. A good example of this is controlling the size of the

particle to control the rate of reaction. When a PRB is being installed, the ZVI should be designed to maintain reactivity for years. To achieve this, micro-sized ZVI is used. If ZVI is being used to treat a source zone, more reactive yet shorter lived nano-sized particles may be better suited (Nurmi et al., 2005).

Perhaps the main shortcoming of ZVI is its inability to break down 1,2-DCA, as well as its limited reactivity with other lower halogenated compounds such as vinyl chloride (Lien & Zhang, 2005). This of course has important implications for any remediation field project (such as the one which is the focus of this study) with the presence of significant concentrations of these compounds.

## 2.4 Bioremediation

The term bioremediation is usually broadly defined as the chemical breakdown of contaminants because of biological activity. This definition includes biotic pathways, as well as abiotic pathways that rely at some point on a biological process. The following section provides short overview of bioremediation as a soil and groundwater remediation tool, a brief introduction into how it works and the most relevant reactions, as well as its advantages and limitations.

### 2.4.1 History

The soil and groundwater remediation industry first saw success using microorganisms in treating petroleum hydrocarbons from gasoline and diesel plumes. It was identified that the rate limiting step was the rate of introduction of the electron acceptor, so by increasing the oxygen content in the subsurface, native microorganism populations and degradation rates could increase by several orders of magnitude (Raymond et al., 1977). Biodegradation of chlorinated compounds under anaerobic conditions was first recognized as early as 1983 (Bouwer & McCarty, 1983). It was also noted that each subsequent reductive dechlorination step was slower than the previous one, and like ZVI, often resulted in the accumulation of more toxic compounds such as vinyl chloride. Researchers finally identified a group of organisms (*Dehalococcoides* spp.) that was able to completely reduce chlorinated compounds to ethene in 1989 (Freedman & Gossett, 1989). With more recent work showing the viability of bioremediation to even treat high concentrations or even

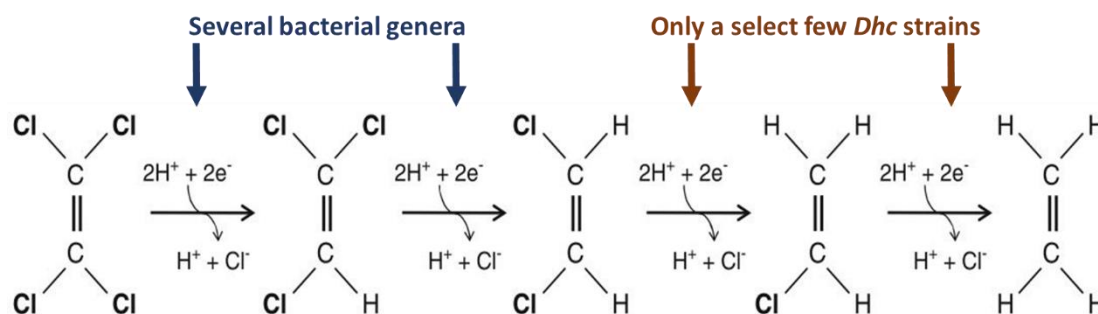
source zones, this has become one of the most commonly used remediation technologies used today (Kueper et al., 2014a).

## 2.4.2 Technical Basis

Many different approaches to bioremediation have been developed since the technology's inception. Aerobic and anaerobic oxidation, aerobic and anaerobic cometabolism, and direct reductive dechlorination can all degrade solvents (Brown et al., 2009). The biochemical reactions listed below include those that are more commonly used by practitioners treating chlorinated solvents in groundwater and soil, together with a brief description of each:

- *Aerobic Oxidation* – This reaction is restricted to dichloroethene (DCE) and vinyl chloride (VC) and is not effective for most parent compounds such as tetrachloroethene (PCE) and trichloroethene (TCE) (Bradley & Chapelle, 2010). Research has shown that VC can biologically oxidize at very low oxygen levels that may appear to be anaerobic.
- *Anaerobic Oxidation* – Again only applicable for DCE and VC degradation, this reaction has been proposed but has proven to be difficult to verify. This process has not been used as the primary bioremediation tool in engineered remediation systems, but may play a minor role in natural systems (Bradley & Chapelle, 2010).
- *Aerobic Cometabolism* – The organisms involved in this process have non-specific oxygenases which fortuitously oxidize chlorinated ethenes to CO<sub>2</sub>, but the process has only ever been reported for TCE and DCE. While it is unlikely to significantly contribute to non-engineered bioremediation, there has been some success in engineered systems (McCarty et al., 1998).
- *Anaerobic Cometabolism* – This process is largely viewed as a side effect when stimulating *Dehalococcoides* bacteria for reductive dechlorination. The rate of dechlorination decreases by an order of magnitude with each chlorine removed, making the process inefficient and unattractive as a remediation tool (Bouwer & McCarty, 1983).

While these approaches have shown the ability to contribute to dechlorination under different circumstances, reductive dechlorination (Figure 2.4) has become the most prominent remedial approach due to its ability to treat all chloroethenes, and has proven to be relatively easy to implement and control under field conditions when compared to other biological approaches (Stroo et al., 2014). Sequential reductive dechlorination takes place when a chlorine is substituted with a hydrogen atom. The anaerobic bacteria that can degrade chlorinated solvents can use them as terminal electron acceptors in their metabolism for ATP synthesis (McCarty et al., 1998). Because of this, the term organohalide respiration is commonly used due to the fact that the organisms are ‘breathing’ the chlorinated ethenes, using them as electron acceptors in the same way mammals use oxygen (Stroo et al., 2014).



**Figure 2.4 Reductive dechlorination pathway leading to detoxification of chlorinated ethenes.** Adapted from Löffler et al 2013.

While researchers continue to search for new organisms capable of organohalide respiration, the current list is short and restricted to a few genera of bacteria, and only strains of *Dehalococcoides mccartyi* (*Dhc*) have been shown to be able to respire DCE and VC (Löffler et al., 2013b). These specialized cells required hydrogen as an electron donor and reduced organic compounds such as acetate as a carbon source. They also rely on other bacteria to supply vitamin B<sub>12</sub> (Stroo et al., 2014). This reliance on other microorganisms has made researchers realize the importance of cooperative functions in microbial communities in addition to the activity of individual species for dechlorination (Bradley & Chapelle, 2010).

In engineered remediation systems, the two categories of active remediation are biostimulation and bioaugmentation. Bioaugmentation refers to the practice of adding organisms to impacted soil or groundwater. Suitable organisms are not always present at contaminated sites, or at concentrations too low to achieve a timely and cost-effective remediation. If the proper environmental conditions are present, studies have shown that organisms introduced can establish and increase the rate of complete dechlorination (Ellis et al., 2000). Biostimulation involves creating optimal conditions for the growth and activity of the targeted microbes. In the case of creating conditions for reductive dechlorinators such as *Dhc*, this typically means neutral pH, potentials  $< -100$  mV, and readily available hydrogen (Stroo et al., 2014). Stimulating reductive dechlorination typically relies on adding organic compounds that are fermented to produce acetate and hydrogen to act as electron donors. During fermentation, an anaerobic environment is created through the consumption of oxygen and other electron acceptors. The more favourable redox environment along with increased levels of the ultimate electron donor for anaerobic bacteria, hydrogen, create the optimal conditions for these bacterial groups to function (Bradley & Chappelle, 2010). The types of substrates that are most commonly used to achieve these conditions can be categorized into soluble substances, such as lactate, molasses, ethanol, methanol; slow release substrates such as emulsified vegetable oil (EVO), hydrogen releasing compounds (HRC<sup>®</sup>); and solid substrates including bark mulch, compost, manure, chitin, and other trademarked mixtures (Henry, 2010). These substrates vary in their ability to distribute in the subsurface and rate at which they degrade or ferment.

### 2.4.3 Advantages and Limitations of Bioremediation

The advantages and disadvantages of using bioremediation need to be well understood by those planning to use the technology. While its use has been increasing, there are important limitations that can significantly undermine its effectiveness. Some of the reasons this technology is attractive to practitioners is its relative low cost, especially when using biostimulation. One post-mortem study of over 200 remediation projects estimates bioremediation to be approximately 50% less expensive compared to zero valent iron and thermal treatment (Mcguire et al., 2016). The same study also found that the performance

of these bioremediation projects was not significantly different when compared to the other remediation technologies.

As described earlier, the reactions that contribute to bioremediation can cause accumulation of partially dechlorinated compounds, most importantly a possible increase in highly toxic VC concentrations (Stroo et al., 2014). This can have serious regulatory implications if these accumulations become long-term trends. Another important aspect that must be acknowledged is the fact that the microorganisms that are responsible for organohalide respiration – and especially *Dehalococcoides sp.* – require very specific environmental conditions and have several sensitivities that can significantly reduce their effectiveness. Duhamel and collaborators have shown that chloroform concentrations of 2.5  $\mu\text{M}$  and 1,1,1-trichloroethane concentrations of 5.2  $\mu\text{M}$  can completely inhibit vinyl chloride degradation to ethene (Duhamel et al., 2002). Work done by Bagley and collaborators have also shown evidence that carbon tetrachloride can completely inhibit PCE degradation at concentrations of 19  $\mu\text{M}$  (Bagley et al., 2000). Research also shows that ORP conditions that promote sulfate reduction or methanogenesis ( $E_h < -200$  mV), and near neutral pH are essential for effective bioremediation (H F Stroo, Major, & Gossett, 2010; Robinson, Barry, Mccarty, Gerhard, & Kouznetsova, 2009). Finally, *Dehalococcoides* spp. are strict anaerobes, and even minimal oxygen exposure will destroy the microorganism (He et al., 2003).

## 2.5 Combining Zero Valent Iron and Bioremediation

The practice of treatment trains, or using multiple technologies either in series or in parallel has become a popular method to combine the most advantageous aspects of various technologies. Combining ZVI with bioremediation has the potential to create both causative, and synergistic advantages (Brown et al., 2009).

Causative interactions can occur when the metabolism of a carbon substrate results in the reduction of iron species capable of mediating abiotic dechlorination reactions. Examples include ferrous iron precipitates, formed by the corrosion of ZVI reacting with chlorinated solvents (Matheson & Tratnyek, 1994). Iron-based reductive chemistry has also been demonstrated in the field by the reactions of naturally occurring, ferrous-containing



minerals (Brown et al., n.d.). In a laboratory column experiment, Shen & Wilson (2007) simulated a passive reactive barrier constructed with plant mulch. Sulfate reduction driven by anaerobic biodegradation produced as much as 100 mg/L of sulfide, which reacted with naturally occurring iron to produce 500 – 2500 mg/L of acid volatile sulfide. The researchers attributed one-half of the TCE removal observed to abiotic reactions with iron monosulfides, and the remainder to biotic reactions (Shen & Wilson, 2007).

Examples of possible synergistic advantages when using these technologies in combination include the more favourable redox conditions created by the ZVI which better supports biotic dechlorination. ZVI also generates hydrogen, which is used by the bacteria as an electron donor (Dolfing et al., 2008). This phenomenon has been demonstrated in bench-scale column experiments testing the degradation of 1,2-DCA (Brown et al., 2009). Typically, treating 1,2-DCA with only ZVI or a source of carbon results in incomplete degradation. Researchers found that when contaminated soil was treated with a combination of controlled-release carbon plus ZVI particles, 99% reduction could be obtained in 98 days compared to 33% in the control column.

## 2.6 Summary

Chlorinated solvents are a very difficult group of contaminants to remediate. The degradation of these solvents by ZVI and bioremediation have been researched for over 25 years. This cumulative body of work indicates that these technologies can be quite effective under the right set of conditions. Moreover, during this time, researchers and practitioners have developed an understanding of the advantages and limitations of their use. Better understanding the possible short and long-term effects of the novel remediation process being investigated in this research project will add to the body of work, and allow future practitioners to make more informed decisions regarding if an ex-situ soil mixing process is best for their remediation efforts.

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## Chapter 3

### 3 Abiotic and Biotic Effects of Zero Valent Iron and Organic Substrates in an Ex-Situ Chlorinated Solvent Contaminated Soil Remediation Project

#### 3.1 Introduction

In the practice of environmental remediation, excavating large quantities of contaminated soil is usually more expensive than treating the soil in-situ (i.e. without the need for excavation). This is the main reason why most remediation projects and research focus on destroying or immobilizing contaminants in-situ (Harkness & Konzuk, 2014). Though in circumstances such as tight project completion timelines, ex-situ remediation techniques may be the most preferable option to ensure clean-up criteria are met on schedule. The specific constraints of the project discussed in this work made ex-situ remediation the most preferable option. Practitioners and managers of the clean-up project also decided that an ex-situ approach provided more confidence in knowing the contaminant concentrations before and after treatment, allowing them to treat large quantities of soil that was predominantly clay based, which also made in-situ technologies less effective. The remediation strategy chosen involved mixing into the soil a proprietary blend of 40-50% micro sized ZVI, and 50-60% dry organic substrate (Figure 3.1).



