Bioremediation of Trichloroethylene by Use of Polymer Bio-beads and Quantification of Diffusion of Trichloroethylene through Various Polymer Membranes

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by

Kirsten E. Hillyer

Major Professor: James Moberly, Ph.D.

Committee Members: Wudneh Admassu, Ph.D., Krishnan Raja, Ph.D.

Department Administrator: D. Eric Aston, Ph.D.

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AUTHORIZATION TO SUBMIT THESIS

This thesis of Kirsten E. Hillyer, submitted for the degree of Master of Science with a Major in Chemical Engineering and titled "Bioremediation of Trichloroethylene by Use of Polymer Bio-beads and Quantification of Diffusion of Trichloroethylene Through Various Polymer Membranes," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____

James Moberly, Ph.D.

Committee Members:_____ Date: _____

Wudneh Admassu, Ph.D

_____ Date: _____

Krishnan Raja, Ph.D.

Department

Administrator: _____ Date: _____

D. Eric Aston, Ph.D.

ABSTRACT

Trichloroethylene is a wide spread carcinogenic contaminate in groundwater, present in roughly 60% America's Superfund sites. Beginning in the early 1900's, TCE was commonly used as an anesthetic, food processing agent, industrial degreaser, and dry cleaning agent. In 1989 the Environmental Protection Agency (EPA) implemented a maximum contaminate level (MCL) on chlorinated solvents such as TCE. Although TCE usage is strictly regulated now, the previous 100+ years of usage has left a significant amount of contamination in the environment worldwide.

TCE is a dense non-aqueous phase liquid (DNAPL), making it particularly difficult to remediate. TCE travels down through groundwater until it reaches a confining layer, resulting in a persistent contamination problem for decades. Bioremediation has moved to the forefront as cost effective treatment technology for the remediation of chlorinated aliphatic hydrocarbons (CAHs), but many of these methods are still in the research phase due to the struggles the environment presents. Elevated dissolved oxygen, low pH, and high concentration of contaminates contribute to the inability of unprotected microorganisms to effectively reduce TCE. Encapsulation of microorganisms into "bio-beads" presents an opportunity to address each of these environmental challenges.

For this thesis bio-beads, were developed utilizing biodegradable polymer and a reductive dechlorinating bacterium consortia known as KB-1, were subjected to various concentrations of TCE. The TCE rate of degradation was monitored to assess the kinetics of TCE reduction and organism viability. To better understand the mass transfer of TCE through the bio-beads, the diffusion coefficient of TCE through the various polymers used to create the beads was quantified. The quantification was completed by casting the polymers in membranes. With the quantification of the diffusion coefficient of TCE through various polymer membranes, development of the bio-beads can be modified in the future to improve the reaction kinetics of the bioremediation of TCE.

ACKNOWLEDGMENTS

First, I would like to acknowledge my advisor and major professor, Dr. James Moberly, and my contacts, Jen Webb and Jeff Roberts at SiREM, without whom I would have never been able to pursue my thesis research for my master's degree. SiREM generously offered their support and donated KB-1 bacterium consortia and microorganism growth media for these experiments. These experiments would not have been able to be started or completed without their bacteria and media donation.

Secondly, I would like to acknowledge my committee, Dr. Wudneh Admassu, Woody, and Dr. Krishnan Raja, for all of their input and helping me get started in the research and design aspects of my thesis. A special thanks to Woody for his wise words of encouragement to help me stay motivated and complete my thesis and degree.

In addition, I would like to thank Dave MacPherson, Charles Cornwall, and Aaron Babino, the glass blower. I would not have been able to start the diffusion experiments without D-Mac's determination and building of my diffusion apparatus. I am eternally grateful for him converting my hand drawings into solid works. Thanks to Charles for machining the various parts for the diffusion apparatus and his patience with my lack of technical wording knowledge. A special thanks to Aaron Babino for making not one, but two, electrode assemblies for the diffusion apparatus and fixing the diffusion cell when I broke it.

I would also like to thank and acknowledge my parents, Tom and Gigi Hillyer, and my brother Bobby, for all their love, support, and wise words of encouragement. I would not be where I am today without them.

DEDICATION

I would like to dedicate this thesis to my grandfather Robert Morris Hillyer, for his encouragement, humor, and persistence to make me succeed. I would not have pursued graduate school had he not given me such wise words of advice to follow my dreams and see the bigger picture. Thank you for being practically perfect and inspiring me to be the same.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Nomenclature

1,1-DCE	1,1-dichloroethylene
AC	Activated Carbon
$CaCl_2$	Calcium Chloride
CAH	Chlorinated Aliphatic Hydrocarbon
cis-DCE	cis-1,2-dichloroethylene
CTC	Carbon tetrachloride
DC	Direct Current
DI	Deionized
DNAPL	Dense non-aqueous phase liquid
EPA	Environmental Protection Agency
GCMS	Gas chromatograph mass spectrometer
$KMnO_4$	Potassium permanganate
M	molar
MCL	Maximum Contaminant Level
NaOH	Sodium Hydroxide
OSHA	Occupational Safety and Health Administration
PCE	Tetrachloroethylene
PCL	Polycaprolactone
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PHV	Polyhydroxyvalerate
PLA	Ploylactic Acid
PVAc	Polyvinyl Acetate
ppb	Part per Billion
ppm	Part per Million
PVA	Polyvinyl Alcohol

SA	Sodium Alginate
TCA	1,1,1-Trichloroethane
TCE	Trichloroethylene
trans-DCE	trans-1,2-dichloroethylene
v/v	volume/volume
w/v	weight/volume
ZVI	zero valent iron

CHAPTER 1: INTRODUCTION

HISTORY AND USE OF CHLORINATED SOLVENTS IN THE UNITED STATES

The production of chlorinated solvents in the United States started when World War I broke out. Before World War I there was no production of synthetic organic chemicals except for those derived from coal tar in the United States. Many chemicals were imported from Europe, most commonly from Germany. Therefore, when the war broke out all European chemical exports were halted, which led to the demand of the United States needing to produce their own chemicals. In 1917, the Trading-with-the-Enemy Act allowed the United States to confiscate the German plants and patents for production of chlorinated solvents. This then led to three major producers of chlorinated solvents. The four main chlorinated solvents used and produced in the United States were carbon tetrachloride (CTC), tetrachloroethylene (PCE), trichloroethylene (TCE), and 1,1,1-trichloroethane (TCA). The first major producer of chlorinated solvents was the Dow Chemical Company with production facilities scattered across the United States in Michigan, Texas, California, and Louisiana. The second major producer is Du Pont. Du Pont started as a manufacturer of gunpowder and explosives, but became involved with the production of chlorinated solvents when they acquired Roessler & Hasslacher Chemical Company, which specialized in the electrochemical processes used in production of chlorine and halogenated chemicals. The last major producer of chlorinated solvents is the Warner-Klipstein Chemical Company and its successors, Westvaco Chlorine Products Corporation, Food Machinery and Chemical Corporation, and FMC Corporation. Westvaco and its successors produced CTC, PCE, and TCE[1].

Following WWI the demand for chemicals drastically decreased until the late 1920s when the lacquer and rayon industries expanded into the use of synthetic chemicals. This then led to the boom in production of TCE and other chlorinated solvents. Slowly, the dry-cleaning industry began using PCE to have a safer working environment. Prior to using PCE, dry-cleaners commonly used gasoline which led to many explosions, and the clothes to have a terrible odor. Dry-cleaners favored PCE to gasoline due to its relatively low toxicity, good

cleaning properties, low flammability, high stability, and moderate cost[1]. When PCE is produced, TCE and CTC are produced as co-products. Therefore, when PCE became widely popular in dry-cleaning, uses for TCE was found. As PCE became more popular in the dry-cleaning industry the demand and uses for TCE grew, which led to the steady increase of TCE production until the late 1960s, as seen in Figure 1.1. TCE was found to be a highly effective cleaning and degreasing agent due to its volatile and low flammability[2]. TCE had many other uses such as in electronics, automotive, food processing, shoe, textiles, and dry-cleaning industries. In addition, TCE was used as a refrigerant, low-temperature heat transfer medium, an extraction agent in the decaffeination of coffee, and a cleaner for optical lenses. TCE was an ingredient in inks, elastomers, paints, varnishes, lubricants, pesticides, household cleaners, and adhesives. A pharmaceutical grade of TCE was developed and used as a general anesthetic for short surgical procedures such as childbirth and dental extractions, as well as for veterinary medicine anesthetic for pigs, dogs, and cats[2]. With the varied consumer and industrial uses of TCE, it is easy to understand how it was easily introduced into the environment. For example, improper disposal for paints, household cleaners, shoe polish, or degreaser would contribute to the contamination issue of TCE in the water table and in the atmosphere.

Starting in the early to mid-1930's the toxicity of TCE came into question; however, very little was done to investigate this[2]. In the 1960s, years after these major producers started producing chlorinated solvents in the United States, awareness of the effects of chlorinated solvents started to increase. The first study of these hazardous chemicals came into effect in 1963 with the Clean Air Act[1]. Then in 1970, Clean Air Act Amendments were put into effect to control the emissions of TCE and PCE. As seen in Figure 1.1 below, control of the emissions of TCE and PCE happened at the peak of TCE production in the United States. This then led to the decline of production of chlorinated solvents. The Safe Drinking Water Act was signed into effect in late 1974 to establish regulations for public water supplies. However, it was not until 1989, the Environment Protection Agency (EPA) implemented a maximum contaminant level (MCL) on chlorinated solvents and chlorinated aliphatic hydrocarbon (CAH) wastes, such as CTC, PCE, TCE, and TCA. Unfortunately, by that

time the amount of incorrectly disposed CAHs were extremely high and had impacted groundwater, surface waters, soils, and sediments.

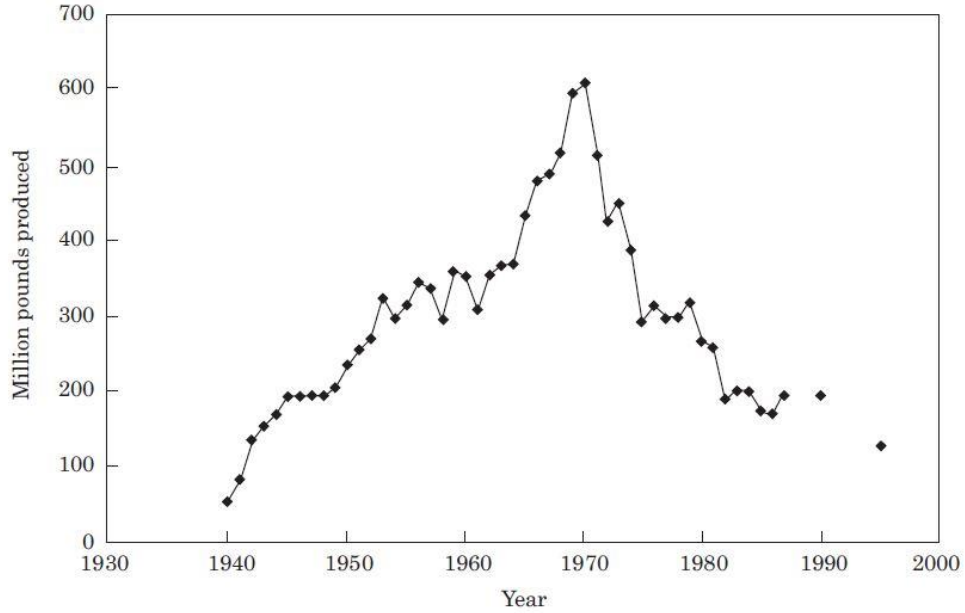

Figure 1.1: Production of trichloroethylene (TCE) in the United States[2]

CHEMISTRY, CHEMICAL HAZARDS, AND PATHWAYS FOR BREAKDOWN

TCE was classified as a human carcinogen in 2011 by the EPA because of the serious health effects it causes in small concentrations[3]. Some of these effects are nervous system effects, liver and lung damage, abnormal heartbeat, coma, cancer, and in some cases death. Due to serious health effects these chemicals cause the EPA has put an maximum contaminant level (MCL) of 5 parts per billion (ppb) in drinking water, and the Occupational Safety and Health Administration (OSHA) set an exposure limit of 100 ppm time weighted average (TWA) in the air for a standard work week [4, 5]. Due to the carcinogenic nature of this chemical and the large amount of contamination, it is important to degrade and remediate these TCE plumes into less toxic forms without releasing large concentrations of TCE into the atmosphere. Natural degradation does occur slightly, however, due to the small amount of reducing and oxidizing agents in the soil and groundwater aquifers, but the majority does not degrade.

Tetrachloroethylene (PCE), the precursor to TCE is highly volatile and has a low solubility in water. If PCE is present in soil near the atmosphere it is likely to volatize into the atmosphere rather than dissolve into the groundwater. However, if PCE partitions to the soil, it will stay in the soil causing a contamination problem in the aquifer. PCE can degrade to TCE in a strong reducing environment. When chlorinated reducing agents, such as reductive dechlorinating bacteria, are introduced to the system the degradation reaction will start. The more reducing agents in the environment, the longer the reduction reactions continue, eventually leading to mineralization of CAHs[6]. The mechanism and kinetics of the dechlorination of chlorinated hydrocarbons by use of Geobacter lovleyi SZ, a dechlorinating anaerobic bacteria, was thoroughly studied by Cretnik et al[6]. The main focus of their study was to determine which di-chlorinated form was the most likely to be synthesized during the reduction of TCE and why it formed during bioremediation. Geobacter lovleyi SZ is an anaerobic dechlorinating bacterium that uses acetate and hydrogen as electron donors and metabolically reduces a variety of electron acceptors[7]. A strain SZ-like bacterium was detected in the bioaugmentation consortium KB-1, which is encapsulated in polymer for the experiments to follow. As shown in Figure 1.2, there are three possible options for the dechlorinated form: 1,1-dichloroethylene (1,1-DCE), cis-1,2-dichlorothylene (cis-DCE), and trans-1,2-dichloroethylene (trans-DCE). This step is crucial to know because of the activation energy required to further reduce the compound to vinyl chloride. In addition, Figure 1.2 shows there are two possible results, either ethene/ethane or complete mineralization where there is no presence of chlorinated hydrocarbons at the end of the reduction reactions.

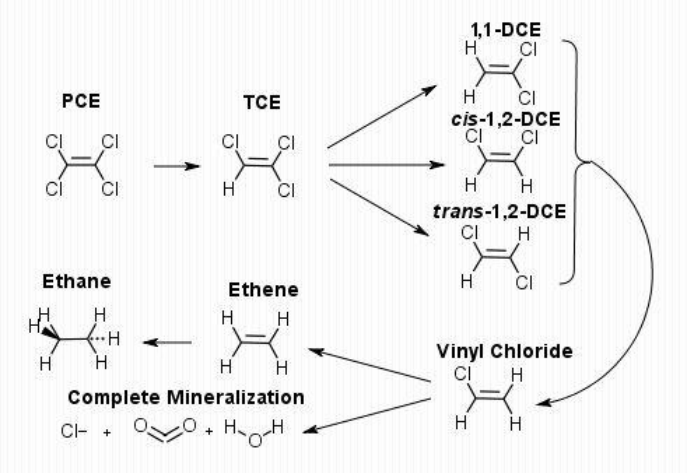

Figure 1.2: Overall Reaction Mechanism for Degradation Starting at PCE

From experiments using Geobacter lovleyi SZ, it was found that the bioremediation of TCE to DCE exclusively formed cis-DCE. Figure 1.3 shows all possible routes the bioremediation may take to degrade TCE into cis-DCE. The first possible path of dechlorination is nucleophilic substitution, the top path in Figure 1.3. This is shown by the addition of cobalt and chlorine. The next method was nucleophilic addition, the middle path in Figure 1.3, shown by adding cobalt and a hydrogen proton. Nucleophilic addition has the ability to create both trans-DCE and cis-DCE. The last method was a single electron transfer, the bottom path in Figure 1.3. Single electron transfer was done by adding an electron to the system to replace a single chlorine in TCE. This method had the potential to produce both cis-DCE and trans-DCE. However, trans-DCE production was not observed and only cis-DCE production occurred[6].

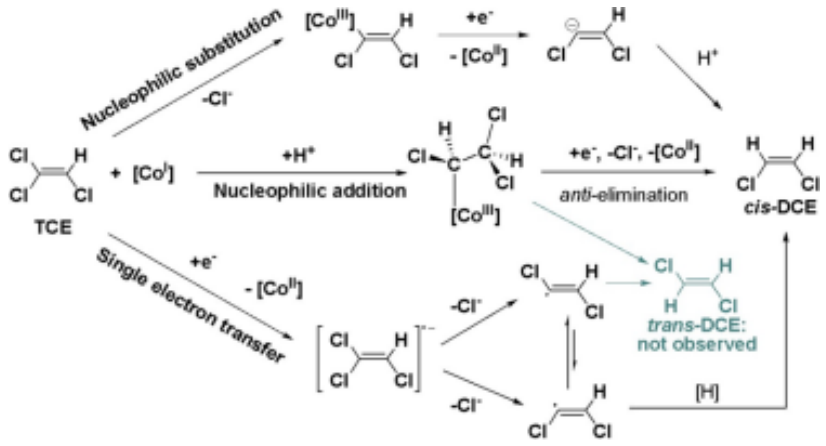

Figure 1.3: Degradation Mechanism of TCE to DCE[6]

Cretnik, et al, then explored each route individually by evaluating the isotopes present in TCE and cis-DCE products. Upon evaluation of the isotopes present, it was determined that nucleophilic substitution is not a possible pathway for the bacteria to use because it was discovered that both α positioned chlorines are reactive, shown in Figure 1.4. Since both α positioned chlorines are reactive and knowing that the β positioned chlorine is unreactive because 1,1-DCE is not observed, nucleophilic substitution is no longer a possibility because it eliminates the selectivity needed for nucleophilic substitution to occur.

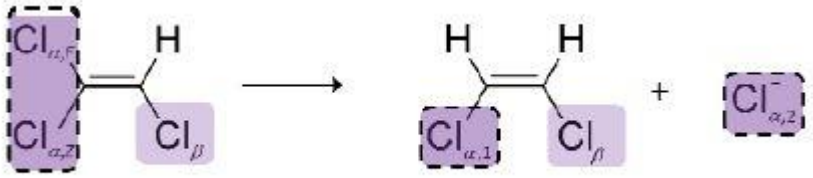

Figure 1.4: Schematic of Chlorine Reactivity[6]

Because both α positioned chlorines are reactive, that still leaves the possibility of nucleophilic addition and single electron transfer as the pathway for bioremediation from TCE to cis-DCE. However, the schematic in Figure 1.2 does leave out what is happening to the chlorine once it has been taken off of the carbon. Hydrogen protons are likely being produced because Geobacter lovleyi SZ uses hydrogen as electron donor which could lead to acidifying the environment[7].

The key component in these degradation reactions is having enough of the reducing agents, such as reductive dechlorinating bacteria or protons, to remediate CAHs completely and not become “stuck” at a specific stage. “Stuck” is defined as when the reaction stops progressing in the forward direction towards complete remediation of TCE due to the lack of reducing agents or a shift to an acidic pH. A shift to an acidic pH causes the reducing agents to stop working efficiently, and could cause a dramatic effect on the environment and local ecosystem. A small shift in pH can have a large effect on the plants and animals that utilize the water from the aquifer.

OCCURRENCE AS A CONTAMINANT

TCE is known as a dense non-aqueous phase liquid (DNAPL). A DNAPL is a chemical whose density is greater than that of water and is only slightly soluble in water, which results in TCE migrating down through an aquifer until it encounters a confining layer, for example, bedrock. When TCE containing solutions such as degreasers or dry-cleaning solutions are ill-properly disposed of, they migrate downward into the water table. This migration then causes substantial volumes of TCE to pool due to the presence of non-uniform soil textures[8]. These substantial volumes are known as plumes. The downward mobility makes the plume a particularly prevalent problem as a TCE plume remains “out-of-reach” as it continues to contaminate an aquifer as it migrates and dissolves over decades. The solubility of TCE in water, approximately 1000 parts per million (ppm), causes TCE to slowly dissolve into the flowing water, thus causing a long term contamination problem. One of the largest plumes in America is located in Mancelona, Michigan, which contaminates 13 trillion gallons of water and moves at a rate of 300 feet per year. It has reached the Cedar River, which flows into a chain of lakes that feed into Lake Michigan[9]. Even though TCE is no longer widely used as an industrial solvent, it remains a challenging problem due to plumes like the one in Mancelona. Because of its use as a dry-cleaning agent and industrial degreaser, TCE contamination is widely distributed in urban areas[1]. Michigan alone has about 300 TCE-contaminated sites and roughly 60 percent (852 out of 1430 in 1997) of Nation Priority List sites contain TCE. Thus making TCE one of the most commonly found contaminants in

America's Superfund sites [2, 9]. Due to contamination sites such as the one in Michigan and others across the United States many remediation technologies have been researched and developed to resolve this problem.

CURRENT REMEDIATION STRATEGIES AND TECHNOLOGIES

A wide variety of oxidizing and reducing technologies have been developed to degrade chlorinated hydrocarbons, in particular TCE. This is due to TCE having a stronger presence in groundwater aquifers in urban areas that are now contaminating large sources of water[9]. Many of these technologies utilize the reducing properties of various biological forms.

The first remediation technology is phytoremediation. Phytoremediation is the process of using plants and their associated rhizospheric microorganisms to remove, degrade, or contain chemical contaminants located in the soil, sediments, groundwater, and even the atmosphere[10]. This technology has gained traction with remediation of TCE because of the poplar tree. The poplar cells are capable of transforming and mineralizing TCE without the involvement of microbial metabolism[11]. Phytoremediation is a highly attractive technology because of its economic, aesthetic and environmental benefits. At first glance, phytoremediation technology is a promising solution for clean-up of TCE. However, it does have several limitations. It is limited to shallow soils, streams, and groundwater, high concentrations can be toxic to plants, and the identity and toxicity of degradation products are not known, which could potentially contaminate the groundwater and enter the food chain through animal consumption[10]. Despite all these disadvantages, phytoremediation has proved to be a reliable remediation strategy for near surface contamination issues, because it does clean up the environment in an extremely environmentally friendly manner. The use of poplar trees is considered an in situ technology that requires very little man power. However, this technology is not a good solution for aquifer contamination, because the contamination issue is too deep in the ground for the trees to reach.

The next technology is an in situ three-stage treatment train system [12]. The first stage of this system is groundwater and biodegradable surfactant flushing. In this stage a

biodegradable surfactant and laboratory made contaminated groundwater were placed into a reactor and flushed. The TCE removal was monitored during the flushing. The second stage is potassium permanganate ($KMnO_4$) oxidation. This stage involves injecting $KMnO_4$ into the enclosed reactor after the flushing of the surfactant. Samples were taken to monitor TCE, $KMnO_4$, manganese oxides, and chloride concentrations. The third stage is an enhanced bioremediation phase where an aerobic microorganism is introduced to the system to further remediate the ground water. This process proved to be extremely successful in remediating TCE from the ground water. The total TCE removed after the first stage was 87.6%, the second stage removed 10.7%, and the third stage removed 1.7%. This technology was able to fully remove TCE from the groundwater. The main downfall of this technology is that the groundwater would have to be pumped out of the ground in order for this process to be utilized and then the clean water would have to be pumped back into the ground. This would make the technology highly expensive and possibly economically infeasible.

The next technology utilizes methane oxidizers associated with wetland plant roots[13]. The wetland plant roots provided aerobic bacteria that catalyzed the oxidation reaction to degrade the chlorinated hydrocarbons present in the water. The experiments were run in a soil free environment testing the remediation of TCE and cis-DCE. Figure 1.5 below shows how this process was accomplished. As seen in the diagram the roots' bacteria are able to fully degrade the chlorinated hydrocarbons from the water. However as a side effect of degrading chlorinated hydrocarbons, it does create a slight acidification of the soil and water. The results of the experiment showed a removal of about 46% of TCE and 90% of cis-DCE from the water. Another downfall of this remediation technology is that is unable to be utilized for deep aquifer contamination and only for contamination near the surface.

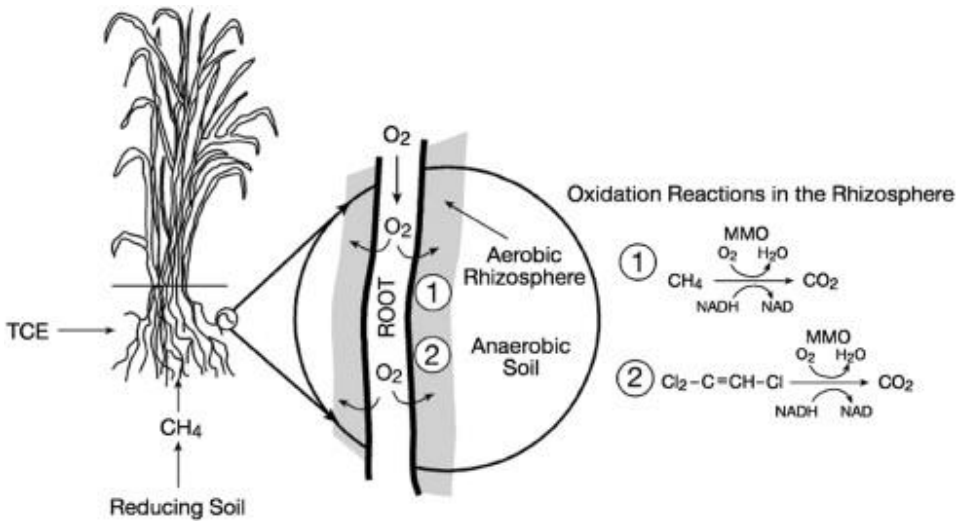

Figure 1.5: Schematic of Wetland Plant Root Remediation[13]

The next remediation technology is a zero valent iron and direct current system [14]. This system contains an electro-enhanced permeable reactive barrier using zero valent iron (ZVI) and direct current (DC). The electro-enhanced permeable reactive barrier is able to remediate TCE by causing an electron transfer between the ZVI and the chlorines on the TCE. The DC charge to the membrane causes the electrons to transfer more willingly than without the charge. The barrier still works without the charge; however, it is not as effective. The experimental set up is shown in Figure 1.6. The apparatus is a completely closed system which is necessary for the health and safety of the person running the experiment.