**Figure 3.1** Picture of the amendment used to treat chlorinated solvents ex-situ.



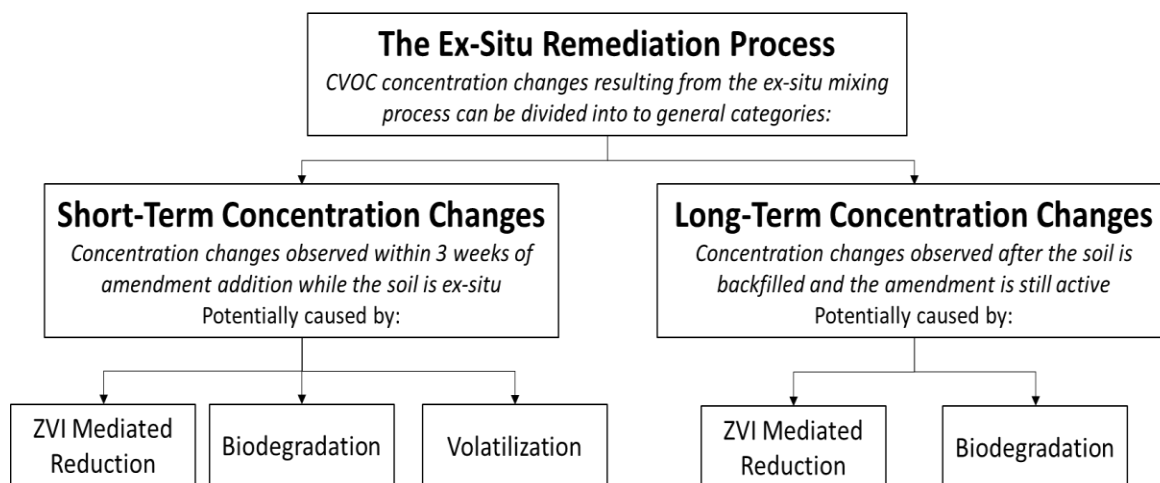
ZVI is a powerful reducing agent, and research has shown it is capable of reductive dechlorination (Gillham & O'Hannesin, 1994; O'Carroll et al., 2013), while organic substrates have proven to enhance biodegradation reactions by helping to create optimal conditions to enhance population growth and activity of targeted microorganisms (Bradley & Chapelle, 2010; Stroo et al., 2014)

The ex-situ soil mixing process begins by removing contaminated soil from the ground and placing it in long piles, or windrows, approximately 4 m wide and 1.5 m high. An excavator then moves along the pile placing the ZVI/organic substrate mix (or amendment) at approximately 2% by soil weight on top of the pile using its bucket. A machine most commonly used to mix compost piles called a windrow turner is then used to mix in the substrate and break the soil down to smaller pieces. The process of adding and mixing in the substrate is repeated until the CVOC concentration of the soil in the pile meets the project's remediation criteria (usually within three weeks). Once these criteria are met, the remediated soil is backfilled in the area it was originally excavated from. The reagent is advertised to have rates of ZVI reduction and organic substrate decomposition that allow for both components to remain active for five or more years due to the size of the ZVI particles and the initial low bioavailability of the fibrous organic substrate (A.G. et al Seech, 2000).



**Figure 3.2** Pictures of the of the remediation process used to treat chlorinated solvent impacted soil ex-situ. *The impacted soil is excavated (top left), then the amendment is mixed into the soil (top right and bottom left) before being backfilled to its original location (bottom right).*

This remediation process creates two distinct periods in time that can contribute to changes in contaminant concentration. These two periods are categorized into the contaminant concentration changes during the time the soil was excavated and mixed with the amendment ex-situ (which from now on is referred to as short-term concentration changes), and the contaminant concentration variations that occur after the soil is returned to the ground and the amendment is still abiotically and/or biotically active (which will be referred to as long-term change). The possible processes that will have the greatest effect on contaminant concentration during the short-term and long-term are summarized in Figure 3.3. The experimental design conducted for this research project aims to test the importance of each short-term, and long-term processes hypothesized to have the greatest contribution to CVOC concentration changes.



**Figure 3.3** Flow chart describing the most probable processes impacting CVOC concentrations.

### 3.1.1 Short-Term Concentration Changes

On average, the contaminated soil was treated ex-situ for approximately three weeks before being returned to the ground. The changes in contaminant concentration observed in this three-week period were hypothesized to be most impacted by ZVI mediated reduction, bioremediation, and volatilization. The effect these processes had on CVOC concentrations in laboratory scale batch reactor experiments were used as a proxy to test the importance of these processes in the full-scale remediation project.

### 3.1.2 Long-Term Concentration Changes

As previously stated, the amendment is designed to actively contribute to CVOC degradation for up to 5 years. In this time, it is hypothesized that bioremediation along with ZVI mediated reduction will continue to impact contaminant concentrations, while volatilization will no longer be a contributing factor. As such, ZVI mediated reduction, and bioremediation will be the focus of research to better understand the long-term concentration changes.

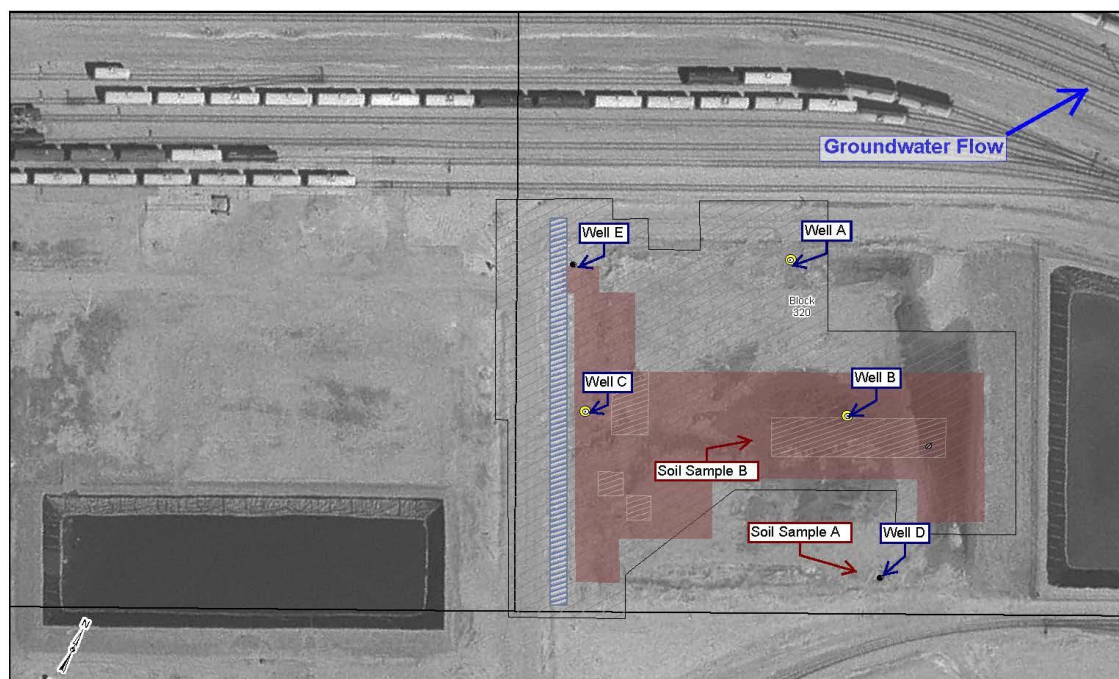
Since this research began four years after the ex-situ remediation project took place. Continuous sampling of CVOC concentration changes after the soil was returned to the

ground did not take place. Because of this, abiotic and biotic changes to the soil that may be a result of the ex-situ mixing process are assessed to investigate the likelihood the amendment contributed to long-term dechlorination.

## 3.2 Site Description

The ex-situ mixing process took place at two chemical production facilities between 2012 and 2015. A variety of chlorinated ethenes, ethanes, and methanes existed in the treatment areas, but the two most common CVOCs encountered were 1,2-DCA and PCE.

The ex-situ mixing process was first used on a now decommissioned 1,2-DCA storage area in Fort Saskatchewan, Alberta between 2012 and 2013 (Figure 3.4). The area is covered by glacial sediments, including till, clay, silt, and sand. The bedrock in the area is of Late Cretaceous age and consists of marine and non-marine shales, sandstones, and siltstones. Buried pre-glacial valleys are eroded into the bedrock surface and contain sand and gravel deposits of the Empress Formation which are in hydraulic connection with the regional river systems. The geometric mean value of hydraulic conductivity of the site before it was remediated was  $4.9 \times 10^{-8}$  m/s (URS Corporation, 2003). The 1,2-DCA Plant was first commissioned in 1979 and remained operational until 2006. The area historically consisted of a 1,2-DCA storage area with three 13,600,000 kg capacity steel aboveground storage tanks, a transfer pump station, and a spill contingency pond. 1,2-DCA contamination was found in an approximately 6000 m<sup>2</sup> area to a depth of up to 6 m below ground surface encased mostly in a lacustrine sediment unit. All soil and groundwater samples used for experiments and analysis were collected from this project site; the locations of which are depicted in Figure 3.4.



**Figure 3.4** Aerial photo of remediated area. The area highlighted in red shows the excavation extent. Arrows point to the locations where soil cores were taken, and to monitoring wells where groundwater samples were collected.

The second remediation project took place between 2014 and 2015 in Sarnia, Ontario at a former chemical production facility that was decommissioned approximately 20 years ago. Remediation took place at five distinct areas, treating a total of 70,000 m<sup>3</sup>. The subsurface comprises of a 1 to 1.5 m layer of fill (clay and granular material) as a result of past development. The fill layer is underlain by brown silty clay till to varying depths of 3.5 to 5.5 m. This unit contains the majority of the contaminated soil and has a hydraulic conductivity of  $2.1 \times 10^{-9}$  m/s (Husain et al., 1998). The fractures in the brown silty clay till allow the unit to be hydrogeologically active, and have shown to provide contaminant migration pathways. The brown silty clay till is underlain by a massive grey silty clay till, which has acted as a barrier to vertical contaminant migration. The area most pertinent to this work historically contained three tanks either containing carbon tetrachloride, PCE, or 1,2-DCA. All three tanks were decommissioned and removed in 1999. The area has been the focus of previous remedial efforts including permeable reactive barriers, as well as

pneumatic soil fracturing and injection of emulsified vegetable oil, which was piloted in a small portion of the site.

### 3.3 Materials and Methods

#### 3.3.1 Chemicals

Tetrachloroethene (PCE) (99+%, Alfa Aesar), and 1,2-Dichloroethane (1,2-DCA) (99+%, Sigma Aldrich) were used as received. Daramend<sup>®</sup> Reagent (40-50% iron, 50-60% organic amendment) (Peroxychem) was used as the remediation amendment in field and laboratory experiments. Gas Mix (5% H<sub>2</sub> balance Ar, PRAXAIR), and nitrogen (Ultra High Purity, PRAXAIR) were used in the anaerobic glove box. Hydrochloric acid (HCL) (37%, Sigma Aldrich) was used for the ZVI digestion experiments.

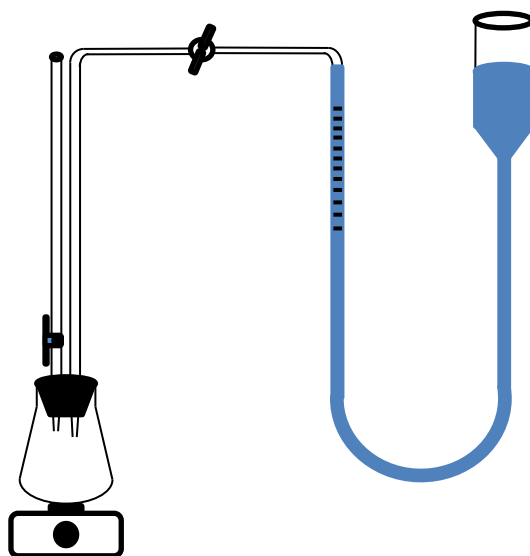
#### 3.3.2 Experimental Systems

##### 3.3.2.1 Hydrogen Production Experimental Setup

The proportion of iron in the zero valent state was measured using a gas volumetric based method. 10 mL of 32% HCl was added to 0.1 g of the iron taken from the amendment, producing hydrogen gas (Equation 3.1).



An air tight seal attached a flask containing the acid and iron to a eudiometer to measure the volume of water displaced by the H<sub>2</sub> gas (Figure 3.5). The displaced water volume is assumed to be equal to the produced volume of H<sub>2</sub>. From this, the total moles of zero valent iron can be calculated using the ideal gas law.



**Figure 3.5 Experimental setup used in ZVI hydrogen production experiments.**

### 3.3.2.2 Batch Reactor Experimental Setup

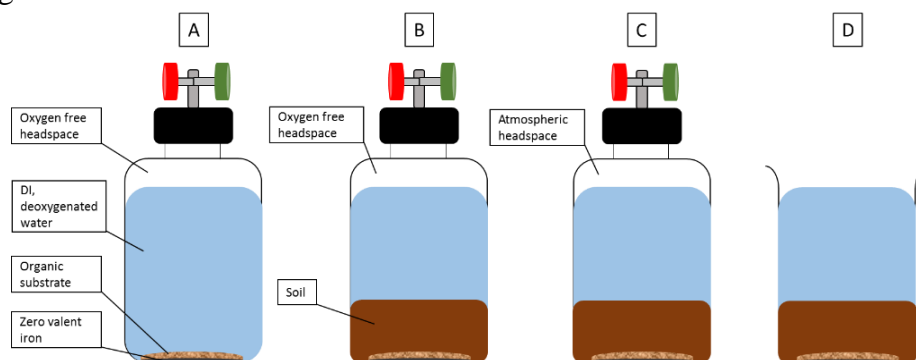
Reactivity experiments were conducted at room temperature in either 150 mL beakers, or 120 mL amber bottles sealed with Mininert valves. All experiments used deoxygenated DI water, and those carried out using amendment contained 3.0 g of the solid. Experiments with only the organic substrate contained approximately 1.4 g and experiments with only ZVI contained approximately 1.6 g. These weights represent the proportion of the organic substrate or ZVI in 3.0 g of the mixed amendment. When needed, the organic content and ZVI were separated using a magnet. The CVOCs were added to the reactors using a gas-tight syringe. The sealed reactor experiments were carried out using an orbital shaker (Thermo Scientific MAXQ 4000) set to 200 rpm.