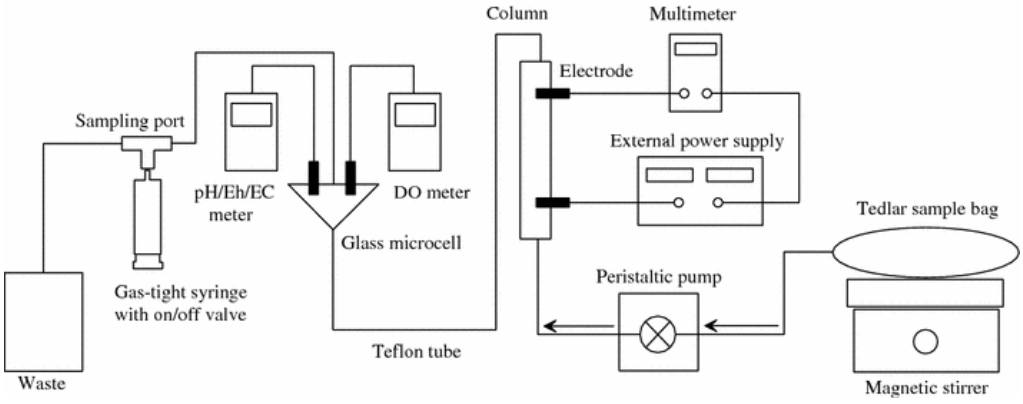

Figure 1.6: Experimental Set-up of ZVI and DC System[14]

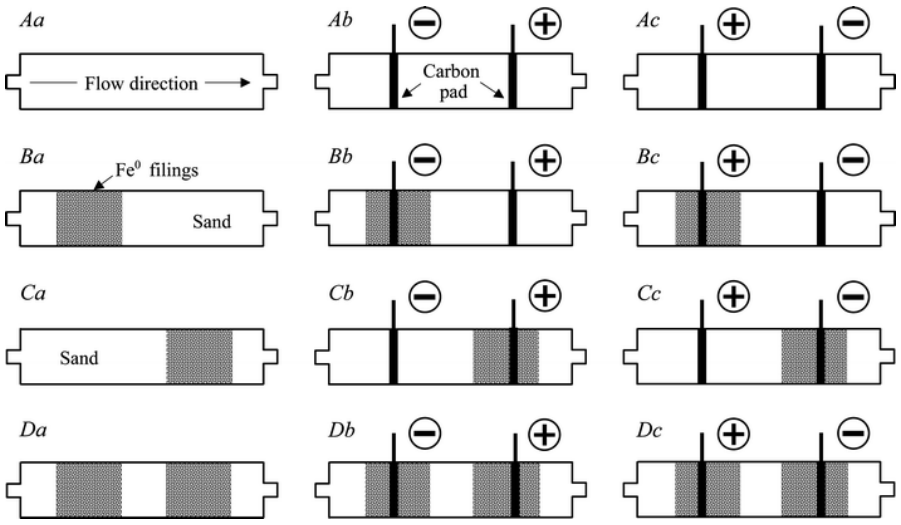

Figure 1.7: Various Column Packing Designs Tested[14]

This permeable reactive barrier was developed by placing one or two zones of ZVI in a sand packed column and running contaminated groundwater through it while varying the DC charge and placement. This was done to determine the optimum set up based on the amount of TCE remaining in the waste solution after flowing through the column. Figure 1.7 shows the different column packing designs that were tested. It was found that column packing design Dc provided the highest electron transfer, so therefore the highest amount of TCE remediated in the column. This technology has the same major downfall as the previous technology. It will be challenging for the permeable reactive membrane to be incorporated into aquifers. Water will have to be pumped out of the ground to be treated and then pumped back into the ground once treated. Upon doing so, the technology may become economically infeasible.

One of the most basic forms of utilizing biology to remediate ground water is a permeable reactive barrier filled with plant mulch, also known as a bio-wall, developed by Lu, et al[15]. A pilot-scale of the bio-wall was installed at the Altus Air Force Base in Oklahoma, and has successfully degraded the TCE to concentrations below the MCL enforced by the EPA. However, there remains a high concentration of vinyl chlorides in the wall. Vinyl chloride is extremely hazardous, toxic, and has a high flammability rating of 4. With such a high flammability rating, it makes removing the bio-wall extremely hazardous.

Vinyl chloride has a low boiling point (-13.4°C) causing liquid vinyl chloride to undergo flash evaporation upon its release to atmospheric pressure forming a dense cloud, thus creating a significant risk of a subsequent explosion or a fire. Vinyl chloride, the last de-chlorination step in remediation, tends to be the most common step to become “stuck” at due to the system running out of reducing agents to complete the remediation. Vinyl chloride is also the most toxic of the chlorinated hydrocarbons and has a MCL of 2 ppb. Therefore, it is highly undesirable to run out of reducing agents at this stage of reduction. Thus, creating a major design flaw for this technology.

The idea of a permeable reactive barrier then progressed from the use of free floating biological matter like plant mulch, to the development of bio-beads, where biological matter and zero-valent iron (ZVI) is encapsulated in polymer. An example of this technology is bio-beads developed to remediate 1,1,1-trichloroethane (TCA) in ground water[16]. The bio-beads utilized immobilized anaerobic bacteria, zero-valent iron (ZVI), and activated carbon (AC) powder. The polymer used to form the bio-beads was polyvinyl-alcohol (PVA) and alginate mix. The bio-beads were then packed into a column creating a permeable reactive membrane, shown in Figure 1.8. During the experiments it was discovered that the amount of AC used in the production of the beads significantly impacted the results. The study showed that the beads were able to successfully dechlorinate TCA. The main downfall to this technology is that the ZVI would leach out of the bio-beads and could lead to a high level of iron in the aquifer. It may also cause an acidic shift in pH to the aquifer because of the production of hydrogens and chloride ions.

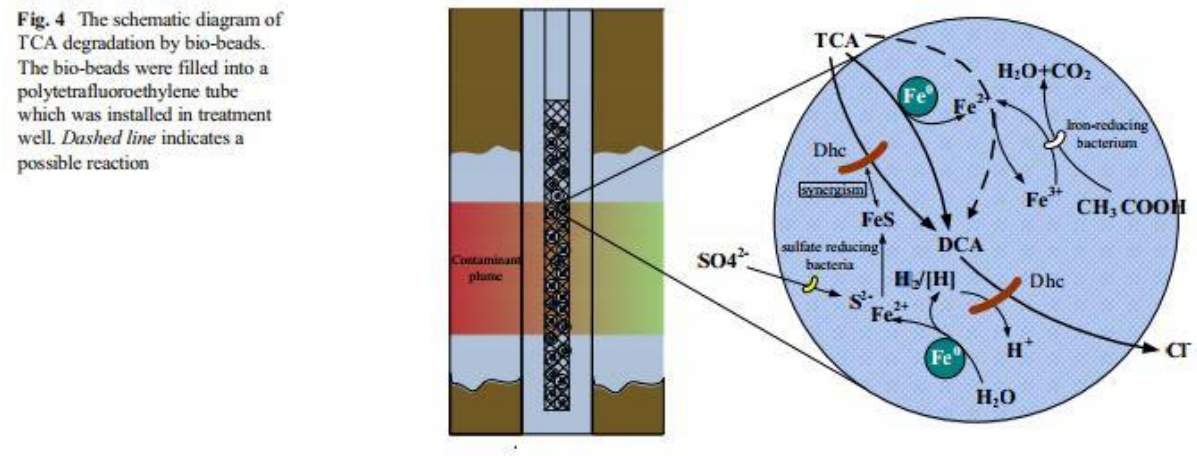

Figure 1.8: Schematic of Packed Permeable Reactive Barrier Using Bio-Beads[16]

This leads to the present study of the bioremediation of TCE. In this study bio-beads will be created using biodegradable polymers to encapsulate an anaerobic bacterium consortium called KB-1. KB-1 and media solution to run the experiments was provided by SiREM. The ideal experiment would take place in a simulated groundwater solution to imitate environmental like conditions. However, SiREM did not permit the usage of a simulated groundwater solution while using KB-1 in experiments. The KB-1 consortia members and the contents of the media solution are proprietary information of SiREM. Therefore, KB-1 and the media solution were shipped to the University of Idaho using FedEx overnight shipping in a cooler. The materials are overnighted to ensure that KB-1 and the media solution had limited exposure to an oxygen rich environment. Due to the anaerobic nature of KB-1, the bioremediation experiments needed to be run in an anaerobic chamber to have an oxygen poor atmosphere. The idea and composition of the bio-beads came from the previous technologies described in this section; however, the goal is to have an environmentally friendlier and more effective bio-bead than those previously developed. The bio-beads that are developed in this study will only contain biodegradable polymer or polymer mixture and KB-1. KB-1 has been proven to be an effective reductive dechlorinating bacterium for TCE and other chlorinated solvents; however, it has sensitivity to high concentrations of chlorine. The hope is that encapsulating KB-1 will increase the effectiveness of KB-1 in higher concentrations of TCE because it will be less exposed to the

high concentrations of chlorine produced during the reduction of chlorinated solvents. The reaction rates of the bioremediation will be quantified based on decreasing TCE concentration over time.

In addition to the bioremediation experiments involving KB-1 encapsulated in various biodegradable polymers, the diffusion of TCE will be quantified through the same various polymer membranes. Knowing the diffusion coefficient of TCE through various polymers will help better understand the mass transfer effects of TCE on the bio-beads. Quantifying diffusion of TCE can then be used in the future for the bio-beads. The diffusion of TCE in the polymer of the bio-bead has a direct effect on the necessary size of the bio-beads. This way the bio-beads can be modified based on system properties that they will be implemented into. Some of these properties could be TCE plume concentration, plume and water flow rate, and the length of time for the remediation to occur.

INTRODUCTION TO TCE DIFFUSION QUANTIFICATION

The diffusion coefficient was studied for one of the most common polymers used in cell-immobilization studies [16-24], polyvinyl alcohol, used in studies for the remediation of TCE. However, the liquid being observed in the study was not TCE or any chlorinated hydrocarbon[21]. The design of the diffusion chamber, as shown in Figure 1.9, was found to be useful for designing the type of diffusion apparatus needed for the experiments to obtain the diffusion coefficients of TCE through the various polymers.

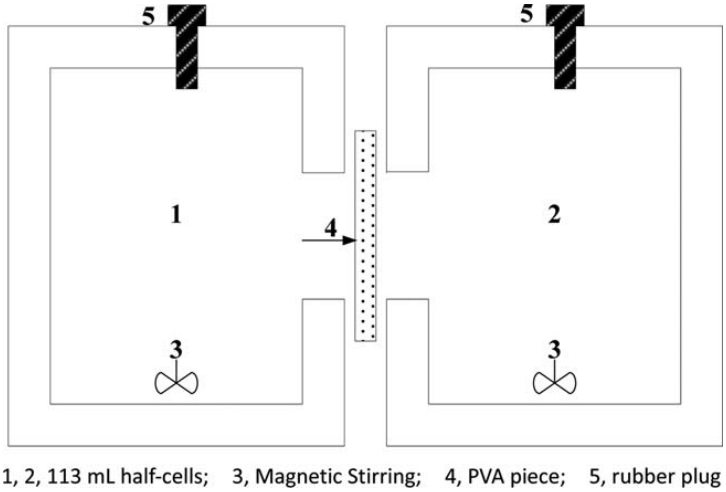

Figure 1.9: Sketch of Diffusion Chamber[21]

Even with a diagram of a diffusion chamber used, many challenges still presented themselves due to the chemical nature of TCE. TCE degrades many types of plastics such as acrylic and has a strong affinity towards the ones it does not degrade, such as Teflon [25, 26]. Only recently, in May 2015, the diffusion coefficient of TCE in water has been experimentally quantified to be $8.16\pm0.06x10^{-6}$ by Rossi, et al[25]. The quantification of diffusion experiment was completed in a flowing water experimental set-up. Due to the slow flowing nature of TCE plumes, molecular diffusion, similar to the study by Li et al[21], should be quantified rather than the flowing diffusion quantified by Rossi, et al[25]. Before the diffusion coefficient can be quantified and the bioremediation kinetics can be studied, polymers for encapsulation and the experiments must first be selected.

CHAPTER 2: POLYMER SELECTION AND METHODS

Polymers considered for microorganism encapsulation for the bioremediation of TCE, and the diffusion measurements had to meet a certain prescreening criteria before continuing on for further evaluation. The prescreening criteria for polymers considered for the experiments were: 1) encapsulation and polymerization process must maintain microorganism viability and reproduction, 2) polymers must be TCE resistant in order for them to not dissolve prematurely in the environment, 3) the polymer must be biodegradable so it does not cause further contamination in the environment, and 4) polymers must be capable of forming a bead and a membrane. A list of polymers considered for the experiments are shown in Table 2.1 with an explanation for why each one did not meet the criteria. Once it was discovered a polymer did not meet a criterion, it was no longer evaluated for any further criteria. For example, if the polymer did not meet the first major criterion (organism viability), then the polymer was not considered further and no information was collected.

Polymer	Criteria				Reason for Elimination
	1	2	3	4	
Polylactic Acid (PLA) [27]	N	-	-	-	Temperature too high to sustain bacteria, Not TCE resistant
Polyhydroxybutyrate (PHB) [27]	N	-	-	-	Temperature too high to sustain bacteria
Polycaprolactone (PCL) [27]	N	-	-	-	Thermoplastic with high set temperature
Agar/Agrose [20]	Y	N	-	-	Not TCE resistant
Polyvinyl Acetate (PVAc) [28]	Y	Y	N	-	Not soluble in water/biodegradable
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [29]	N	-	-	-	Temperature too high to sustain bacteria
Polyhydroxyvalerate (PHV) [29, 30]	N	-	-	-	Temperature too high to sustain bacteria
Polyhdyroxyalkanoate (PHA) [31]	N	-	-	-	Made with microorganisms so contamination may be an issue
Sodium Alginate (SA) [16, 32, 33]	Y	Y	Y	Y	
Pectin [34]	Y	Y	Y	Y	
Polyvinyl Alcohol (PVA) [21, 22, 24]	Y	Y	Y	Y	
Chitosan [35]	Y	Y	Y	Y	

Table 2.1: Summary of Polymers Considered (N-Did not meet criteria, Y-Did meet criteria)

First and most importantly, the polymer must be able to sustain microorganism growth and livelihood during the bead formation and bacteria encapsulation process. Extremes in temperature and pH are likely to damage or denature cell membranes and proteins leading to cell death. Due to this many thermoplastics were removed from consideration. In addition, microorganisms find certain chemicals involved in polymer precursors or cross-linking agents highly toxic. If the polymer construction uses such chemicals, the polymer can no longer be considered. Secondly, the polymers must be TCE degradation resistant. This is important because the beads need to withstand the solvating properties of TCE in order for the encapsulated bacteria to remain inside the polymer structure. If the selected polymer is not resistant to TCE then it will break down rapidly and bypass the purpose of encapsulation.

Third, the selected polymers need to be bio-degradable. With the future goal of these beads to be utilized to remediate contaminated groundwater aquifers, having these beads degrade into harmless byproducts over time is highly important. Natural degradation would be ideal so they would not have to be removed from the aquifers at a later date. Not only would removing the beads at a later date be extremely difficult, it is probable that it would make the bioremediation solution economically infeasible. Lastly, the polymers selected must be able to form a bead, a membrane, and encapsulate bacteria. The polymer membranes are used for measuring the rate of diffusion of TCE through the polymer to better understand the mass transfer of TCE through the polymer. From these criteria, polymers were screened and a final selection of polymers was made. Polyvinyl alcohol, chitosan, sodium alginate, and pectin were selected for to be tested for these experiments. These powdered polymers were obtained through Sigma Aldrich.

Each polymer will need to take two forms during the course of the experiments, the first being a spherical bead for the real world application experiments of bioremediation in aquifers, and the second being a membrane for diffusion experiments. In order for these two sets of experiments to be relatable the molecular structure of both polymeric structures need to be as close to identical as possible. In order for that, the cross-linking process must be the same for both the bead formation and the membrane formation.

FORMATION OF POLYMER BEADS

The polymeric bead-like structures were formed by dripping uncross-linked polymer solution into a cross-linking solution. Uncross-linked polymer solution or liquid polymer solution is called molten polymer solution for the remainder of this thesis. The rate at which the polymer solution drops into the cross-linking solution determines the size of the bead. This method was chosen because it gave the most control over bead size. The slower the rate of the polymer solution being dripped into the solution, the larger the bead; the faster the rate of the polymer solution being dripped into the solution, the smaller the bead. A faster rate is desired because then the surface area to volume ratio is higher on smaller beads, thus increasing the amount of surface area for the encapsulated bacteria to have

access to the TCE. To produce a repeatable drip rate for multiple polymers of various viscosities a repeat pipette with constant volume dispensing was used in the formation of beads. Each method used to construct polymer beads for the selected polymers will be discussed in sequence in the following paragraphs.

Over the course of testing bead formation it was found that pectin was not a suitable polymer for further evaluation. Pectin was unable to maintain a spherical bead structure when dropped into its cross-linking solution. Pectin molten polymer solution was created by mixing 5 grams of powdered pectin in 100mL of type 1 water. The cross-linking solution used for the molten pectin polymer solution was 10% weight/volume (w/v) calcium chloride ($CaCl_2$) solution[34]. When the pectin molten polymer solution was dropped into the cross-linking solution the polymer bead did not hold its shape and broke apart into polymer shards.

Molten sodium alginate polymer solution was made by mixing 4 grams of powdered sodium alginate in a 100 mL of type 1 water. The powder was gradually poured into the mechanically agitated water. A key step in the process of dissolving sodium alginate powder was to gradually pour the powder into the center of the vortex of the stirred water. This step prevented the formation of a non-homogenous mixture or a chunky film on the water surface. If this key step is skipped it results in extreme difficulty in making the solution homogenous once this occurs. The solution, once it became homogenous, needed to be de-gassed. The stirring process introduced air bubbles into the solution due to the molten polymer solution's high viscosity. After the molten sodium alginate polymer solution was homogenous and de-gassed, it was ready to be dropped into the cross-linking solution. The cross-linking solution for the molten sodium alginate solution was a 5% weight/volume (w/v) calcium chloride ($CaCl_2$) solution. The molten polymer solution was drawn up in a VWR Scientific Products Repeating pipette with interchangeable piston-style pipette tips using a 1.25mL tip. The pipette was set to the smallest dispensing volume setting (1), the volume dispensed with each click was 25µL. Molten polymer solution was dispensed into a beaker of cross-linking solution where the polymer rapidly cross-linked forming sodium alginate beads. When fashioned according to this method the beads formed are of consistent size of

approximately 20-30 mm in diameter and a spherical shape. An image of these beads can be seen below in Figure 2.1. For best results, the beaker was placed on a black colored surface to better see the beads due to their transparent color. The beads initially floated on the surface when dropped into the cross-linking solution, and as they completed their cross-linking process they sank to the bottom of the beaker. Once the sodium alginate beads maintained a position at the bottom of the beaker, approximately ten to fifteen minutes after being dropped, they are fully cross-linked and ready to be filtered from the cross-linking solution and rinsed with deionized (DI) water. Sodium alginate beads need to be stored in DI water to remain saturated and preserve their gel like nature.

Figure 2.1: SA Beads

The third polymer examined was chitosan. Chitosan molten polymer solution was formed by first preparing a 100 mL of 2% volume/volume (v/v) acetic acid solution in a beaker. Gradually 2 grams of chitosan powder was added into the acetic acid solution.[35] This mixture was modified from Barreiro-Iglesias, et al, method to create a slightly more viscous molten polymer solution, therefore when dropped into the cross-linking solution a more uniform bead shape would form. Similar to the sodium alginate molten polymer solution it was an important step to add the chitosan powder into the center of the vortex. If this is not done, the powder will stick to the side of the beaker and not go into solution.

When the molten polymer solution was homogenous, the solution was needed to de-gas from the stirring process due to its high viscosity. The cross-linking solution for the molten chitosan polymer solution was a 1.5 molar (M) sodium hydroxide (NaOH) solution. The chitosan beads were formed by drawing the chitosan molten polymer solution into the repeat pipette. The repeat pipette was set to the number 1 setting to drop the molten polymer solution into the 1.5M NaOH cross-linking solution. When the chitosan molten polymer solution was dropped into the cross-linking solution, the beads initially floated on the surface, similar to how the sodium alginate beads behaved, and then sank to the bottom of the beaker when they were fully cross-linked, this took approximately 30 to 45 minutes. A picture of the chitosan beads can be viewed below in Figure 2.2. Once the chitosan beads were fully cross-linked they were filtered from the NaOH cross-linking solution and thoroughly washed with DI water. The beads needed to be stored in DI water to preserve their gel saturated form.

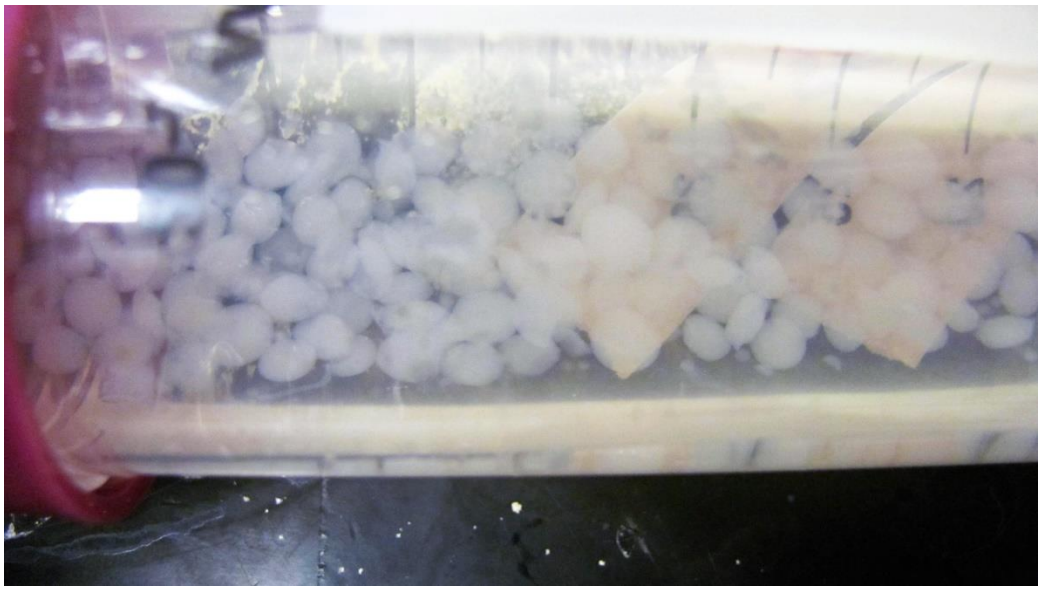

Figure 2.2: Chitosan Beads

The last polymer evaluated was a polyvinyl alcohol sodium alginate (PVA/SA) polymer mixture. The first step was to heat a 100 mL of type 1 water to 80°C so the polymer powders will go into solution and become homogenous. 2 grams of sodium alginate and 10 grams of

PVA were both stirred in to the heated water[21]. Once again it was important to add them gradually and in the center of the vortex to avoid chunks and films from forming in the beaker. The molten polymer solution took approximately one to two hours, to become homogenous. Do not rush the molten polymer solution to become homogenous by cranking up the heat. This causes the polymer to boil and a film of hardened polymer to form on the surface. It is very important to ensure all the PVA powder has become homogenous in the solution or the solution becomes extremely thick with a marbled texture, thus making it extremely difficult to make consistent size beads. Once the solution has become homogenous, the molten polymer solution needed to be set aside to let cool and de-gas from the stirring due to its extremely high viscosity. The cross-linking solution for the PVA/SA beads was a saturated boric acid solution with 2% (w/v) $CaCl_2$ solution. Preparation of the cross-linking solution was accomplished by measuring out an appropriate amount of DI water then combining the necessary amount of $CaCl_2$ crystals to the water. Next, the solution was heated and boric acid was added until the solution became saturated. The cross-linking solution was cooled and the boric acid was allowed to fall out of solution, thus insuring it was a saturated solution. Once the cross-linking solution has cooled the molten PVA/SA polymer solution was dropped into the cross-linking solution using a repeat pipette at the 1 setting as described earlier in the formation of sodium alginate and chitosan beads. This method produced beads approximately 40-50 mm in diameter. The beads were translucent as they were dropped into the cross-linking solution and as they complete the cross-linking process they turn white. Periodically, the cross-linking solution beaker was swirled to disperse the saturated boric acid to provide all the beads with excess boric acid. Once the beads were white and no longer floating on the surface, the cross-linking process was complete. An image of the PVA/SA beads can be seen below in Figure 2.3. It was common for some of the beads to not drop to the bottom of the beaker because they are prone to capturing air-bubbles in them which cause them to float. Beads with air bubbles in them were not desired for the purpose of the experiments and should be separated and discarded. Once the beads are fully cross-linked they needed to be filtered and rinsed

thoroughly several times to ensure any excess boric acid was no longer on the beads. The beads should then be stored in DI water to preserve their hydrated foam like nature.

Figure 2.3: PVA/SA Beads

ENCAPSULATION OF ANAEROBIC BACTERIA

The bio-remediation experiments were run in cooperation with SiREM. SiREM provided a dechlorinating bacterium consortium known as KB-1 and a media solution to support their growth and to run the experiments in. KB-1 was designed to dechlorinate various chlorinated chemicals including PCE, TCE, DCE, and vinyl chloride under anaerobic conditions. Due to the proprietary nature of KB-1, SiREM would not disclose the microorganism members which comprise KB-1 or how to make the media solution. SiREM shipped KB-1 cultures overnight on ice in 160mL serum bottles and the appropriate quantity of media in 1L bottles in a cooler to the University of Idaho to avoid contamination and KB-1 dying during the transportation process.

The first step of encapsulation of KB-1 was to sterilize the polymers. The sterilization method used was dry low temperature extended tyndallization to sterilize the molten polymer solutions prior to encapsulation to avoid contamination of other microorganisms. Low temperature extended tyndallization is the process where the molten polymer solutions were repeatedly heated in an oven for eight hours at 80°C and then cooled at room

temperature for one hour. Standard tyndallization occurs at the boiling point of the substance being sterilized. The heating stage involves steam heating for only one hour and then the cooling process is for five to eight hours to allow microorganism spores to germinate. Both of these tyndallization techniques are done for a total of three cycles. The reason for the lower temperature tyndallization is to avoid changing the molecular structure of the molten polymer solutions. After completing tyndallization, the molten polymer solutions are transferred into the anaerobic chamber to remove oxygen prior to the addition of KB-1.

After tyndallization, prior to being combined with KB-1, each of the molten polymer solutions chitosan, PVA/SA and sodium alginate were dropped into their respective cross-linking to verify that they still cross-link and form beads correctly after tyndallization. Both PVA/SA and the sodium alginate molten polymer solutions cross-linked properly. However after tyndallization, the chitosan molten polymer solution became less viscous during the tyndallization process. Due to the change in viscosity, the solution could no longer form beads. Since the chitosan molten polymer solution could no longer form beads it was removed from both the bioremediation and diffusion experiments.

For encapsulation of microorganisms, KB-1 was incorporated into the molten polymer solution prior to encapsulation in an anaerobic chamber. The anaerobic chamber contained an atmosphere of <5% H_2 with the balance of nitrogen gas. KB-1 was first centrifuged into cell pellets. The cell pellets of KB-1 were then gently mixed in to the molten polymer solution. The ratio of KB-1 to molten polymer solution was one mL KB-1 to five mL molten polymer solution. For every 10 mL of polymer solution 2 mL of KB-1 were centrifuged into a pellet. KB-1 had a cellular density of 1.0×10^9 cells per mL, therefore the density of KB-1 in molten polymer solution was 2.0×10^8 cells per mL of polymer. Once KB-1 and molten polymer solution was homogenous the beads were then cross-linked in their respective cross-linking solutions for the molten polymer solution, as shown in Table 2.2 below. The sodium alginate beads were no longer clear in color when encapsulating KB-1. The incorporation of KB-1 turns the sodium alginate molten polymer solution black thus causing the beads to become transparent black/grey in color as seen in Figure 2.4. KB-1 when mixed

with PVA/SA molten polymer solution turns the solution a clear purple/black color causing the beads to become a grey/purple color as they complete cross-linking instead of the white as seen in Figure 2.5.

Molten Polymer	Concentration	Cross-linking Solution
Sodium Alginate	4 g SA 100 mL type 1 water	5% (w/v) $CaCl_2$
PVA/SA	10 g PVA 2 g SA 100 mL type 1 water	Saturated Boric Acid 2% (w/v) $CaCl_2$

Table 2.2: Summary of Polymers and Their Cross-Linking Solutions

Figure 2.4: Sodium Alginate beads with encapsulated KB-1

Figure 2.5: PVA/SA beads with encapsulated KB-1

FORMATION OF POLYMER MEMBRANE

The formation of a polymer membrane was used in the quantification of diffusion of TCE through the various polymer selected for testing in the bioremediation experiments. For there to be relevance between the quantification of diffusion and the reaction rates from the bioremediation, the formation of a polymer membrane and polymer beads must be as identical as possible. The cross-linking solutions were the same for each of the molten polymer solutions as described in the formation of polymer beads section and previously shown in Table 2.2.