Experiments testing for ZVI mediated reduction were sealed while inside an anaerobic glove box to ensure the headspace was void of oxygen (Figure 3.6, A). These experiments contained 100 mL of water and the required substrate. Samples of the aqueous phase were taken for CVOC analysis using a gas-tight syringe.

Experiments testing for biodegradation used bottles with 70 mL of water and 30 g of soil. The bottles testing for anaerobic biodegradation were sealed while inside an anaerobic glove box to ensure the headspace was void of oxygen (Figure 3.6, B). Experiments testing for aerobic biodegradation were sealed in atmospheric conditions to supply the reactors with oxygen (Figure 3.6, C). Samples of the aqueous phase were taken for CVOC analysis using a gas-tight syringe.

Experiments testing for volatilization used beakers with 70 mL of water and 30 g of soil which were open to the atmosphere within a fume hood (Figure 3.6, D). To collect a sample most representative of the CVOC concentration in the entire beaker, the protocol used in the sealed reactor experiments could not be used. The aqueous sample collected from the top of the reactor contents would be more affected by volatilization, and result in an over-estimate of its effects on CVOC concentration change. Therefore, samples of the reactor slurry taken while mixing the reactor were used for CVOC analysis.

The sealed beaker experiments and the open beaker experiments do not observe CVOC concentration changes in the same phase. The volatilization experiments will include CVOCs sorbed to the soil and amendment. This fact is taken into consideration when analyzing the results.



**Figure 3.6 Experimental setup used in the batch reactor experiments.**

### 3.3.2.3 Groundwater and Soil Field Sampling Procedure

Field groundwater samples were collected from six wells located up-gradient, down-gradient, and within the treatment area (Figure 3.4). The samples were collected during a



single sampling event in the summer of 2017, which was approximately 4 years after the site was remediated. Samples were collected using low-flow methodology where a Spectra Field Pro II peristaltic pump moved water from the well through a flow-through cell with a multimeter attached. The multimeter measured pH, temperature, oxidation/reduction potential, dissolved oxygen, and specific conductance. Samples for DNA analysis were collected using a Sterivex 0.22  $\mu\text{m}$  filter (Millipore, Billerica, MA) unit once the groundwater parameters changed by less than 10% over a 15-minute period.

Soil samples were collected from two locations up-gradient, and within the treatment area (Figure 3.4). Soil was collected from a depth of approximately 2 meters below ground surface using a manual auger.

### 3.3.3 Analytical Methods

#### 3.3.3.1 Zero Valent Iron Characterization Analysis

X-ray diffraction (XRD) was used to characterize the iron in the amendment being tested in laboratory experiments. XRD characterization was performed using a Rigaku RPT 300 RC diffractometer with Co source and measuring K- $\alpha$  ( $\lambda = 1.78890 \text{ \AA}$ ) radiation, with a  $0.02^\circ$  step size, in the  $2\theta$  range between  $10^\circ$  and  $70^\circ$ . The XRD patterns are shown in Cu K $\alpha$  ( $\lambda = 1.54059 \text{ \AA}$ ) radiation. The size distribution of the ZVI was determined by measuring the light scattering pattern using a Mastersizer 2000. Surface morphology of the iron particles was characterized using a scanning electron microscope (SEM, Hitachi S-4500 N, 10kV).

#### 3.3.3.2 Chlorinated Solvent Analysis

Chlorinated ethene sampling was conducted by transferring 250  $\mu\text{L}$  aqueous aliquots from the batch reactors to 2 mL GC vials containing 1 mL of hexane for CVOC extraction. The vials were vortex mixed for 10 seconds and allowed to equilibrate for two hours before extracting the hexane to be injected into the gas chromatograph (GC).

Chlorinated solvent concentrations were obtained using an Agilent 7890 Gas Chromatograph equipped with a DB-624 capillary column (75 m x 0.45 mm x 2.55  $\mu\text{m}$ )

and an electron capture detector (ECD). The experimental conditions were adapted from the EPA method 8021.

### 3.3.3.3 DNA Extraction and Analysis

Sterivex filters were cut into small squares approximately 0.5 cm x 0.5 cm in size using sterile blades. DNA was then extracted from the pieces of filter paper using DNeasy PowerSoil Kit (MoBio Laboratories, Inc.) following the procedure given by the manufacturer. The extracted DNA was eluted with 50uL of sterile DNase/RNase free water and stored at -80°C.

A quantitative Polymerase Chain Reaction (qPCR) thermocycler (BioRad) was used to measure the total abundance of 16S rRNA in the DNA samples. Set up for qPCR was performed in a UV chamber with qPCR designated pipettes. The chamber, pipettes and all equipment handling the sample were UV treated for 30 mins prior to setting up qPCR. Outside of the chamber, a dilution series using a *Dehalococcoides* plasmid of known concentration was made to create a standard curve. A standard curve efficiency of greater than 85% was ensured before other reactions were set up. All dilutions were made with sterile DNase/RNase free water. Reactions were performed in the UV chamber after all dilutions were completed.

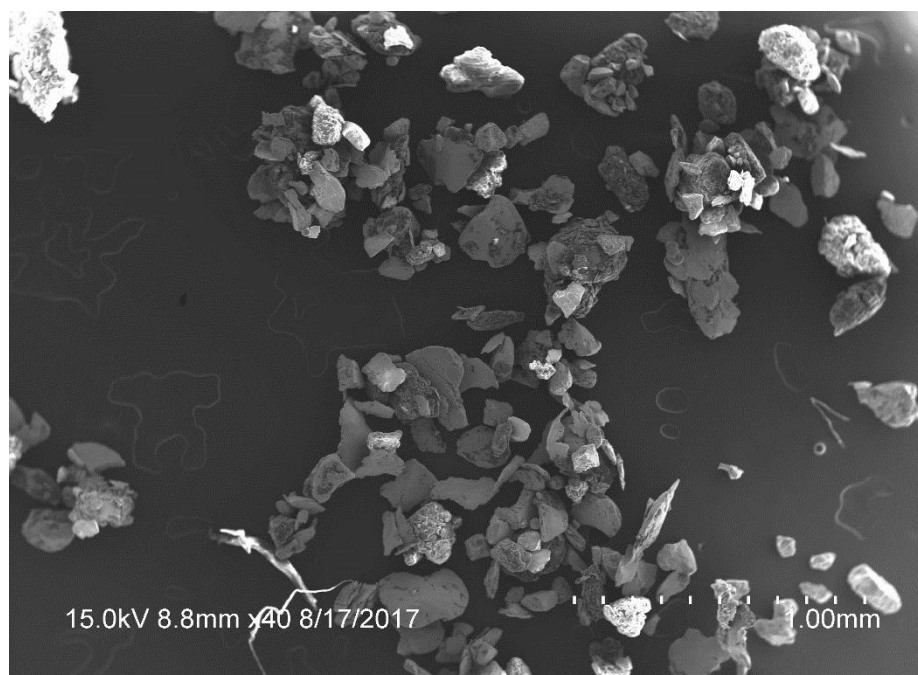
A Master Mix containing UV treated DNase/RNase free water, a reaction mixture (SsoFast EvaGreen Supermix from BioRad), and 10uM forward and reverse general bacteria primers. 2uL of diluted DNA sample or standard and 18uL was created and added to each well of the plate along with the DNA samples. DNA samples were run in triplicates while standards were run in duplicates. Each plate also contained a minimum of 2 blanks containing only Master Mix. The prepared plate was loaded in the BioRad thermocycler and a predefined protocol specific to general bacteria was run to obtain the general bacteria quantities in the sample. The general bacteria concentration in the groundwater for each sample was determined using the quantity measured after performing qPCR, the dilution ratio and the volume of groundwater filtered. The limit of quantification was determined as the lowest value of the standard curve or the highest quantity measured in the blanks.

DNA samples were prepared following the 16S Metagenomic Sequencing Library Preparation protocol for the preparation of 16S ribosomal RNA gene amplicon for the Illumina MiSeq system (Illumina Part # 15044223 Rev. B). After the preparation of the 16S library, Illumina MiSeq is used to sequence the pooled sample library. Analysis is then performed on BaseSpace (Illumina) with the 16S Metagenomics App which performs a taxonomic classification of the 16S rRNA amplicon reads.

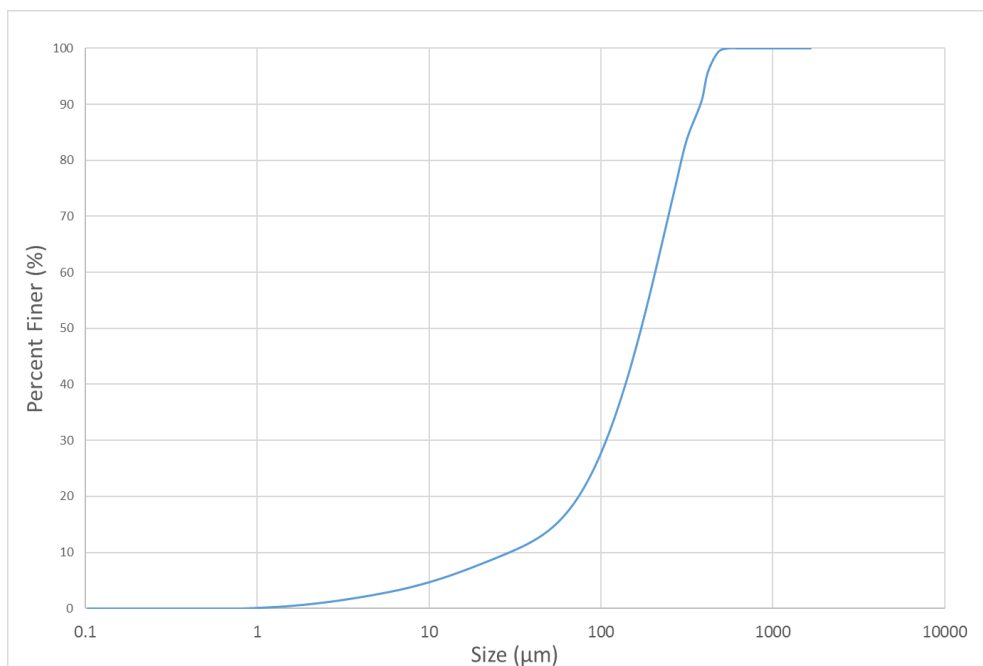
## 3.4 Results and Discussion

### 3.4.1 Amendment Characterization

As a surface mediated reaction, the size, or more specifically available surface area of the ZVI particle influences the rate of reaction with CVOCs (Zhang & Wang, 1997). The size of the iron in the amendment being investigated was examined using electron microscopy (Figure 3.7) and light scattering techniques (Figure 3.8).



**Figure 3.7** Electron microscope image of ZVI particles from the amendment.

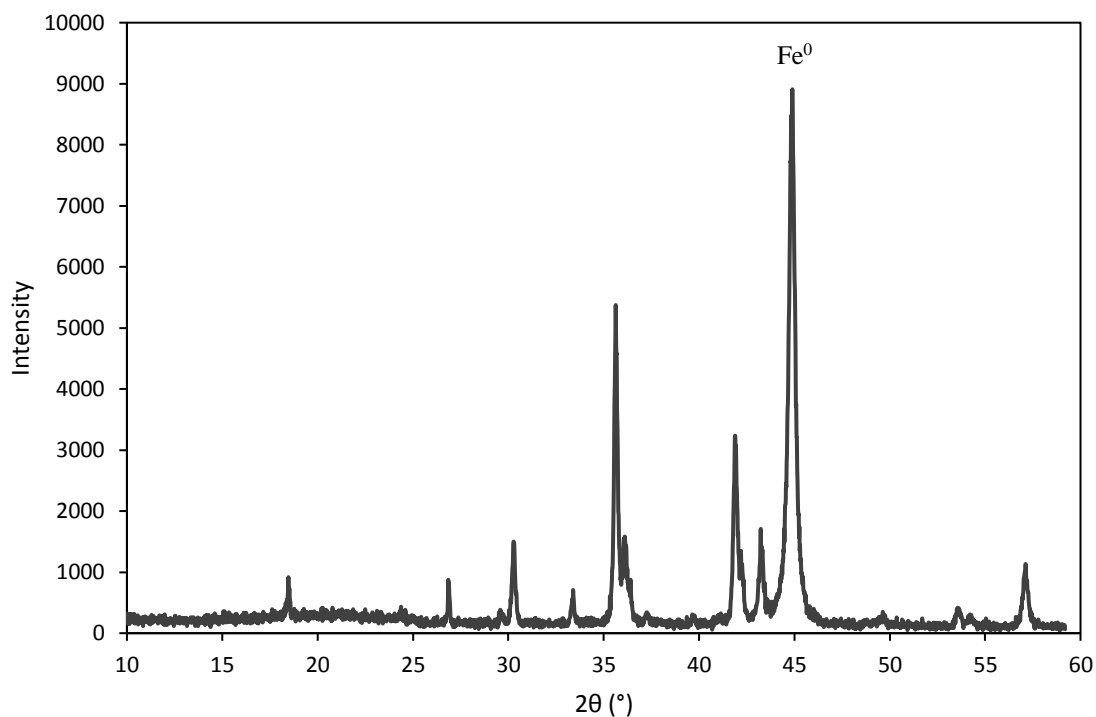


**Figure 3.8 Size distribution of ZVI from the amendment**

Results from the light scattering experiments suggest the size of the ZVI particles have a volume weighted mean of 185.7  $\mu\text{m}$ . For comparative purposes, this size is typically smaller than the ZVI most commonly used in permeable reactive barriers which usually range from 250-2000  $\mu\text{m}$  (ITRC, 2011).