A significant effort was made to produce a smooth membrane. A smooth membrane with consistent thickness was desired for the quantification of diffusion because it produces the most accurate data and the surface area and thickness have a direct impact on the diffusion coefficient determined. Polymer membrane supports were machined out of Teflon in order to reduce the potential of TCE degradation and give the membrane something to be supported by, as seen in Figure 2.6. The support will be further described in chapter 3 during the diffusion apparatus description section.

Figure 2.6: Picture of Empty Teflon support

The first attempted method of forming a polymer membrane was to pour molten polymer solution into the polymer support and then pour the cross-linking solution over the molten polymer solution inside the polymer support. This method was unsatisfactory with the finished cross-linked polymer having an uneven thickness and a divot or gaping hole in the center of the membrane wherever the cross-linking solution was poured over the molten polymer solution. The divot or hole was repeatedly observed irrespective of how slowly the cross-linking solution was poured.

The next attempted method of cross-linking a polymer membrane formation was to pour the molten polymer solution into a disposable petri dish and to gently pour the cross-linking solution over the dish and cut out a circle of polymer from the fully cross-linked polymer sheet to fit into the polymer support. This method had similar issues to that of the previous method and frequently the finished polymer product would not be smooth or have a consistent thickness. However, some portions of the membrane were able to be cut out that had a smooth surface and consistent thickness. When the cut polymer membrane was

placed in the support it was discovered that it would leak and become dislodged from the membrane support.

The third method attempted to create polymer membranes capable of diffusion measurements was similar to the second method except for pouring the cross-linking solution over the molten polymer solution in the petri dish. The petri dish was instead gradually dipped into the cross-linking solution and left to finish the cross-linking process. This method ended as poorly as the other previous two methods. The polymer formed was uneven in thickness and contracted within the petri dish resulting in a membrane resembling more of an unstable blob rather than creating a smooth membrane.

The fourth and successful method attempted finally produced a membrane with a smooth, uniform surface with a consistent thickness throughout the membrane. To achieve this, a small quantity of molten polymer solution, approximately 4mL, was poured into the polymer support to cover the bottom evenly. A 5mL repeat pipet tip was employed for filling the support to ensure the polymer solution was evenly distributed within each of the small holes of the support without entrapping air bubbles. When removing the air bubbles from the holes of the support they would float to the surface but not burst upon doing so. The support was then placed in the refrigerator, to allow the bubbles to burst. The polymer support required a weight to be placed on top of it to seal the support to the petri dish so the polymer would not leak out the bottom. The polymer and the support remained in the refrigerator for approximately 4 hours. Once the polymer and the support were removed from the refrigerator, the polymer was gently misted with the cross-linking solution using a spray bottle and gently patted with a gloved hand to ensure it would form a membrane with a consistent thickness and smooth non-leaking surface. Multiple iterations of spraying and patting are required to provide the molten polymer with enough cross-linking solution to become fully cross-linked into a hydrogel. The sodium alginate membrane can be viewed in Figure 2.7 and the PVA/SA membrane in Figure 2.8. Excess cross-linking solution was thoroughly rinsed off the polymer membrane with DI water. The polymer membrane was stored in a beaker of DI water to keep membranes hydrated until used.

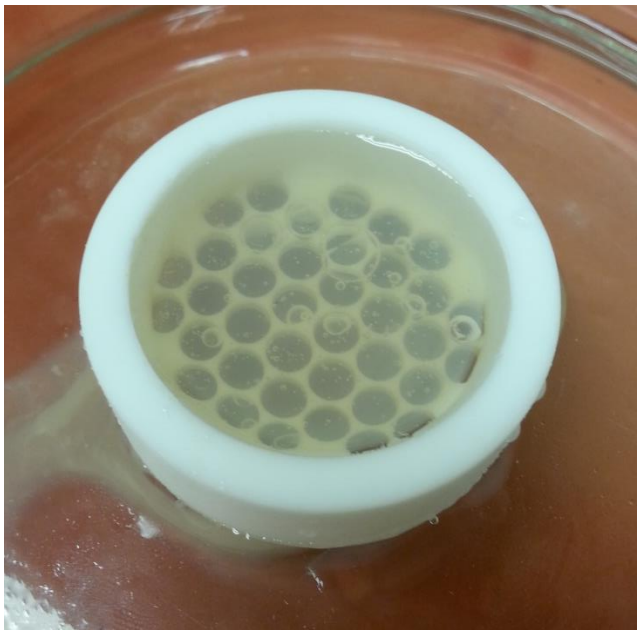

Figure 2.7: Picture of Sodium Alginate Membrane

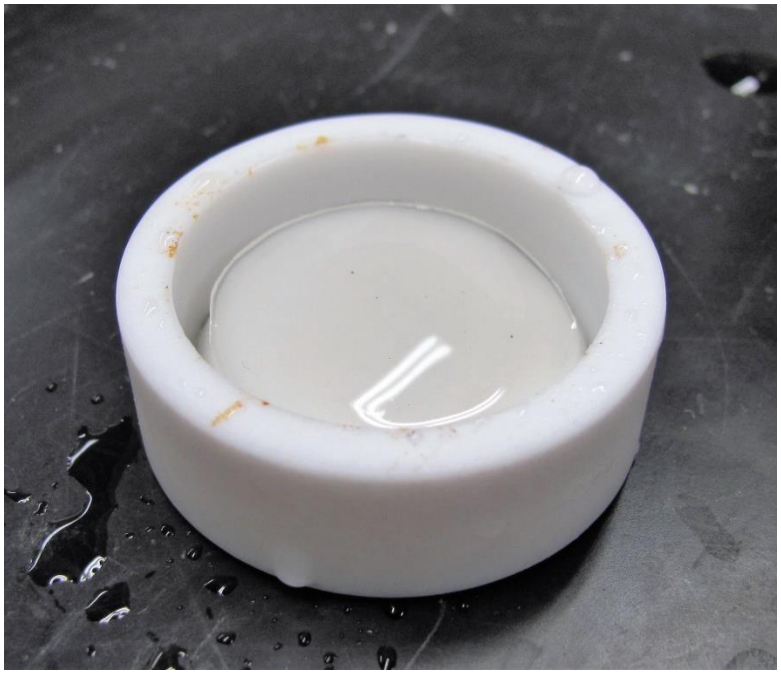

Figure 2.8: Picture of PVA/SA Membrane

CHAPTER 3: DIFFUSION OF TRICHLOROETHYLENE

To better understand the mass transfer of the bio-degradation occurring within the polymer bio-beads, the diffusion of TCE through the polymer bead structure must be studied. Knowing the diffusion coefficient will help determine optimum bead size so the encapsulated bacteria will not be overwhelmed with a high contaminate concentration. Only recently the diffusion coefficient of TCE in water has been experimentally quantified and published in May 2015. Prior to May 2015, the diffusion coefficient has been assumed using a theoretical estimation through the Wilke and Chang equation (3.1) or the Hayduk and Laudie equation (3.2)[25, 36].

$$D_{AB} = 7.4 * 10^{-8} \frac{(\varphi_B M_B)^{1/2} T}{\mu_B V_A^{0.6}} \quad (3.1)$$

$$D_{AB} = 13.26 * 10^{-5} (\mu_B^{-1.14} V_A^{-0.589}) \quad (3.2)$$

Where,

D_{AB} – is the diffusivity of A in dilute solution of B ($cm^2\ s^{-1}$)

φ_B – is the association parameter for the solvent

M_B – is the molecular weight ($g\ mol^{-1}$)

T – is the temperature (K)

μ_B – is the viscosity of the solution (cP)

V_A – is the molal volume of the solute at normal boiling point ($cm^3\ g^{-1}\ mol^{-1}$)

From the use of these equations a theoretical diffusion coefficient for TCE of 1.0×10^{-5} $cm^2\ s^{-1}$ at 25°C was obtained and used in most models [25]. The diffusion coefficient in water was found to be $8.16 \pm 0.06 \times 10^{-6}$ $cm^2\ s^{-1}$ at 25°C, increases linearly with temperature and was independent of concentration [25]. Comparing the experimental value to the theoretical value obtained from the Wilke and Chang equation, it is apparent that the diffusion coefficient for the polymers should be experimentally obtained due to lack of accuracy the equations provide for TCE.

CONSTRUCTION OF DIFFUSION CELL

Careful consideration had to go into construction of the diffusion cell and many criteria had to be met. First and foremost the diffusion cell needed to be completely air tight. Since TCE was the component being measured it needed to remain contained in the chamber without the option volatilizing and escaping. The second criterion was that it needed to be constructed out of materials that are TCE resistant and preferably transparent. This proved to be a challenging task because TCE degrades and wears down many standard materials rapidly. The transparency of the material was desired so that one could visually observe what is happening inside the chamber and if there were air bubbles present. Third, the volumes on either side of the membrane support needed constant mixing to avoid concentration gradients forming and ensure the system was homogenous. Forth, there must be both sampling and electrode ports on each side of the membrane. These ports also need to meet the first and second criterion of being air tight and have minimal reactions with TCE.

Many materials were considered to build the diffusion cell including acrylic, PVC, Teflon, and glass. Acrylic and PVC were immediately discarded because they have poor TCE resistance. Teflon became undesirable because TCE has a strong affinity towards it[26], it is not transparent, and it has a high cost. Glass was decided upon as the final material because it was readily available, transparent, and highly TCE resistant. A glass tube was made by the glassblower at University of Idaho. The end caps of the diffusion cell were decided to be created out of Teflon because of its TCE resistance and easy ability to machine both a sampling and electrode port on each one. The sampling ports were then sealed with Teflon coated septa that could easily be replaced. The electrode ports were fitted with a stainless steel yor lok fitting where the electrode could fit inside and then be sealed off from the outside with an O-ring and a fitting. The end caps were then attached to the glass tube with two stainless steel plates per end cap. The first plate fit around the glass tube and the second plate fit on the Teflon end cap. The two plates were then tightened and pulled towards each other while compressing the Teflon end cap to the end of the glass tube making an air tight seal. The completed diffusion apparatus is shown below in Figure 3.1. Magnetic stirrers, seen below the glass diffusion cell in Figure 3.1, were built with high

power magnets attached to a rotary motor which was controlled by a dial speed controller. This allowed for both sides to be stirred continuously at the same rate for the duration of an experiment.

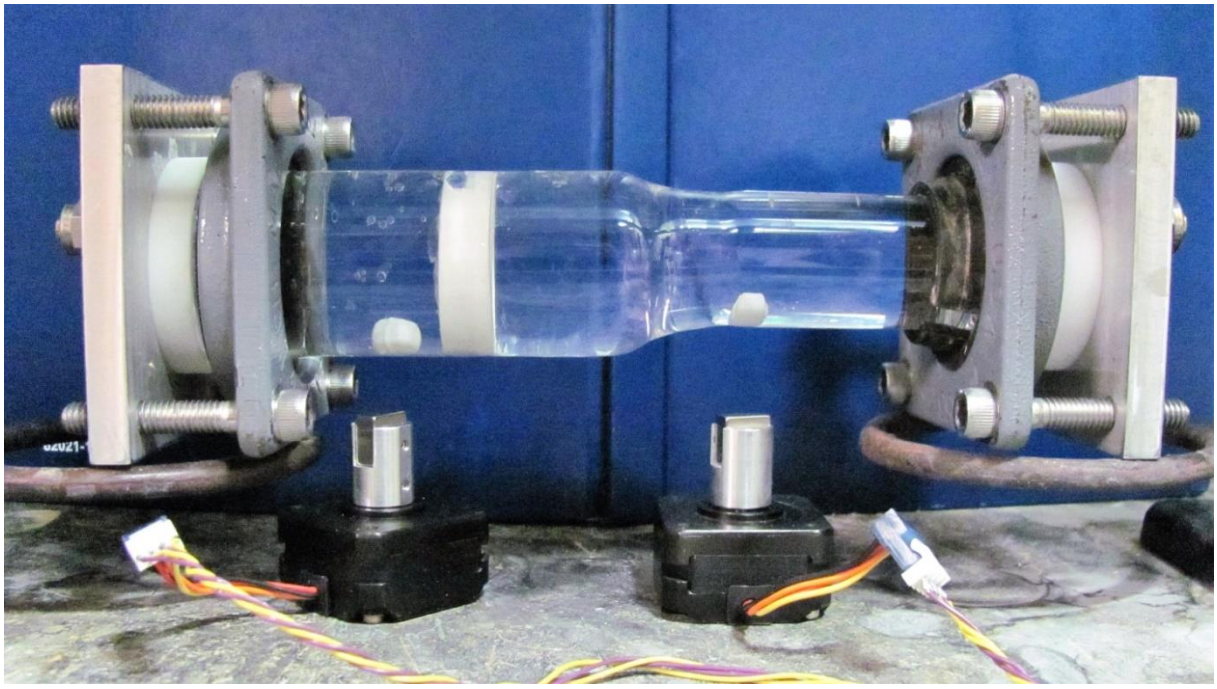

Figure 3.1: Diffusion cell apparatus

EXPERIMENTAL PROCEDURE AND MEASUREMENTS

Due to the highly volatile and toxic properties of TCE the concentration and the diffusion of TCE through the polymer membrane was attempted to be measured electrochemically. The first electrode assembly constructed was composed of a stainless steel rod for support and conductivity with a copper mesh attached to the end with a screw and an insulated copper wire running through the middle of the stainless steel rod. The wire running through the center of the rod had to be sealed into position with polydimethylsiloxane (PDMS) to keep water and TCE from leaking out of the diffusion cell. This electrode assembly is shown in Figure 3.2. A potentiostat was used to run the electrode assembly. To start the experiments the electrode was first tested that it could split water to ensure it was functioning properly and that the system had enough electrolytes present.

Then the potential for which TCE reacts at, must be determined. This was done by cyclic voltammetry. Once the potential was obtained then chronoamperometry was run at the determined potential for TCE. Then the change in current observed represents TCE diffusion through the system. Many solutions for inside the diffusion were attempted to achieve the best results. These solutions include: type 1 water with $CaCl_2$, pipes buffer solution, potassium phosphate, sulfuric acid, and hydrochloric acid. At first, the copper electrode assembly seemed to be measuring TCE correctly. However, as the electrode testing progressed, the potential that the electrode needed to be run at kept gradually drifting making the experiments unrepeatable and an inaccurate measure of how much TCE diffused at what point in time. The extensive attempts to collect data from these experiments can be seen in Appendix A.