Before conducting the batch reactor experiments, XRD analysis was performed on the iron from the amendment to confirm the presence of ZVI and investigate the presence of other iron species (Figure 3.9).

The diffractogram obtained is consistent with the presence of mainly metallic iron (main diffraction peak at  $44.5^\circ$ ) (Sohrabi et al., 2016). Other peaks identified are attributable to iron oxides, most notably magnetite, and hematite with main diffraction peaks of  $30^\circ$ ,  $35.5^\circ$ ,  $43^\circ$ ,  $54^\circ$ , and  $57^\circ$  (Boparai et al., 2010).



**Figure 3.9 XRD results of the ZVI from the amendment**

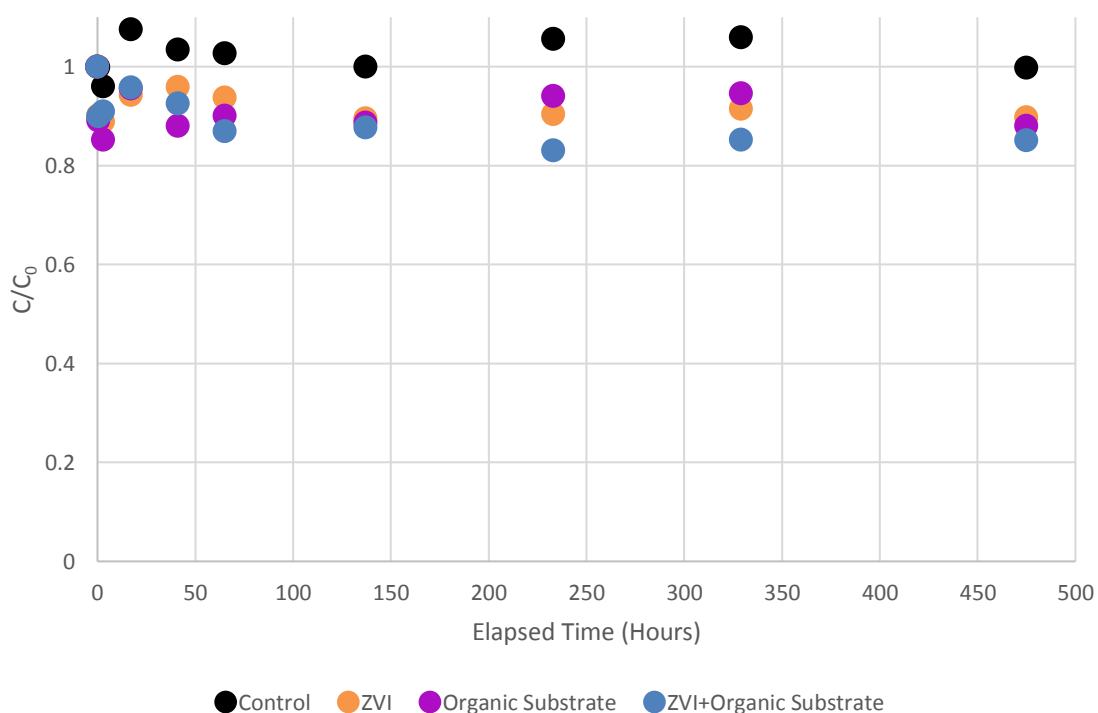
The contents of the organic substrate is proprietary and was not characterized. As stated in the patent, the organic content consists of “fibrous organic matter capable of supporting bacterial growth”. The organic matter is generally derived from plant matter preferably with high nitrogen content, and can be supplemented with both fibrous simple carbon sources, as well as complex organic matter (A. G. et al Seech, 2000).

### 3.4.2 Short-Term Changes

#### 3.4.2.1 Abiotic Effects

Both the ZVI and the organic substrate which make up the amendment used in the remediation project, are reported to have abiotic effects that can contribute to CVOC concentration changes. ZVI has been shown to be a versatile remediation tool, capable of reacting with many priority groundwater contaminants (O’Carroll et al., 2013). The technology is widely used in treating chlorinated compounds, though numerous studies have shown that it is not effective at degrading 1,2-DCA (Lien & Zhang, 2005; Song &

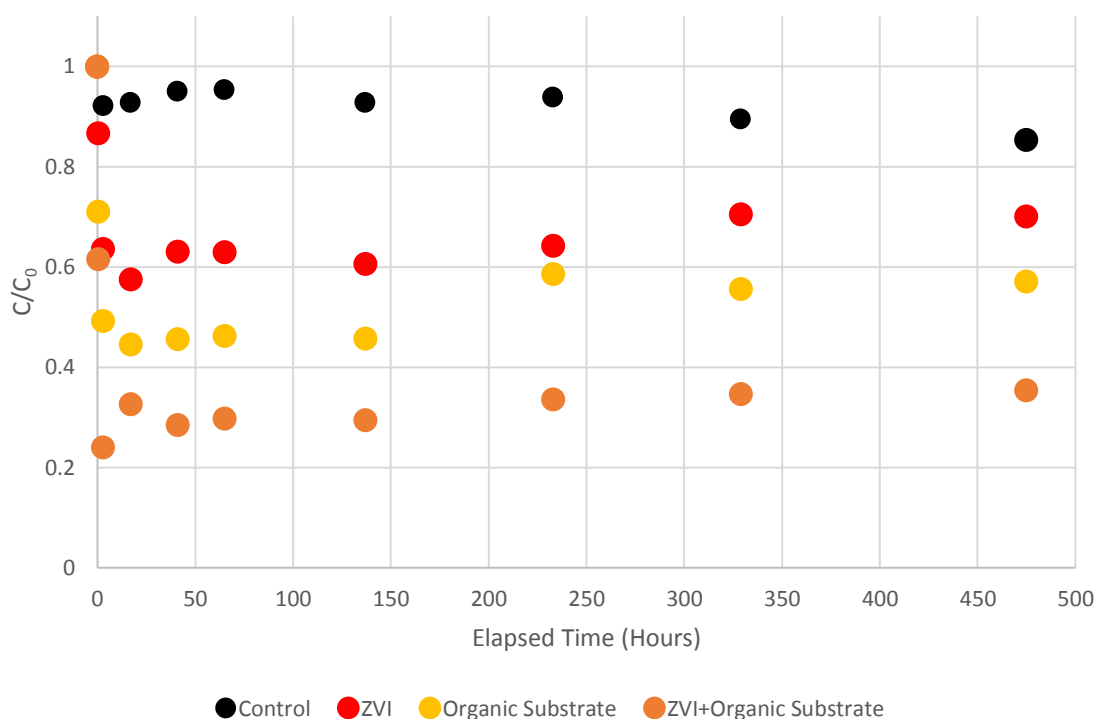
Carraway, 2005). The organic content of the amendment is designed to enhance the biodegradation caused by pre-existing microorganisms in the soil, though suppliers also state that the fibrous nature of the organic substrate in the amendment permits absorption of halogenated organic chemicals (A. G. et al Seech, 2000). The reactor experiments in the absence of soil were designed to remove the effects of biodegradation, allowing for a better understanding of the abiotic processes imparted by the ZVI and organic substrate. The results of experiments testing the short-term impact the ZVI and the organic substrate have on 1,2-DCA concentrations without the presence of soil are summarized in Figure 3.10.



**Figure 3.10 Effect of the amendment components on 1,2-DCA concentrations when combined and separated.** *Black points represent the control which did not contain amendment. Blue points contained both ZVI and the organic substrate. Orange points contained ZVI, and purple points contained the organic substrate.*

These results suggest that the ZVI and organic substrate, both separately and combined, had minimum impact on 1,2 DCA concentrations. 1,2-DCA conversion levels were less than 20 percent within 20 days.

Previous research suggests that PCE can be rapidly dechlorinated by ZVI, and of all the chlorinated ethenes, shows the highest rate of degradation (Cwiertny & Scherer, 2010; Song & Carraway, 2005). The results of experiments testing the short-term impact the ZVI and the organic substrate have on PCE concentrations without the presence of soil are summarized in Figure 3.11.



**Figure 3.11 Effect of the amendment components on PCE concentrations when combined and separated.** *Black points represent the control which did not contain amendment. Orange points contained both ZVI and the organic substrate. Red points contained ZVI, and yellow points contained the organic substrate.*

From the experiments testing the abiotic effects on PCE concentration, reactors with only ZVI suggest iron can decrease PCE concentrations 30 to 40 percent. The rate of degradation caused by the ZVI appears to plateau in this relatively short timeframe as the surface of the particles passivate due to hydrolysis (Lee & Batchelor, 2000). Reactors containing only the organic substrate show evidence of being able to reduce PCE concentrations 40 to 50 percent. Since the absence of soil should inhibit biotic reactions, the reduction in PCE concentration in the organic substrate reactor is hypothesized to be the result of sorption to

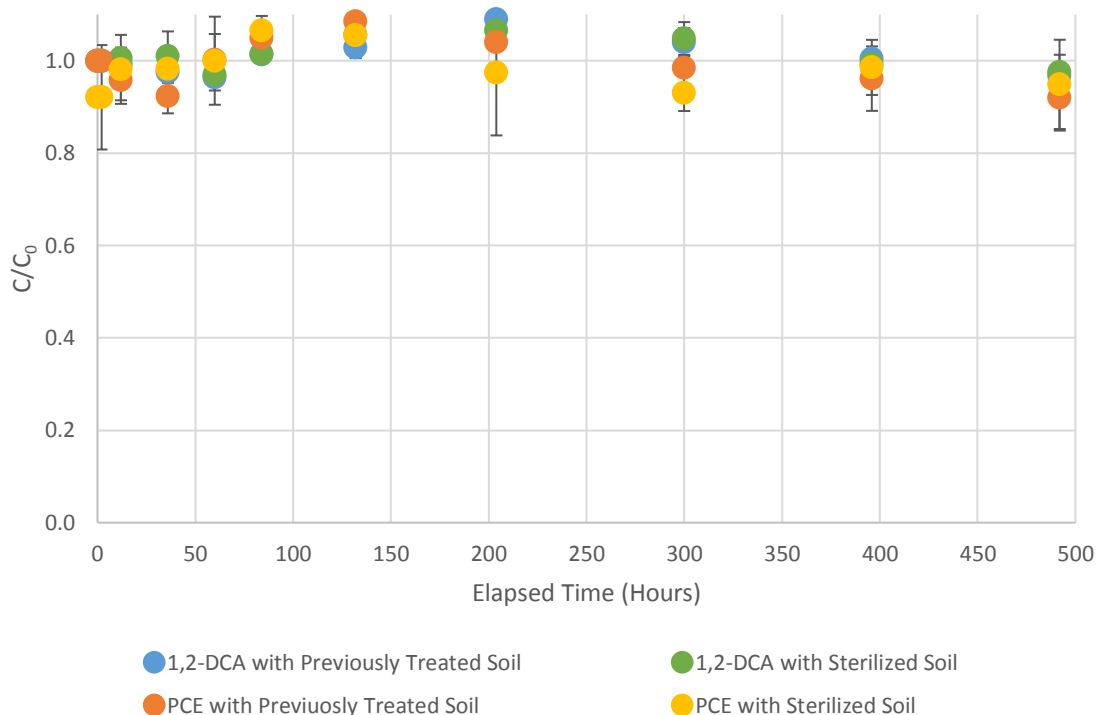
the organic substrate. The octanol/water partition coefficient ( $\log K_{OW}$ ) can be a proxy for a solvent's affinity or sorb to organic matter in the subsurface. The greater  $K_{OW}$  of PCE (2.88) compared to 1,2-DCA (1.48) may explain the greater PCE concentration change compared to 1,2-DCA in reactors containing organic substrate. The effects of the ZVI and the organic substrate also seem to be additive, demonstrated in the reactors with both ZVI and the organic substrate where PCE concentrations decreased between 65 and 70 percent, likely by an additive combination of both sorption and chemical dechlorination.

### 3.4.2.2 Biotic Effects

While both anaerobic and aerobic reactions can degrade chlorinated solvents, most remediation practitioners try to harness anaerobic biodegradation due to ease of implementation in-situ (compared to aerobic reactions), and ability to treat a wide range of chlorinated hydrocarbons (Stroo et al., 2014). While anaerobic degradation has been shown to degrade all chloroethenes, literature suggests that the rate of reductive dechlorination of PCE will be greater than 1,2-DCA (Christ et al., 2005; Löffler et al., 2013b). Furthermore, researchers have suggested that biodegradation can appear to stall at lower chlorinated solvents like 1,2-DCA due to the difficulty many microorganisms have in degrading it (Bradley & Chapelle, 2010). To test for the impact anaerobic biodegradation can have on PCE and 1,2-DCA concentrations in the laboratory experiments, soil collected from a contaminated site that was previously treated using the ex-situ mixing process 4 years prior to sample collection was added to the next iteration of batch reactor experiments. In these experiments, the effect of the amendment on biotic degradation was tested using the undivided amendment; the ZVI and organic substrate were not analyzed individually as in the previous experiment. Anaerobic reactors either contained soil collected from within the area previously treated to incorporate microbial activity to the reactors, or soil from the same location that was first autoclaved to control for the abiotic effects of soil addition. The results of these experiments, testing for the combined abiotic and biotic effects of the amendment on the anaerobic degradation rate of 1,2-DCA and PCE, can be seen in Figures 3.12 and 3.13. The change in 1,2-DCA and PCE concentrations over time in soil slurry reactors without the amendment present can be seen in Figure 3.12. The effects of the

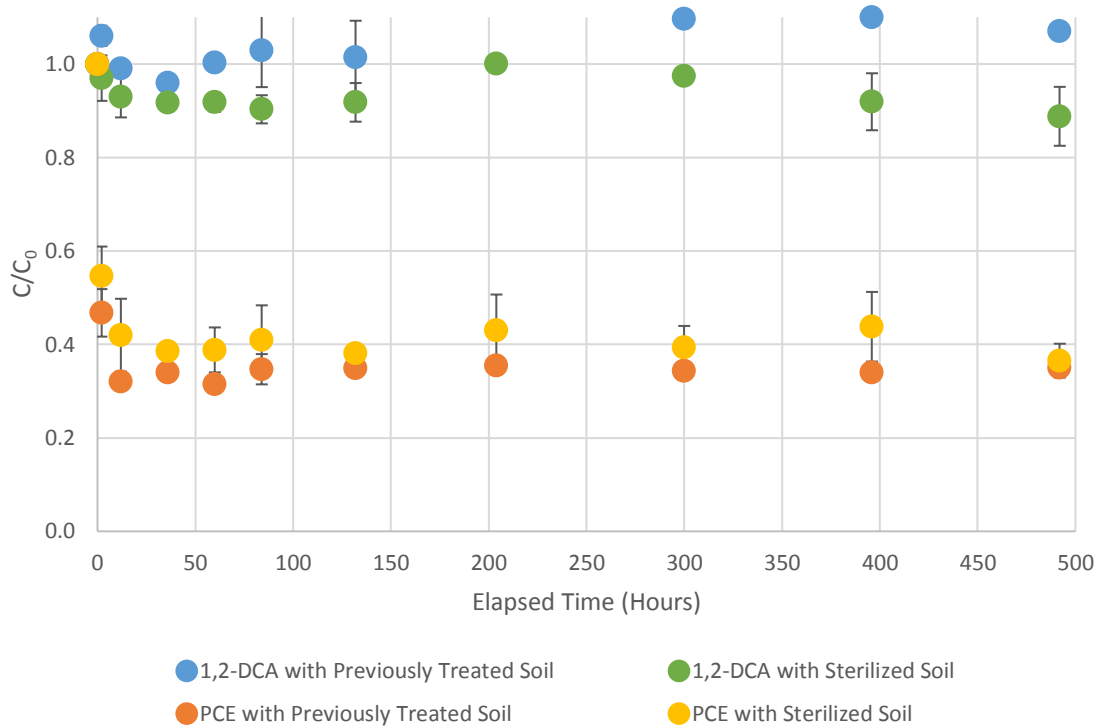


amendment in the presence of both treated and sterile soil on CVOC concentrations can be seen in Figure 3.13.



**Figure 3.12 Effect of soil addition on CVOC concentrations in an anaerobic environment in the absence of amendment.** Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols.

Figure 3.12 shows that without amendment present, neither 1,2-DCA, or PCE concentrations change more than 10 percent within 3 weeks, suggesting that the microorganisms present from the addition of soil previously treated using the ex-situ mixing technology cannot significantly biodegrade the CVOCs under anaerobic conditions without amendment. The reactors containing sterilized soil also show the soil does not abiotically affect 1,2-DCA or PCE concentrations.



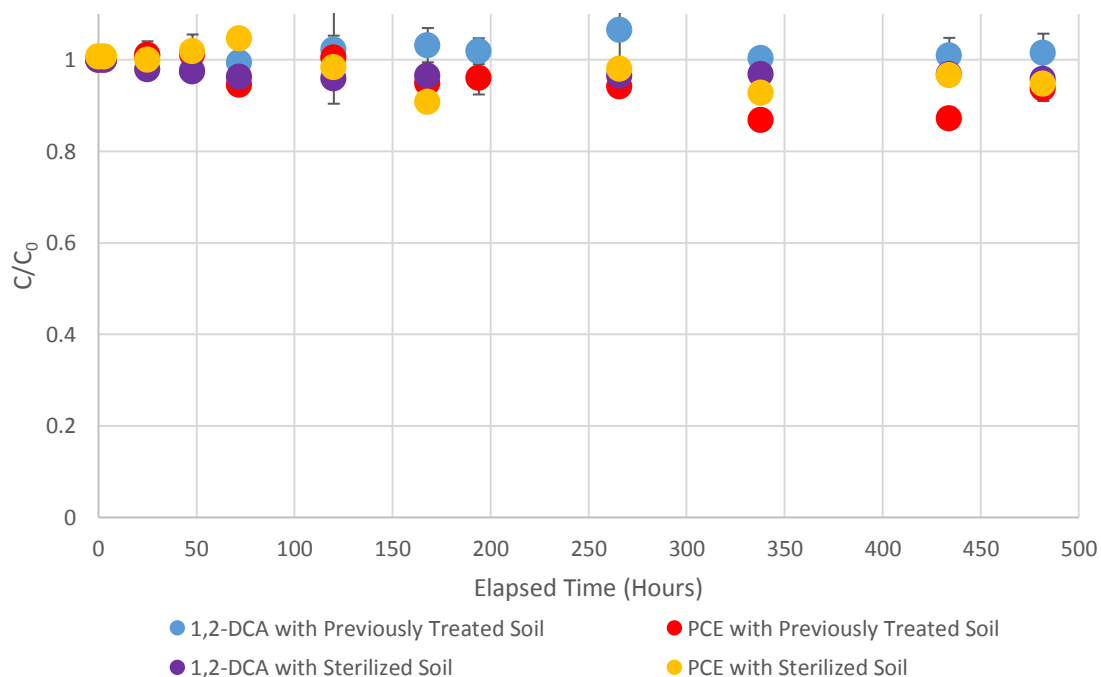
**Figure 3.13 Effect of soil addition on CVOC degradation in an anaerobic environment with amendment present.** Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols.

When the amendment is added to the anaerobic reactors, 1,2-DCA concentrations still do not change more than 10 percent within 3 weeks (Figure 3.13). An approximate 40% reduction in PCE concentration is consistently observed in all anaerobic batch experiments regardless of the soil being sterilized or not, indicating that the cause of the chemical reduction is not biotic. Furthermore, the 60% reduction is comparable to the reduction seen in the reactors only containing the amendment, and not soil (Figure 3.11). This suggests that the reduction in PCE concentration observed in Figure 3.13 is also due to adsorption to the organic content in the amendment and is not an effect of microbial activity.

The amendment used in the ex-situ mixing process is designed to work best in anaerobic conditions, where the ZVI can reduce competing oxidants and create low ORP conditions promoting reductive dechlorination (A. G. Seech et al., 1995). Although, the ex-situ mixing process likely means that while above-ground, the soil environment was aerobic. As such,

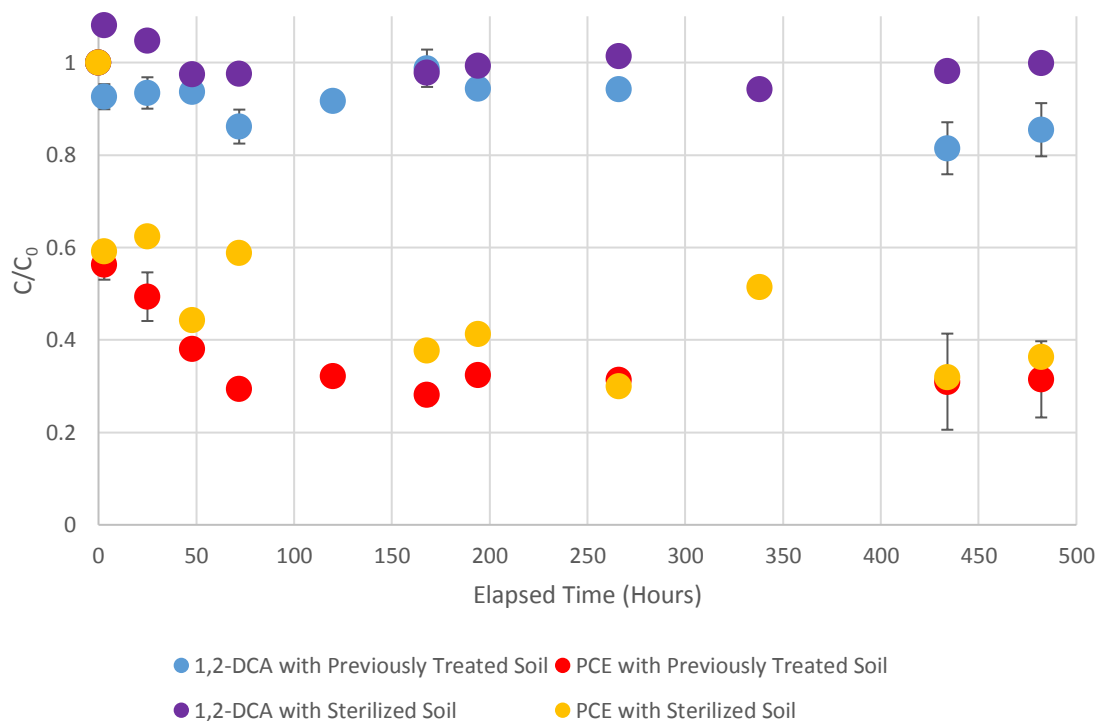
aerobic bioremediation may have been a more realistic process that could lead to dechlorination in the 3-week period the soil was ex-situ. In reducing environments, PCE is more readily degraded, however, under aerobic conditions microorganisms can rapidly dechlorinate lesser chlorinated species like VC and 1,2-DCA, but have never been shown to degrade higher chlorinated ethenes such as PCE (Field & Sierra-Alvarez, n.d.; Le & Coleman, 2011).

To test for possible effects aerobic degradation may have on 1,2-DCA and PCE concentrations, the batch reactors were sealed in atmospheric conditions to allow for aerobic activity. Experiments testing for the combined abiotic and biotic effects of ZVI and the organic substrate on the aerobic degradation rate of 1,2-DCA and PCE can be seen in Figures 3.14 and 3.15. The change in 1,2-DCA and PCE concentrations over time in soil slurry reactors without the amendment present can be seen in Figure 3.14. The effects of the amendment on CVOC concentrations in the soil slurry reactors can be seen in Figure 3.15.



**Figure 3.14 Effect of soil addition on CVOC degradation in an aerobic environment in the absence of amendment.** *Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols.*

Figure 3.14 shows that without amendment present, 1,2-DCA and PCE concentrations change less than 20 percent within 3 weeks. This suggests that the microorganisms present in the soil previously treated in the ex-situ mixing project cannot significantly biodegrade the CVOCs under aerobic conditions when amendment is not present.

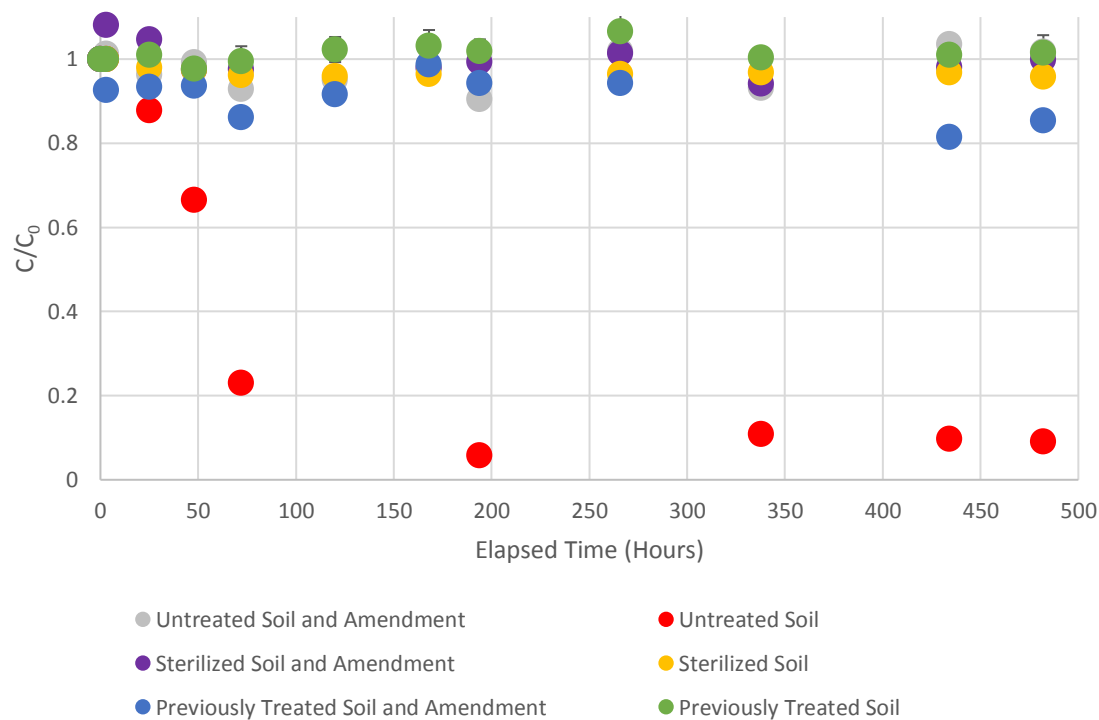


**Figure 3.15 Effect of soil addition on CVOC degradation in an aerobic environment with amendment present.** Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols.