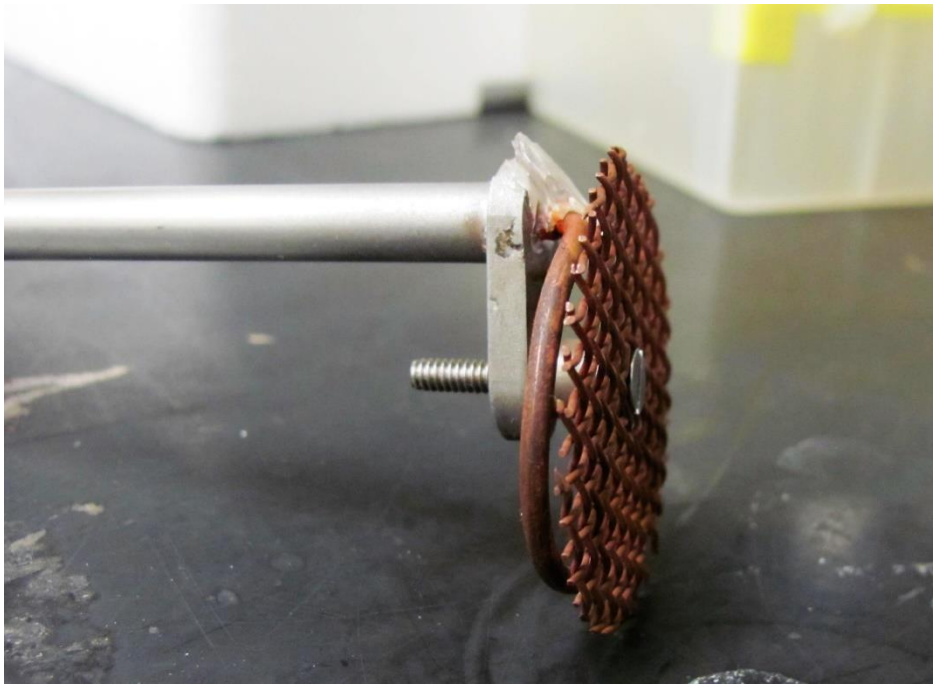

Figure 3.2: Copper Electrode Assembly

The constant change in potential was caused by several design flaws in the electrode assembly. The first major design flaw was the lack of a reference electrode. This causes the potential of the system to gradually drift and be inconsistent. The second was the mixing of

metals on one electrode. The copper mesh electrode did not only consist of copper but of stainless steel as well because the current flows from the copper mesh and down the stainless steel rod to where the potentiostat was connected. The third design flaw was the electrodes are composed of metals that pit and whose surface area changes over time. A new electrode assembly was designed and constructed with these design flaws in mind. The recommended metal to construct the electrodes out of was platinum however due to the high cost; this was not possible to do for all the electrodes in the system. Instead platinum was selected for the reference electrode and titanium wire was selected for the working/sensing and counter electrodes. Then instead of a stainless steel rod being used for support, a glass rod was custom made by the glass blower at the University of Idaho to encapsulate the wires for the electrodes. The ends of the titanium wires were then bent into a spiral pattern to achieve a large surface area inside the diffusion cell. Figure 3.3 shows a picture of the titanium electrode assembly.

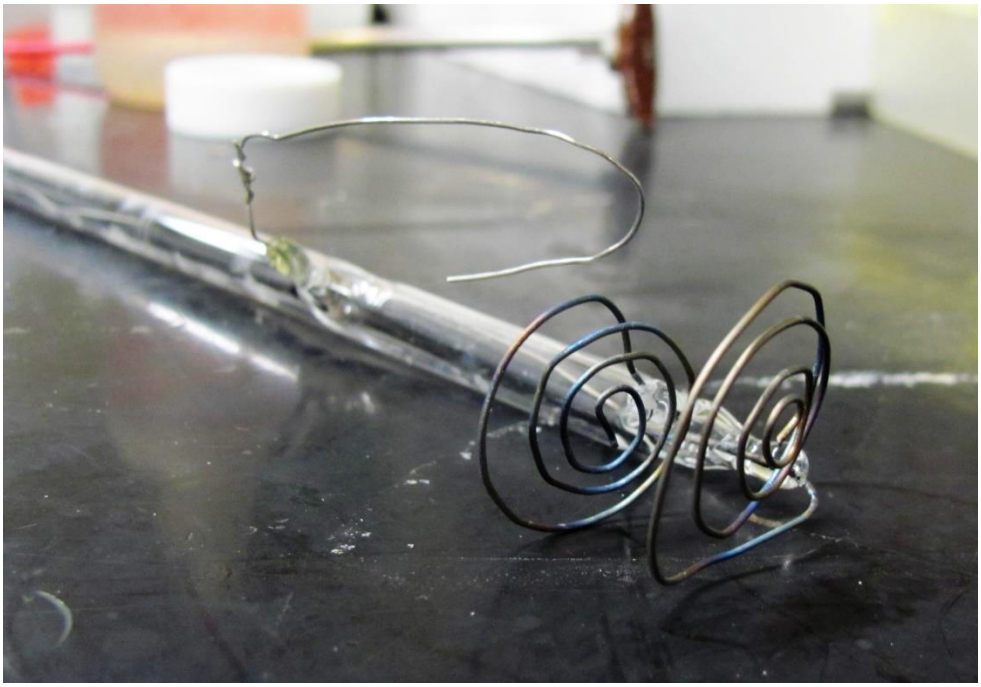

Figure 3.3: Titanium Electrode Assembly

This new titanium electrode assembly was tested the same way the copper electrode was tested. First the titanium electrode assembly was tested that it could split water and there were enough electrolytes in the system. It successfully passed this test. Then the electrode assembly underwent several cyclic voltammetry tests to discover the potential of which TCE reacts. During the cyclic voltammetry tests it was discovered that the titanium electrode did not contain the sensitivity to TCE that was desired. The minimum amount of TCE the electrode was able to sense was 30 ppm which was much too high for the purposes of measuring and quantifying diffusion through polymer membranes. The data from testing the titanium electrode assembly can be found in Appendix A.

In addition, to electrode design flaws and lack of sensitivity, there was a high amount of issues caused by the stir bars in these experiments. The stir bars had a significant effect on the readings of the electrodes. This was discovered during one of the experiments when the stir bar near the electrode stopped and the readings of the electrodes changed dramatically until the issue was resolved. In addition to the stir bar effects, the pressure and the air tightness of the cell also had effects on the data obtained. If the pressure in the diffusion cell got too high the cell would then start to leak from the Teflon septa. When this occurred, it also would greatly affect the reading output of the electrodes.

As a result of the electrode assemblies producing poor data, the method for measuring concentration of TCE had to be modified. The method for measuring data switched to taking liquid samples from the diffusion cell. These samples were then analyzed using a gas chromatograph mass spectrometer (GCMS) to determine the concentration of TCE present. The GCMS samples were placed in 2 mL GC auto sampler vials. In order to prevent TCE volatilizing in the headspace of the vial while awaiting analysis, the vials were filled completely full. This increased the accuracy of the measurement of the TCE concentration. When a sample of diffused liquid was taken from the low concentration side of the diffusion cell, the same volume of the sample was replenished into the diffusion cell with type 1 water. This was done so that the diffusion cell would maintain a constant volume and pressure.

The sampling times for the diffusion experiments were varied to determine the best rate to take samples. The first polymer membrane to be studied was sodium alginate. The sodium alginate membrane was cast in the membrane support according to the method described in chapter 2. The support was then placed in the diffusion cell and the diffusion cell was then filled with type 1 water. When placing the support into the diffusion cell it was important that the bottom of the Teflon support was facing the low concentration side, the side with the smaller volume, of the diffusion cell. The support was needed on the side with the smaller volume so that during the filling and sampling process the membrane was not damaged or displaced from the support. When the diffusion cell was filled, the larger side of the diffusion cell should have been filled first and then the smaller side filled second. Once the diffusion cell was filled with minimal air present inside the cell, the diffusion cell was then spiked with a known volume of TCE from 950 ppm TCE stock solution. A stock solution was decided to be used for these experiments because concentrated TCE would have to dissolve into the water during the course of the experiment which would greatly affect the results of the experiments. 950 ppm was chosen for the concentration of the stock solution because it is near the saturation point for TCE in water. Therefore, it is near the highest concentration a stock solution can be at which is best for the experiments. The higher concentration of the stock solution the smaller the volume needed to add to the diffusion cell in order to obtain the desired concentrations.

The first diffusion experiment run was to verify that diffusion was indeed happening and how fast it was happening. Samples were taken from the low concentration side of the diffusion cell every hour for 6 hours. This experiment verified that diffusion was occurring and the first sign of TCE appeared within the first hour. The second diffusion experiment was to narrow down the time window for which the first sign of TCE appeared in the low concentration side. This experiment showed that the first measurable concentrations of TCE could be observed within the first 30 to 45 minutes after the diffusion cell was spiked with TCE. From this data, the first experiment could be run. It was decided that it was best to do rapid sampling over two hours. The rapid sampling would begin 40 minutes after the TCE had been introduced into the system and a sample would be taken every five minutes for

two hours. This type of sampling was done twice. Once for a starting concentration of 152 ppm on the high concentration side of the diffusion cell and once for a starting concentration of 79.8 ppm on the high concentration side of the diffusion cell. The data from these two experiments can be seen below in Figure 3.4. These two concentrations, 152 ppm and 79.8 ppm, were picked because it required a volume of 20mL and 10mL, respectively, to be injected into the system from the 950 ppm stock solution. The first rapid sampling experiment, 152 ppm, has a poor fitting trend line for the data because of the large amount of human error in the rapid sampling process. The human error took place when the sample was taken from the diffusion cell and the same volume of the sample had to be replenished at the exact same time in order for the pressure and volume inside the diffusion cell to remain constant. The second rapid sampling experiment, 79.8 ppm, contained less human error from this because of the experience gained in replenishing and taking volume from the diffusion cell from the first experiment. Then from the data of both rapid sampling experiments it was determined that rapid sampling might be causing too much error in the concentration measurements. Therefore, it was then attempted to sample every 15 minutes for an extended amount of time until the system appeared to reach steady state. However, this experiment went very poorly. The starting concentration on the high concentration side of the diffusion cell was 25 ppm and the amount of TCE to diffuse through the membrane for the diffusion cell to reach steady state was very small and nearly undetectable by the GCMS. Therefore, this set of data had to be rejected from the quantification of diffusion data set. However, it can be seen in Appendix B. From this experiment it was discovered that the minimum starting concentration in the high concentration side of the diffusion cell should be greater than 25 ppm. The next experiment, had a starting concentration of 100 ppm on the high concentration side and it was determined that a sample should be taken every 30 minutes starting at time zero, when the TCE was introduced into the system, for seven hours. The concentration of 100 ppm experiment was run twice. The first experiment was thought to be highly successful because there a distinct time period at which the concentration was increasing and then it could be visually seen that the system reached steady state. However, when this experiment was attempted to be replicated with the

second 100 ppm diffusion experiment, the data was inconsistent with the first experiment's data. The second 100 ppm diffusion experiment data matched up with the data obtained from the 152 ppm and the 79.8 ppm diffusion experiments as seen in Figure 3.4. Therefore, it was concluded that the first 100 ppm diffusion experiment had a leak in the membrane. Thus causing the TCE in the diffusion cell to diffuse through the membrane and reach steady state at a much faster rate than the other experiments.

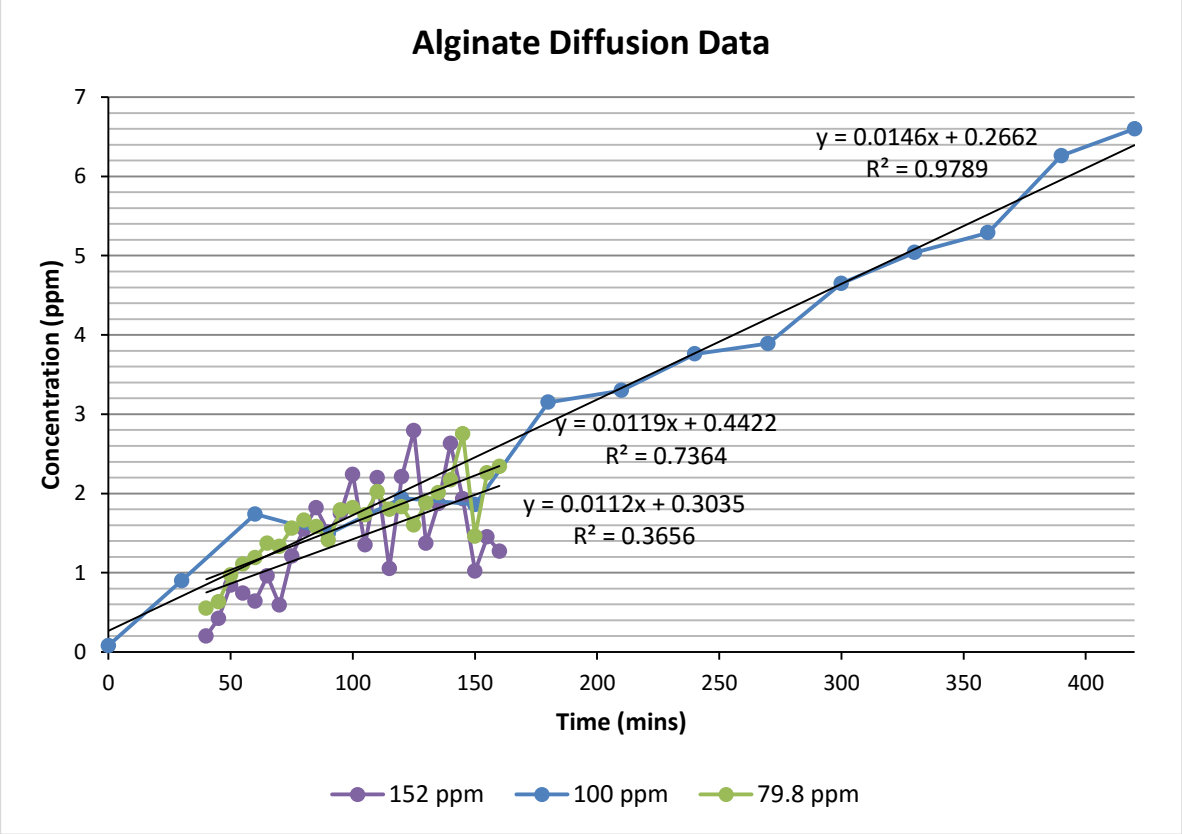

Figure 3.4: Sodium Alginate Diffusion Data

The slopes obtained from the time vs concentration plots from the experimental data were then used to quantify the diffusion coefficient for TCE through the sodium alginate membrane. The thickness of each membrane was measured after each diffusion experiment by measuring the distance from the top of the membrane support to the surface of the membrane. This distance was then subtracted from the total height of the membrane support to find the membrane thickness which was need for the diffusion coefficient

calculations. The membrane thickness needs to be measured after the experiment has finished because the membrane tends to swell over the course of the diffusion experiment. A new membrane needed to be cast for each diffusion experiment in order for the results to be accurate and consistent.