When the amendment is added to aerobic reactors, 1,2-DCA concentrations do not decrease more than 20 percent. PCE concentrations decrease between 50 and 70 percent regardless if the soil is sterilized or not. The trends observed for both 1,2-DCA and PCE are consistent with those found in the anaerobic reactor experiments. This further supports the hypothesis that the reduction in PCE is mediated by an abiotic process, and is not the result of either anaerobic, or aerobic biodegradation.

Previous studies have investigated the impact of oxygen exposure to microorganisms capable of dechlorination – in particular, *Dhc* (Amos et al., 2008). It was hypothesized the

previously treated soil used in the aerobic batch reactor experiments could have undergone a microbial community change that could impact its ability to biodegrade CVOCs. This idea was explored by conducting aerobic batch reactor experiments with contaminated soil collected up-gradient to groundwater flow from the area that was previously remediated ex-situ. This soil has not been subjected to the ex-situ remediation process and is assumed to contain a similar biological community to what would have been present in the treatment area before the remediation project took place. Figure 3.16 shows 1,2-DCA concentration changes when untreated soil was used in the aerobic batch reactor experiments, and compares the results to the other aerobic batch reactors containing 1,2-DCA.



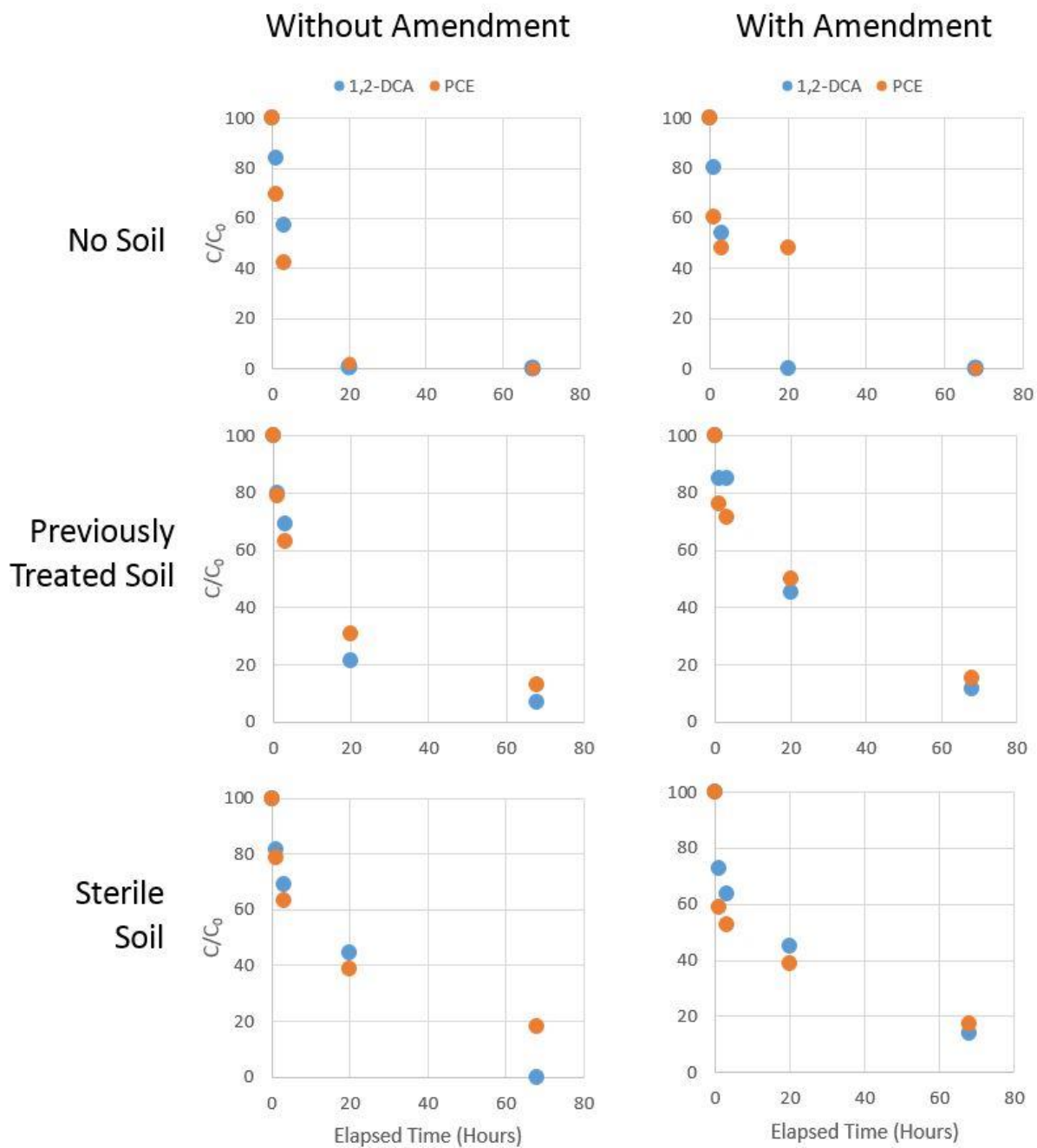
**Figure 3.16 Effect of previously treated and untreated soil on 1,2-DCA concentrations in an aerobic environment.** Symbols represent average concentrations from duplicate reactors or controls. Error bars may be smaller than the symbols.

As previously shown, 1,2-DCA does not degrade more than 20 percent when microorganisms are introduced using previously treated soil. 1,2-DCA concentrations decreased over 90 percent in less than 200 hours when untreated soil was used instead, but did not occur when the untreated soil was first sterilized. This suggests that a difference in the microorganism community between the previously treated soil and untreated soil has

resulted in the treated soil becoming unable to biodegrade 1,2-DCA. When the amendment is also added to a reactor with untreated soil, 1,2-DCA concentrations do not decrease more than 10 percent, demonstrating the amendment may inhibit aerobic biodegradation of 1,2-DCA over a 3-week period. These experiments though do not clarify the mechanism(s) causing this inhibition. One possible hypothesis is the ZVI is reducing oxygen to hydroxides and water causing the reactor to become anaerobic. Future tests should monitor for the presence of oxygen during these experiments.

### 3.4.2.3 Volatilization

The proportion of short-term CVOC losses observed during the ex-situ mixing project that can be attributed directly to volatilization is not known, and attempts to isolate its effect in the context of this remediation technology have not been carried out. With Henry's constant values for PCE and 1,2-DCA of  $26.3 \text{ atm M}^{-1}$ , and  $1.2 \text{ atm M}^{-1}$ , and vapor pressures of 18.1 torr and 79 torr respectively, it is hypothesized that volatilization could measurably affect CVOC concentration changes given the ex-situ mixing process. To evaluate the effect of volatilization on CVOC concentrations in a soil slurry, experiments in open beakers were conducted and are presented in Figure 3.17. Sterilized and unsterilized soil, as well as reactors with and without amendment were used to evaluate the possible impact of abiotic and biotic activity during the experiment.



**Figure 3.17** Effect of volatilization on CVOC concentrations. *Blue points represent 1,2-DCA concentrations, and orange points represent PCE concentrations.*

It can be observed that the trend of CVOC concentration change among reactors with, and without amendment are similar. This suggests that when tested in sterilized soil, the addition of amendment does not impart any abiotic effects such as adsorption or chemical reduction to the rate of CVOC concentration change. Furthermore, when tested in the previously treated soil, it suggests that there are also no biotic effects on CVOC concentrations being created by amendment addition.

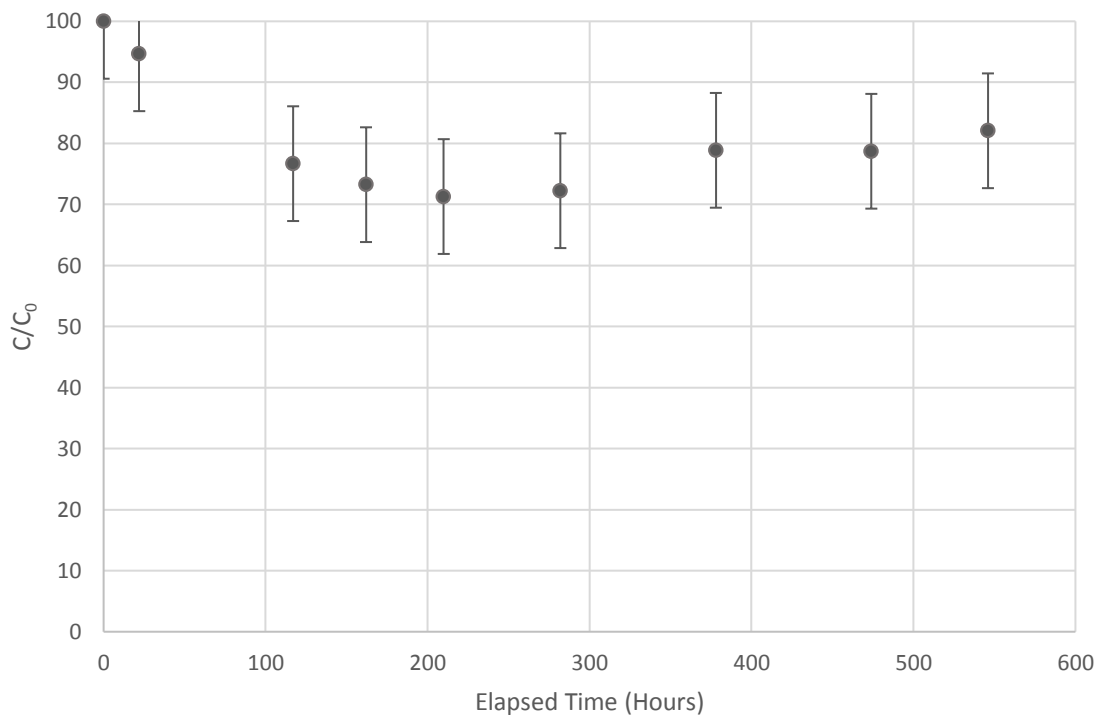
These experiments also show decreases in 1,2-DCA concentrations that differ from the findings observed in the sealed vessel experiments using the previously treated soil, which never showed losses greater than 20 percent. There also appears to be a noticeable difference in the PCE concentrations observed after 70 hours in the volatilization experiments compared to those observed in the sealed reactors with the same contents (Figures 3.11, 3.12, 3.13, 3.14, 3.15). It is hypothesized that the only additional process that could affect CVOC concentration changes in the open beaker experiments versus sealed experiments is volatilization. These results strongly suggest that the main process causing the observed decreases in CVOC concentrations in the field is volatilization.

### 3.4.3 Long Term Changes

#### 3.4.3.1 ZVI Mediated Reduction

Over time, if exposed to the environment zero valent iron will corrode (passivate) as it reacts with oxygen in the atmosphere. While the amendment suppliers report that the product can remain active in the ground for over 5 years, this is dependent in part on the proportion of iron that is in the zero-valent state when it is placed in the ground, and the proportion that is subject to corrosion while above ground. The ex-situ application process used in this field remediation project allowed for the iron to potentially be in contact with the atmosphere for an average of three weeks. If iron passivation is substantial within this time, the iron may not have been able to impact CVOC concentrations in the ground for as long as what is claimed by the amendment manufacturer. Figure 3.18 depicts changes in the proportion of metallic iron in the zero-valent state while being exposed to the atmosphere over a three-week period, as measured by the hydrogen evolution experiments.

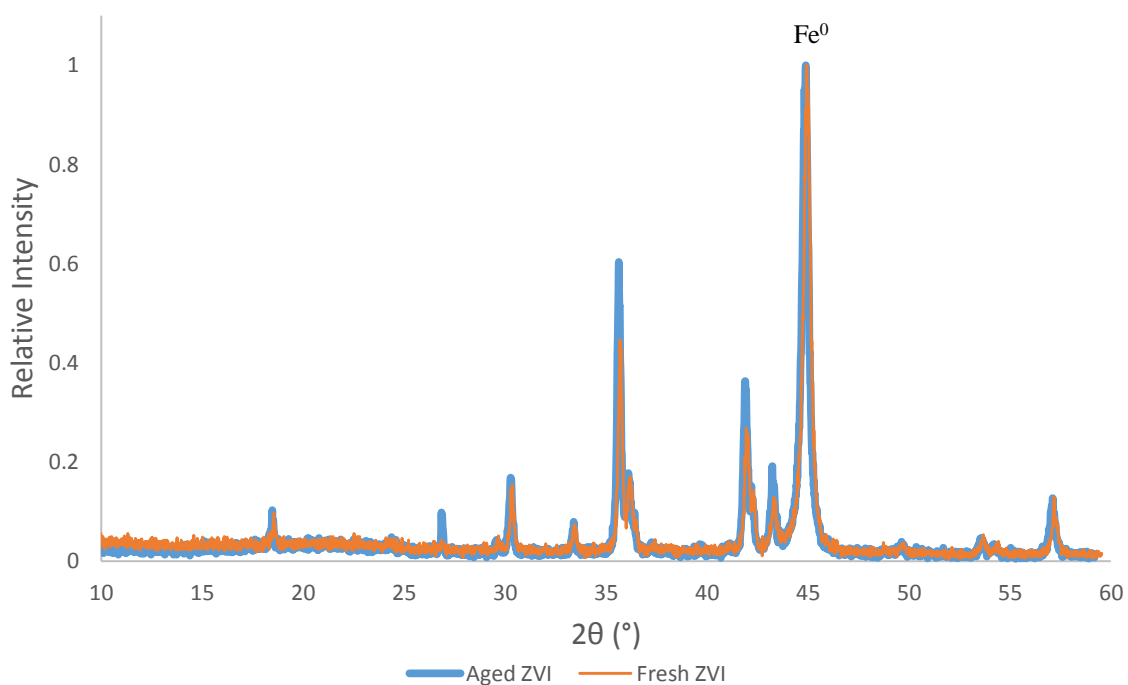




**Figure 3.18 Change in the proportion of iron in the zero-valent state caused by atmospheric exposure.**

The results of this experiment show that the relative proportion of iron in the zero-valent state may decrease approximately 30 percent due to exposure to the atmosphere. It is hypothesized that this may be due to surface passivation on the iron particles (Song & Carraway, 2005). These results also seem to show that after an initial decrease, the proportion of iron in the zero-valent state remains relatively stable over the course of the experiment. This observation may also be explained by surface passivation, which will slow the rate of oxidation of the zero-valent iron core (Zhang & Wang, 1997)

To evaluate the changes in morphology of the iron after being exposed to the atmosphere for 3 weeks, XRD analysis was completed on a fresh ZVI sample, and a sample that was exposed to the atmosphere for three weeks, the diffractograms are shown in Figure 3.19.



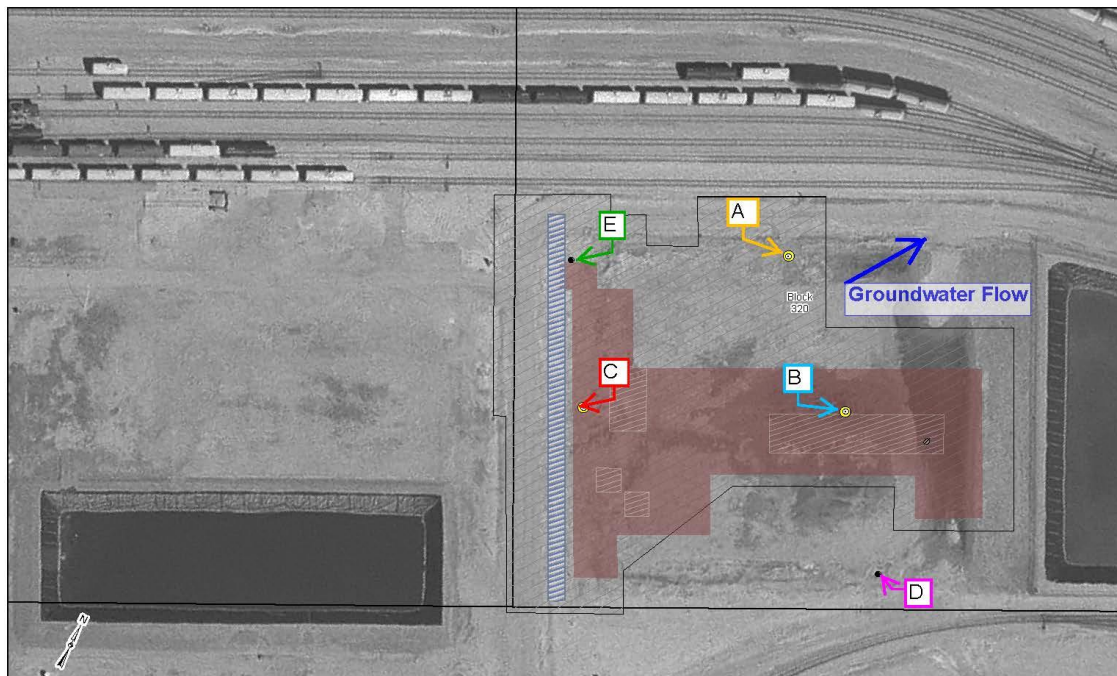
**Figure 3.19 XRD patterns of ZVI unexposed to atmosphere (fresh ZVI), and ZVI that was exposed to atmosphere for 3 weeks (aged ZVI).**

The results clearly indicate that both the fresh, and aged ZVI samples show the characteristic peak of zero valent iron at  $44.5^\circ$  (Sohrabi et al., 2016). The aged ZVI shows similar peaks as the fresh ZVI, likely indicating the most prevalent iron oxide species (magnetite, and hematite) identified in figure 3.9, are dominant in both. The relative intensity of the peaks associated with iron oxide species appear to be larger most notably at  $27^\circ$ ,  $30.5^\circ$ ,  $35.5^\circ$ ,  $42^\circ$ , and  $43^\circ$  in the aged sample, suggesting a greater degree of passivation.

### 3.4.3.2 Biodegradation

Only a small number of microorganisms have been shown to anaerobically degrade chlorinated solvents (Löffler & Edwards, 2006). Of these species, just a fraction of them respire DCE, 1,2-DCA, and VC. This makes the presence of these specific microorganisms in a bioremediation project critically important. To investigate the abundance of these specific microorganisms, and to better understand the broader impact of the ex-situ

remediation process on microbial community structure, groundwater samples were collected from five different locations on a site remediated using the ex-situ mixing technology 4 years earlier (Figure 3.20).

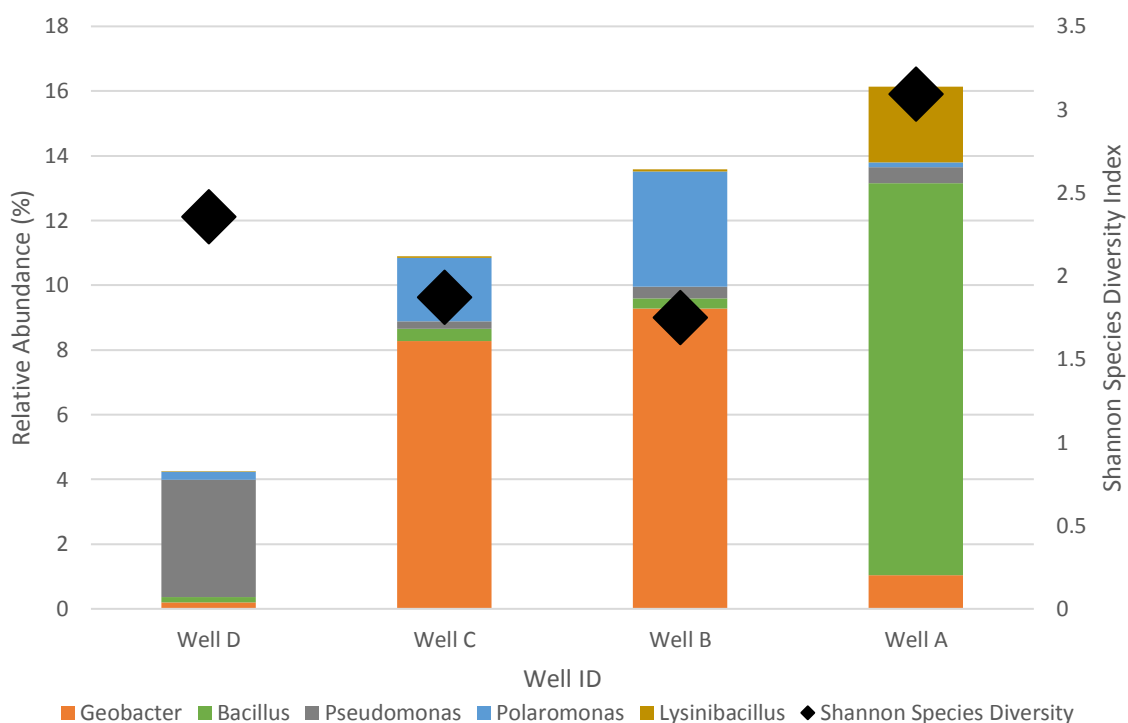


**Figure 3.20** Location of the five monitoring wells sampled for DNA analysis (labelled **A through E**). *Burgundy highlighted area represents the area remediated using the ex-situ mixing process.*

Monitoring well D is located up-gradient of the remediation site. This well therefore represents conditions that were not affected by the remediation process. Monitoring well B and C are installed within the area remediated. Monitoring well A is down-gradient from the area that was treated, and monitoring well E is located outside the area that was remediated using the ex-situ process, but still within the area impacted by CVOCs. Monitoring well E is also the location of a fracture injection study that took place in 2011 where emulsified vegetable oil (EVO) was injected into the subsurface to stimulate biological activity.

Illumina® sequencing was performed on the groundwater samples to understand the change in abundance of each microorganism present at the monitoring well locations.

Figure 3.21 shows the abundance of genera that are capable of degrading chlorinated solvents. The genera included had 2 percent or greater relative abundance in at least one of the 4 monitoring wells up-gradient, within, or down-gradient of the previously remediated site. Figure 3.21 also shows the Shannon Species Diversity Index at each location, which is used as a measure of the community's species diversity.



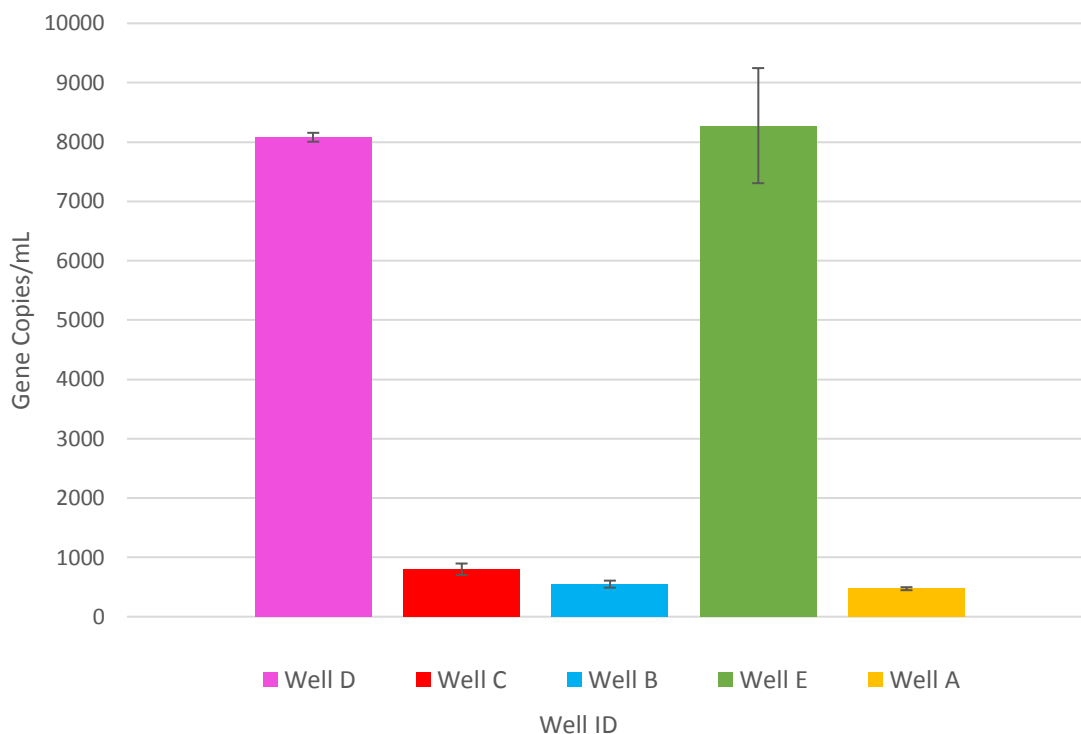
**Figure 3.21 Relative abundance of the most common microorganisms capable of dechlorination as well as the Shannon Species Diversity Index at monitoring wells up-gradient, within, and down-gradient of a previously treated site.**

The Shannon Diversity Index shows higher values outside (up-gradient and down-gradient) of the area treated than within the remediation zone. Looking at the change in relative abundance of the genera capable of dechlorination also shows a distinct community structure within the treatment zone that differs from that both up-gradient and down-gradient of the site.

This community structure shift within the treatment zone is hypothesized to be a result of the ex-situ mixing process, though since samples could not be collected from the treatment

zone before the ex-situ mixing process took place, more indirect evidence is used to support this hypothesis. Historical measurements of CVOC concentrations at monitoring well D have been within the same order of magnitude as groundwater samples collected in the treatment area. The distinct community structure in monitoring wells C and B that differ from monitoring well D provides evidence that the microbial community structure in monitoring well C and B has changed due to the remediation process, and is not a result of contamination concentrations within the treatment zone causing a selective pressure before the remediation project took place. Further evidence is provided by observing that the greatest genera abundance in the wells installed within the treatment area is *Geobacter*. This organism grows chemoorganotrophically with Fe(III) serving as the sole electron acceptor (Mahadevan et al., 2006). The ZVI added to the soil may be the source of ferric iron, and acting as the dominant factor in the population growth of *Geobacter*.

While the relative abundance of microorganisms capable of dechlorinating organic compounds seems to increase from up-gradient to within the treatment zone, none of the genera identified are capable of completely dechlorinating ethenes. Only a select few strains of *Dehalococcoides mccartyi* (*Dhc*) have shown the ability to degrade lower chlorinated species (Löffler et al., 2013b). As such many remediation projects quantify the population of this genera as a proxy for the effectiveness of the microorganism community at degrading chlorinated solvents without stalling at more toxic compounds such as VC (Stroo et al., 2014). *Dhc* population was investigated by analyzing the number of *Dehalococcoides* gene copies from monitoring wells using qPCR analysis (Figure 3.22).



**Figure 3.22 Dehalococcoides gene copies/mL at five monitoring wells sampled on a previously remediated site.** Error bars are the standard deviation of duplicate samples.