Due to the high success of the sampling pattern of every 30 minutes for seven hours for the sodium alginate membrane, it was then used for the PVA/SA membrane diffusion experiments as well. The PVA/SA diffusion experiments were set up the same way the sodium alginate diffusion experiments were set up. The PVA/SA membrane was cast using the method described in chapter 2. The cast membrane and the support were then placed into the diffusion cell. When placing the support into the diffusion cell it was important that the bottom of the Teflon support is facing the low concentration side, the sampling side, of the diffusion cell. Once again this was highly important in order to not damage the membrane during the filling process or during the diffusion experiments. Once the diffusion cell was filled the experiment may begin. The concentrations studied for TCE diffusion through PVA/SA membranes was 152, 100, and 76 ppm. The goal was to study as similar concentrations as those studied for the sodium alginate experiments.

The first concentration measured was 100 ppm. This experiment was deemed as highly successful, as seen in Figure 3.5. This data was then used as a baseline to see how well the other two concentrations data compared. The next experiment measured the diffusion of 152 ppm concentration. While the diffusion cell was being prepared for this experiment, the membrane appeared to be leaking. As the experiment proceeded, it was confirmed that the membrane was indeed leaking. The rate at which the concentration increased in the low concentration side of the diffusion, did not match that of the 100 ppm experiment which furthered the suspicion of the membrane leaking. Therefore, the 152 ppm concentration experiment needed to be re-run in order to verify the membrane was indeed leaking. The data from the new 152 ppm concentration experiment confirmed that the membrane was indeed leaking in the previous experiment. The second 152 ppm experimental data matched up with that of the 100 ppm experimental data which can be seen below in Figure 3.5. The first 152 ppm experimental data did not match up because the slope of the data was an

entire order of magnitude higher than of the rest of the data. The first 152 ppm concentration experiment compared to the rest of the data obtained for the PVA/SA membrane diffusion experiments can be seen in Appendix B. The final experiment with a concentration of 76 ppm was run and the data obtained from that experiment matched up with that of the second 152 ppm experiment and the 100 ppm experiment as seen in Figure 3.5.

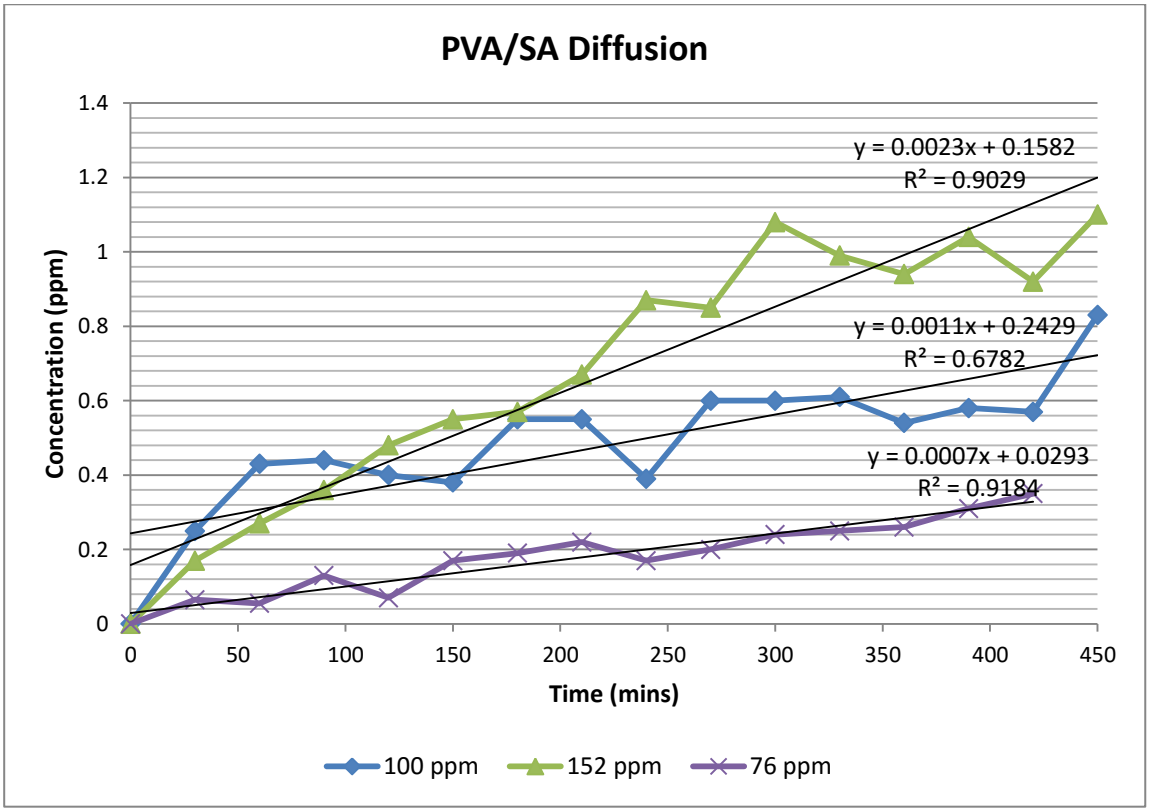

Figure 3.5: PVA/SA Diffusion Data

The slopes obtained from the time vs concentration plots from each of the concentrations are then used to determine the diffusion coefficient for TCE through PVA/SA membrane. The thickness of the membranes was measured using the same method used on the sodium alginate membranes where the distance was measured from the top of the membrane support to the surface of the membrane after the diffusion experiment had been

completed. A new membrane must be used for each experiment in order for the results to be accurate.

QUANTIFICATION OF DIFFUSION

To determine the diffusion coefficient experimentally several assumptions must first be made to determine which law should be applied. Given the diffusion cell apparatus shown in Figure 3.1 the following assumptions can be made:

1. Flow is only in the x-direction
2. D_{AB} is constant and has a linear correlation
3. The system density (ρ) is constant
4. The system is non-flow, the velocity is zero ($\mathrm{v} = 0$)
5. There is no reaction occurring
6. The concentration of TCE in volume 1 (V_1) remains constant

With these assumptions, the one-dimensional form of Fick's first law of diffusion can be used, shown in equation (3.1)[21, 36, 37]. Fick's first law can then be simplified and applied towards the experimental quantification of diffusion coefficients with simple manipulation and substitution as shown in equations (3.2-4)

$$j_{ax} = -D_{AB}\frac{dC_A}{dx}\frac{1}{L} \tag{3.1}$$

$$j_{ax} = \frac{dC_A}{dt}\frac{1}{A} \tag{3.2}$$

$$\frac{dC_A}{dx} = K_P(C_A^* - C_{Ao}) \tag{3.3}$$

$$\frac{dC_A}{dt} = -D_{AB}K_P(C_A^* - C_{Ao})\frac{A}{L} \tag{3.4}$$

Where,

j_{ax} – molecular mass flux

L – thickness of the polymer film (cm)

A – surface area of the polymer film (cm^2)

C_A^* - concentration of TCE in V_1

C_{Ao} – starting concentration of TCE in V_2

K_P – solute partition coefficient

Over the course of the experiment the concentration of TCE that has diffused through the polymer was measured with respect to time. Therefore the slope of the plot (m) of time vs concentration shows the change in concentration over the change in time as seen in equation (3.5). This then ties in the experimental data to Fick's first law. The solute partition coefficient (K_P) can be assumed to be 1[21]. It can be assumed to be one because TCE is completely miscible in water. With this information the diffusion coefficient can then be obtained using equation (3.6).

$$m = \frac{dC_A}{dt} \tag{3.5}$$

$$D_{AB} = \frac{mL}{AC_A^* K_P} \tag{3.6}$$

Using the slopes shown in Figures 3.4 and 3.5 and the thickness measurements taken from each membrane, the diffusion coefficient can easily be quantified using equation (3.6). The diffusion coefficients obtained for TCE through sodium alginate for 79.8 ppm, 100 ppm, and 152 ppm are 2.356 ± 0.5444 x 10^{-7}, 2.113 ± 0.1809 x 10^{-7}, and 1.164 ± 0.6651 x 10^{-7} cm^2 s^{-1} respectively. The diffusion coefficients obtained for TCE through PVA/SA for 76 ppm, 100 ppm, and 152 ppm are 1.607 ± 0.1329 x 10^{-8}, 1.548 ± 0.1263 x 10^{-8}, and 2.615 ± 0.2294 x 10^{-8} cm^2 s^{-1} respectively. Sample calculations for how each of these numbers was obtained are shown in Appendix B. As seen from the numbers listed above there is a very small if any dependence of concentration of the diffusion coefficient. The average overall diffusion coefficient for TCE through sodium alginate membrane was determined to be 1.880 ± 0.6610 x 10^{-7} cm^2 s^{-1} at 20°C. The average overall diffusion coefficient for TCE through PVA/SA membrane was determined to be 1.923 ± 0.5089 x 10^{-8} cm^2 s^{-1} at 20°C.

The experiments conducted in this chapter were extremely limited and could use some further work to expand the diffusion coefficients determined. One of these expansions would be to determine the temperature dependence on the diffusion coefficient. The conclusions and future work of these experiments will be further discussed in chapter 5.

CHAPTER 4: BIOREMEDIATION OF TRICHLOROETHYLENE

The bioremediation of TCE experiments involved encapsulating an anaerobic bacteria consortium known as KB-1 provided by SiREM into polymer beads. The procedure for producing the polymer beads with encapsulated bacteria was described in chapter two. All of the experiments that took place needed to be done in an anaerobic chamber due to the anaerobic properties of KB-1. The bioremediation experiments were studied in a media solution provided by SiREM. The media solution contained resazurin. Resazurin is a color indictor dye used in anaerobic chambers to detect the presence of oxygen. The dye remains clear when the environment is anaerobic and when the environment becomes aerobic the dye turns pink. This was an extremely helpful indictor for these experiments because it was critical for KB-1 to remain in an anaerobic environment. KB-1 is a highly sensitive consortium to oxygen. Any oxygen presence in the experiments would cause KB-1 to lyse, thus wreaking havoc on the experiments.

EXPERIMENTAL SET-UP

As previously stated all the experiments took place in an anaerobic chamber for the livelihood of KB-1 and the solution all the experiments were run in was a media solution provided by SiREM. When initially designing the experiments it was desired to run the experiments in a simulated groundwater solution. However, this was not possible because of the legal agreement with SiREM and the proprietary nature of the KB-1 consortia members. The recipe for the media provided by SiREM is also proprietary information of SiREM. Therefore, SiREM mailed the media to the University of Idaho in sealed 1L or 2L glass bottles. These shipments were overnighted to the University of Idaho from SiREM located in Ontario, Canada. The shipments were overnighted to prevent contamination and limit KB-1 and the media's exposure to oxygen. Majority of the bottles of media received from SiREM were still clear in color meaning that they remained free of oxygen during the shipping process. The bottles that were not clear in color instead they were pink in color meaning there was

oxygen present in the solution. Those bottles were placed in the anaerobic chamber until the pink faded and the solution turned and remained clear.

The bioremediation experiments were set up as batch processes. This means that each part of the experiment was run in a 160mL serum bottle sealed with Teflon septa. For the experiments there was the possibility of six different types of bottles to be created. These bottle types were:

1. Bottles with just media
2. Bottles with 2mL planktonic KB-1
3. Bottles with 10mL sodium alginate beads without KB-1
4. Bottles with 10mL sodium alginate beads encapsulating KB-1
5. Bottles with 10mL PVA/SA beads without KB-1
6. Bottles with 10mL PVA/SA beads encapsulating KB-1

Each of these bottles was created in triplicate to have experimental redundancy. Each bottle needed to be replicated and run simultaneously because the age of KB-1 needed to be the same in all the bottles for the experiments. When the bottles were created, the beads not containing KB-1 were created first to prevent contamination. The beads were created in 10mL batches. Each 10mL batch as it was created was placed in a serum bottle. The serum bottle was then filled with 150mL of media solution. After each bottle was filled it was then sealed with a Teflon septum and a crimp. Prior to encapsulation, samples of KB-1 were taken and cell counts were done to know the density of KB-1 that was encapsulated. The bottles containing encapsulated KB-1 were then created next. Once again the encapsulated KB-1 beads were produced in 10mL batches. Then each 10mL batch was added to a serum bottle which was then filled with 150mL of media solution. The bottle was then sealed with a Teflon septum and a crimp. Once all the bottles were created, each of the bottles was then spiked with the necessary amount of TCE to begin the experiments. The time that the TCE was spiked is known as time zero for the experiments. Samples of each bottle are taken and filtered, using a sterile syringe filter and analyzed using a GCMS to determine the concentration of TCE, cis-DCE, and vinyl chloride in each sample. After the experiment is completed each bottle's pH was measured to determine if the environment was acidifying

when TCE undergoes bioremediation. The pH was highly important to the success of the experiments because KB-1 works best in a neutral pH range of 6.5 to 8.5 and once the pH shifts out of that range, below 5.5, KB-1 will lyse. If this occurs the bioremediation reactions will stop and become “stuck”.