The results of this investigation show that the abundance of *Dhc* per milliliter at monitoring wells D and E are an order of magnitude greater than concentrations found at monitoring wells A, B, and C. The higher concentration of *Dhc* gene copies at the background location (monitoring well D) compared to monitoring well B and C, which are within the treatment area is contrary to both what is expected and what is desired. One of the goals of adding the amendment in the ex-situ mixing process is to have the population of *Dhc* become greater than background concentrations. One explanation may be *Dhc* activity being adversely affected by the presence of other chlorinated solvents, as previously reported (Bagley et al., 2000), which could be affecting how substantially the remediation process can increase *Dhc* concentrations within the area treated. Although this hypothesis is not supported by the observation that *Dhc* abundance at wells B and C are an order of magnitude lower than monitoring well D and E, which have both been impacted by CVOCs.

The effect of oxygen exposure on *Dhc* provides another hypothesis contributing to the lower *Dhc* abundance seen within, and down-gradient of the treatment area. Research has shown that exposure to even small quantities of oxygen can irreversibly inhibit *Dhc* dechlorination (Amos et al., 2008; Löffler et al., 2013a). The ex-situ mixing process exposed the soil to oxygen concentrations much greater than the concentrations used to test *Dhc*'s oxygen sensitivity in the referenced literature. The dissolved oxygen content of the monitoring wells at the time of sampling is considered below 1 mg/L, which is the level deemed damaging to anaerobic biodegradation (Stroo et al., 2014) (Table 3.1). This further suggests that if oxygen exposure has negatively impacted *Dhc* abundances, it was a result of past exposure possibly from the mixing process, and not an effect of the dissolved oxygen levels at the time of sampling.

**Table 3.1 Groundwater parameters important to *Dhc* viability at monitoring wells before and after the ex-situ remediation process.**

	Spring 2011 (Pre-Remediation)			Spring 2016 (Post-Remediation)			Spring 2017 (Post-Remediation)		
	Oxidation-Reduction Potential (mV)	Dissolved Oxygen (mg/L)	pH	Oxidation-Reduction Potential (mV)	Dissolved Oxygen (mg/L)	pH	Oxidation-Reduction Potential (mV)	Dissolved Oxygen (mg/L)	pH
Well A	-15.2	1.17	6.4	-29	0.69	7.9	-56.1	0.31	6.7
Well B	-17.1	1.21	6.6	-127.2	0.31	7.6	-23.5	0.41	6.5
Well C	-292.8	0.68	6.5	-3.7	0.22	7.5	-40.8	0.62	6.4
Well D	30.1	0.87	6.8	187.9	0.25	7.8	-53.1	0.42	6.8
Well E	-137.1	0.21	6.3	-128.9	0.35	7.8	-100.1	0.34	6.2

The oxidation-reduction potential (ORP), and pH have also been reported to be important for *Dhc* activity. Measurements of these parameters taken at the time of sampling (Table 3.1) indicate that pH values are within the acceptable range, but ORP values are generally higher than the desired values of < -100 mV (Stroo et al., 2014) at the wells within the area remediated (wells B and C) at the time of microorganism sampling (Summer 2017). As mentioned previously, a key attribute of the amendment that is advertised is its ability to create redox conditions which promote reductive dehalogenation. Field measurements collected at the time of sampling do not support these conditions exist. It is important to

note that these conditions could have existed in the past, but the amendment's effect on the subsurface environment had diminished by the time samples were collected for this study.

### 3.5 Summary

This study used a combined field and laboratory approach to evaluate the efficacy of a novel chlorinated solvent remediation strategy. Overall findings suggest that the strategy did not work as hypothesized by the practitioners.

Laboratory experiments provide a proxy for the three weeks that the soil was above ground and having the amendment mixed into it during the remediation project. Results from the short-term batch reactor experiments show that 1,2-DCA cannot be degraded abiotically, or biotically in a sealed anaerobic system within three weeks. 1,2-DCA may be able to be biodegraded aerobically, but this process may be inhibited by the amendment. The batch reactor tests suggest that the organic substrate may be sorbing up to 60% of the PCE in the system, though this does not necessarily imply degradation. Further studies would need to be conducted to confirm whether the PCE is being degraded or only removed from the aqueous phase. Beyond the hypothesized sorption mediated decrease in concentration, PCE did not show evidence of abiotic or biotic degradation. In reactors open to the atmosphere, PCE and 1,2-DCA concentrations decreased a minimum of 75%, and at similar rates regardless of amendment addition or soil type. This supports the hypothesis that volatilization is the dominant process mediating chlorinated solvent concentration changes. There is little evidence that supports any further abiotic or biotic processes that significantly contribute to CVOC degradation.

Directly measuring the long-term contribution the amendment and the mixing process have on CVOC concentrations once the soil is returned to the ground is very difficult. Understanding that the objective of the remediation process is to anaerobically dechlorinate the CVOCs allows for an investigation into specific parameters that literature suggest are required for successful anaerobic dechlorination. Results from field investigations reveal that the geochemical environment as well as the biological community within an area



previously remediated using the ex-situ mixing process may not support anaerobic dechlorination at a rate considered acceptable for engineered remediation systems. Furthermore, the microbial community within the treatment zone is still showing effects of the ex-situ mixing process and populations of the organisms most desired to be present for complete anaerobic dechlorination have been negatively impacted from the ex-situ remediation project, and have not yet recovered.

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## Chapter 4

### 4 Conclusions and Recommendations

#### 4.1 Conclusions

The ex-situ mixing process is an attractive remediation technology in specific situations such as tight project timelines, or when remediating low permeability soils which make in-situ technologies difficult. Large quantities of soil can be treated in a short amount of time, and there is likely no other process that can disperse an amendment as homogeneously. Though the sampling conducted during the full-scale remediation project left important questions to be investigated if the technology is to be used more frequently. This research explored more closely the effects this novel ex-situ remediation technology has on chlorinated solvent concentrations, as well as its impact on geochemical parameters and microorganisms in treated soil. Changes in PCE and 1,2-DCA concentrations in batch reactor experiments measured under anaerobic and aerobic conditions clarified which mechanisms had the greatest impact on CVOC concentrations. Impacts of the ex-situ mixing process and amendment on the subsurface environment were assessed through groundwater samples collected and analyzed from a site remediated 4 years prior to collecting the samples.

Results from the laboratory batch reactor experiments indicate that:

- The amendment used in the remediation project does not degrade 1,2-DCA abiotically in a closed batch reactor system.
- The native microorganisms from within, or outside of the treatment zone cannot biodegrade 1,2-DCA on their own anaerobically. The environment created by adding the amendment also does not make a measurable difference in 1,2-DCA biodegradation.
- Evidence suggests that 1,2-DCA can be degraded aerobically, but the presence of the amendment may inhibit the solvent's dechlorination. This may be due to the

change in the microorganism community structure resulting from the ex-situ mixing process, though further research is needed to confirm this possibility.

- Batch reactor experiments show the organic substrate used in the amendment may be sorbing PCE. Aside from this, the batch reactor experiments do not suggest that PCE can be further abiotically or biotically degraded regardless of the presence of the amendment in the reactor.
- Volatilization appears to impact the change in both 1,2-DCA and PCE concentrations to a larger degree than all other abiotic and biotic degradation mechanisms.

Results from the field investigation suggest that:

- The REDOX conditions within the treatment area were not within the desired range for anaerobic dechlorination when sampled at three and four years after the remediation project took place.
- Field sampling of the microbial community suggest the ex-situ mixing process caused a shift in the microbial community that is still measurable four years after the remediation project.
- The population of Dehalococcoides which are the only known organisms able to completely degrade chlorinated solvents may have negatively impacted from excessive oxygen exposure during the mixing process.

## 4.2 Recommendations

While the batch reactor experiments provide clear evidence that the amendment had minimal effect on the degradation of 1,2-DCA and PCE, the results should be scrutinized in an experimental system which better resembles field conditions, specifically an open system where losses by volatilization are monitored.

The field samples that were collected for this research provide a snapshot in time of the subsurface environment. Because of this, it is unclear if the amendment was still inducing

an effect on geochemical conditions such as ORP. Future work should attempt to collect field samples at regular intervals starting immediately after a similar remediation project finishes to better understand the conditions created from the mixing-process and the amendment.

The *Dhc* populations in all groundwater samples were low compared to what is desired in a bioremediation system. A set of experiments designed to understand how the ex-situ mixing process may affect larger populations of *Dhc* would be beneficial.

Lastly, this information provides important insight into the sacrifices that will need to be made to use it in the future. While this work brings to light some important shortcomings involved in the ex-situ technology, it successfully remediated soil arguably few other technologies would be able to. Additionally, all remediation strategies have their own shortcomings and pitfalls that need to be acknowledged. These results are not believed to invalidate the use of the ex-situ technology, but should be made available to future practitioners, allowing them to make better planning decisions and make the technology itself more successful going forward.



## Appendices



**Appendix A Photo of batch reactors used in the bench scale experiments.**

**Appendix B Results for qPCR duplicate samples.** *Gene copies/mL was determined by multiplying the extracted DNA concentration by dilution and total extracted volumes.*

Name	Cq	Total Gene Copies	Standard Deviation	Average Gene Copies	Gene Copies/mL
Well A	33.7	114.27	25.31	132.16	472.01
	33.27	150.06			
Well B	30.86	699.89	58.74	658.36	548.64
	31.06	616.83			
Well C	30.23	1045.74	96.86	959.05	799.21
	30.54	854.51			
Well D	26.74	9642.54	75.27	9695.76	8079.8
	26.73	9748.99			
Well E	26.81	9242.11	969.98	9930.66	8275.55
	26.53	11039.97			

**Appendix C Standard curve statistics for qPCR analysis.**

Sample	Efficiency %	R <sup>2</sup> Value
Field Study Groundwater	89.22	0.938

**Appendix D Percent unclassified, and the top 50 most abundant genera identified at each groundwater well location through Illumina Sequencing.**

Genus	Well A	Well B	Well C	Well D	Well E
% Unclassified	20.98	31.67	36.44	20.72	21.26
Geobacter	1.04	9.29	8.27	0.20	33.96
Leuconostoc	1.47	10.93	6.49	2.01	0.45
Deinococcus	0.56	1.35	1.12	7.80	1.84
Geobacillus	0.87	1.53	2.29	5.38	0.99
Bacillus	12.11	0.31	0.39	0.16	0.04
Flavobacterium	0.37	2.61	2.12	3.61	1.83
Streptomyces	8.88	0.29	0.62	0.29	0.04
Demequina	0.11	1.23	1.14	4.39	0.01
Acidovorax	0.49	4.14	2.10	2.07	0.01
Mycoplasma	0.49	1.07	1.38	3.77	0.45
Ancylobacter	0.01	2.23	3.13	2.67	0.02
Rhodoferax	0.10	2.79	1.45	2.00	0.01
Pedobacter	0.83	1.14	0.85	2.48	1.34
Pseudomonas	0.50	0.36	0.23	3.63	0.05
Chryseobacterium	0.26	1.44	1.25	1.96	1.19
Weissella	0.24	2.83	3.62	0.13	1.15
Thermomonas	0.01	0.00	0.00	3.70	0.00
Tenacibaculum	0.15	0.16	0.07	3.24	0.02
Bifidobacterium	0.59	0.40	0.87	2.07	0.29
Hydrogenophaga	0.96	1.97	1.23	0.90	0.02
Polaromonas	0.13	3.56	1.97	0.24	0.00
Clostridium	0.90	1.65	1.72	0.21	0.41
Desulfovibrio	0.15	0.08	0.07	0.17	2.48
Salinibacterium	1.04	0.38	0.41	1.18	1.53
Treponema	0.27	1.34	1.36	0.31	1.56
Longilinea	1.08	0.22	0.24	1.20	0.16

Curvibacter	0.35	1.22	1.48	0.66	0.01
Jiangella	3.18	0.08	0.05	0.05	0.01
Acholeplasma	0.22	0.10	0.11	0.10	3.27
Polaribacter	0.03	0.08	0.11	1.46	0.01
Desulfosarcina	0.03	0.20	0.88	0.00	0.92
Syntrophomonas	0.03	0.02	0.00	0.05	3.69
Janthinobacterium	2.36	0.06	0.03	0.06	0.00
Mycobacterium	1.50	0.07	0.14	0.13	0.38
Lysinibacillus	2.34	0.06	0.04	0.02	0.00
Sphingomonas	0.29	1.28	0.81	0.09	0.21
Desulfitobacterium	0.04	1.17	1.34	0.06	0.03
Brevibacillus	0.01	0.09	0.06	1.08	0.00
Microbacterium	1.18	0.07	0.36	0.10	0.25
Simplicispira	0.00	0.00	0.00	1.06	0.00
Skermanella	1.37	0.05	0.06	0.02	0.00
Brachyspira	0.12	0.09	0.12	0.70	0.23
Bellilinea	0.61	0.39	0.44	0.06	0.25
Arthrobacter	0.57	0.18	0.49	0.26	0.01
Thauera	1.61	0.02	0.02	0.01	0.00
Rhodobacter	0.60	0.03	0.03	0.24	0.49
Paucibacter	0.15	0.27	1.06	0.14	0.00
Sulfurospirillum	0.00	0.01	0.01	0.01	2.08
Candidatus Phytoplasma	0.19	0.13	0.29	0.38	0.39
Alkaliphilus	0.05	0.74	0.58	0.05	0.17

## Curriculum Vitae

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**Post-secondary Education and Degrees:** Queens University  
Kingston, Ontario, Canada  
2008-2012 B.Sc.

The University of Western Ontario  
London, Ontario, Canada  
2016-2018 M.E.Sc.

**Related Work Experience** Teaching Assistant  
The University of Western Ontario  
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Environmental Scientist  
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