EXPERIMENTAL PROCEDURE AND MEASUREMENTS

The first experimental set up was preliminary experiment to verify that KB-1 could survive the encapsulation process. Each of the six bottle types was prepared in triplicate, and color coded. The concentration of TCE decided upon for these experiments was 100 ppm. A stock solution of 950 ppm was created using autoclaved type 1 water. A stock solution was used for this experiment to eliminate the time needed for TCE to dissolve into the media solution during the experiment. Each bottle was spiked with 16.8mL from the TCE stock solution to create a concentration of 100 ppm in each bottle. The bottles were monitored over the course of 80 hours. A sample was taken from each bottle every eight hours starting at time zero after TCE had been added to all the bottles. A 2mL sample was taken from a bottle using a syringe and then filtered using an in-line syringe filter into a 2mL GC vial. It was necessary to filter all the samples taken because any particulates in the sample can harm the GCMS and affect the TCE concentration after the sample was taken. The sample was then analyzed using a GCMS to evaluate the TCE concentration present in the sample. During this experiment only the TCE concentration was monitored and none of the lesser chlorinated forms of TCE were monitored. This led to many issues upon evaluating the concentration data obtained from the GCMS.

At first glance of the data obtained from the preliminary experiment, appeared to be excellent data because the concentration of TCE in the bottles containing KB-1 decreased over time and at the end of 80 hours there was minimal TCE presence. However, when looking at all the bottles, involved in the experiment the concentration decreased in all them, as seen in Figure 4.1. The time vs concentration data for each individual bottle can be found in Appendix C. This raised several questions and concerns of what happened during the experiment to cause all the bottles to have a decrease of TCE concentration. Had the

lesser chlorinated forms, cis-DCE, and vinyl chloride, concentrations been obtained during the experiment it would have provided a clearer picture if the TCE was volatizing from the bottles or if the TCE underwent degradation. The pH data obtained from the preliminary bead experiment did not clarify the reason for all the bottles decreasing TCE concentration, as seen in Figure 4.2. The pH in the bottles containing sodium alginate (SA) and PVA/SA beads pH dropped a greater amount than that of the pH of the media and the planktonic KB-1. Therefore this data does not provide any indication if the decrease in TCE concentration correlates with TCE bioremediation.

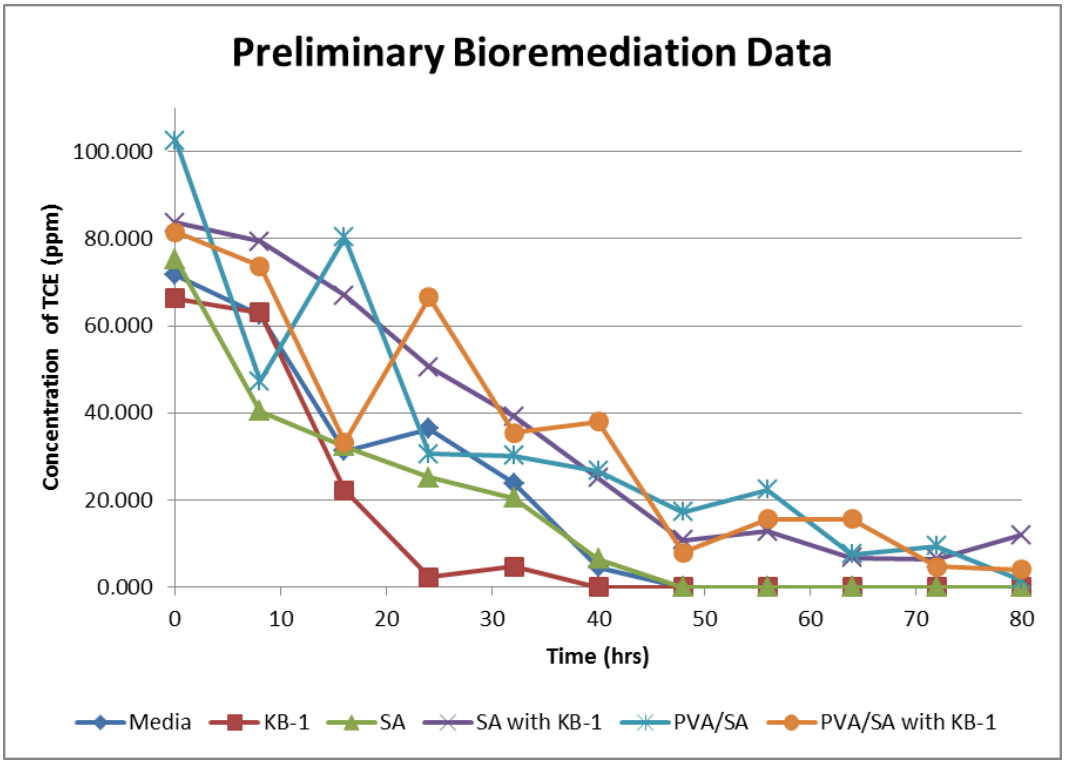

Figure 4.1: Preliminary Bioremediation Data

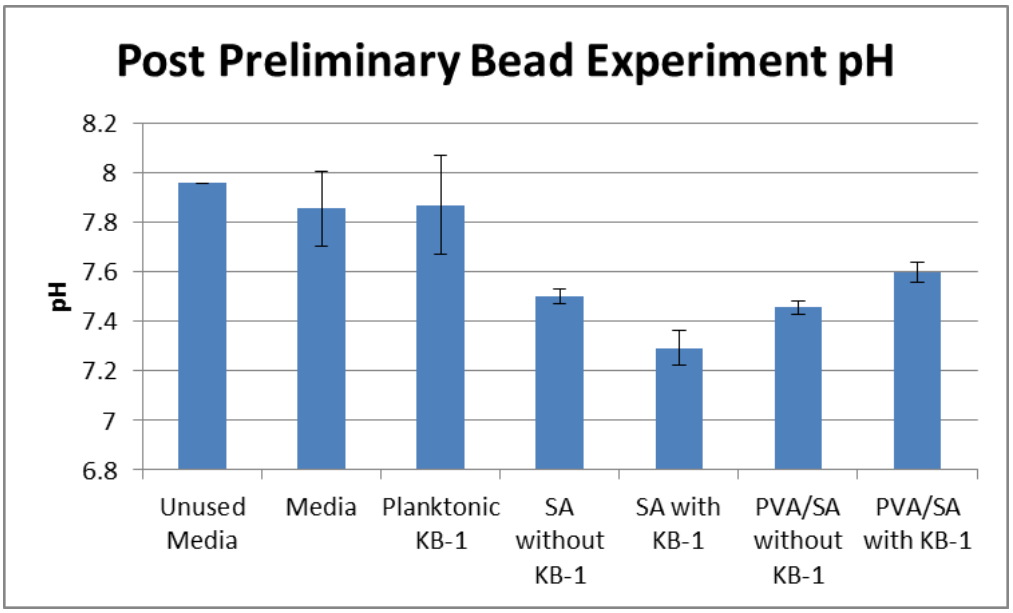

Figure 4.2: Post Preliminary Bead Experiment pH Data

The main concern for why all the bottles showed a decrease in TCE concentration was that there was contamination present in the bottles. To determine if it was a contamination issue from the media solution provided by SiREM, the media was looked at under a microscope to see if there was any microorganisms present in the solution. It was discovered that the media solution had been contaminated at some point of time and that there was microorganisms present in the media solution. This then indicated that the media solution added to each of the bottles, provided microorganisms to the bottles that were supposed to be microorganism free. The contamination of the media could be the reason there was a decrease in concentration in all the bottles in the experiment. From this preliminary experiment it was learned that future media solutions received from SiREM needs to be checked for contamination prior to starting the experiment. Another possible explanation for the concentration decreasing in all of the bottles is the single Teflon septa and crimp did not seal the bottles completely.

Using the contaminated solution from the preliminary experiment it was decided to test the effect of filter sterilizing the solution on the TCE remediation. This was done to test if filter sterilizing the solution would remove the contamination issue seen in the preliminary experiment. This experiment was completed in ten autoclaved anaerobic culture “Balch-

type" tubes. The "Balch-type" tubes were each sealed with two Teflon septa and flushed with nitrogen and carbon dioxide mixed gas. Two Teflon septa were used in this experiment to better seal the tubes and prevent leaking. The tubes were then autoclaved to sterilize them. Once they had completed the autoclaving process, the sterile "Balch-type" tubes were then placed in the anaerobic chamber. The contaminated media solution was then filter sterilized into the "Balch-type" tubes using sterile syringe filters. All ten of the "Balch-type" tubes with the sterile filtered media were spiked with 3mL of 900 ppm TCE stock solution so the overall concentration in the "Balch-type" tubes was 100 ppm. 1mL of KB-1 was added to half, five, of the "Balch-type" tubes. The remaining five did not receive KB-1. Once all the components were in each of the "Balch-type" tubes, each of the tubes were inverted a couple of times to thoroughly mix the contents. 24 hours after the TCE was introduced into the "Balch-type" tubes, samples were taken from each tube to be analyzed by the GCMS. The purpose of the samples was to verify if the TCE had been reduced into cis-DCE. The results from the GCMS showed that only the tubes with KB-1 had reduced TCE to cis-DCE. In addition, the results showed that the tubes with no KB-1 had lost some TCE however, none of the TCE reduced into cis-DCE. Thus proving that filter sterilization of the media could prove to be a viable option for future contaminated media solution.

Knowing there could be an easy resolution for contaminated media solution, the next experiment was designed. The next experiment to be run was decided to be sodium alginate beads with encapsulated KB-1 and planktonic KB-1 in media solution. This was decided because the sodium alginate data obtained from the preliminary experiment contained the most consistent data, the polymer was easier to work with, and the cross-linking process was the least likely to kill KB-1. The purpose of this experiment was to push the concentration limit of KB-1 and observe at which concentration KB-1 would no longer be an effective dechlorinator. KB-1 had a limit of the amount of TCE it can withstand of roughly 620 ppm. At the concentration of 620 ppm KB-1 would lose its ability to effectively reduce TCE and begin to lyse. The concentrations studied in this experiment were 400, 800, and 1600 ppm.

When the media solution arrived samples were taken to check for contamination. The media showed signs of contamination therefore, the media was then filter sterilized. The filter sterilization process was done in the anaerobic chamber in order for the media to remain anaerobic. The process involved pouring the contaminated media solution into a vacuum sterile filter connected to an autoclaved bottle. The vacuum was created by using a hand vacuum pump. The filter sterilized solution did show signs of oxygen being present after filtering by turning pink. The anaerobic chamber underwent air cycling to refresh the anaerobic gas inside the anaerobic chamber. The filter sterilized media solution was then left to sit in the anaerobic chamber until it lost its pink color and turned clear. The anaerobic media was then used to fill each serum bottle with 130mL for the bottles that would contain sodium alginate beads and 158mL for the bottles that would only contain KB-1. Each of the bottles was then sealed using two Teflon septa and a crimp. Each of the bottles was then dosed with the necessary amount of pure TCE to make the desired concentration with a volume of 160mL. Pure TCE was used in this experiment instead of a stock solution because the concentrations involved in the experiments were too high to use a stock solution. After each bottle was dosed, it was mixed thoroughly and sat for a couple of days while the TCE dissolved into solution. While the TCE dissolved into the filtered media solution, the sodium alginate beads encapsulating KB-1 were produced in 10mL batches. The beads were made using the same method as the preliminary experiment and the method described in chapter two. Each 10mL batch of beads was then stored in a sterile 50mL centrifuge tube with 20mL of filtered media solution. When the beads were added to the filter media solution, a strange white powder formed in the centrifuge tubes covering the beads. Once the TCE had mostly dissolved into the filtered media solution in the sealed serum bottles that would contain the beads, the crimp and the Teflon septa were removed. The sodium alginate beads with encapsulated KB-1 and the filtered media solution they were placed in were then added to the serum bottle. The strange white powder that formed unfortunately made it into the serum bottles as well because it had covered the beads and become somewhat attached to the beads during the transition process. The serum bottle was then re-sealed with two Teflon septa and a crimp. The process of adding the sodium alginate beads was done as

quickly and carefully as possible in order to not spill any filtered media, beads, or let too much TCE escape from the serum bottle. The serum bottles not containing beads were then dosed with 2mL of KB-1 soon after. The addition of the KB-1 to the TCE contaminated filtered media solution was then noted as time zero for the experiment. This experiment ran for roughly 408 hours or 17 days.

Unfortunately, all of the data except for the last samples taken from the sodium alginate encapsulating KB-1 and planktonic KB-1 experiment were unable to be quantified for TCE, cis-DCE, and vinyl chloride concentrations. The explanation for the inability to quantify the data was the improper set up of the GCMS. The improper set up involved a GC and MS method that produced poor peak shape and poor mass spectrometer readings. These two methods, the GC method and the MS method, were then over-written on the software during the experiment. The over-writing occurred during a GCMS training session where the methods were then corrected. The corrected method produced ideal peak shape, of a sharp clean point at the tip of the peak, and proper mass spectrometer readings, which involved correcting the solvent delay. Due to the GC and MS methods being changed during the experiment, the standards that were need for quantification of the samples taken during the experiment could not be analyzed using the same method the samples were analyzed with. Thus causing the issue of being unable to quantify the TCE, cis-DCE, and vinyl chloride concentrations present in the nearly all the data obtained for the experiment. The data obtained from the last samples taken from the experiment are shown in Figure 4.3. In addition, to the last time data point the pH for each bottle was recorded at the end of the experiment. The pH of unused sterile filtered media was also recorded as a reference point to observe the change compared to media used in the experiments as seen in Figure 4.4. The complete data set obtained from this experiment is shown in Appendix D.

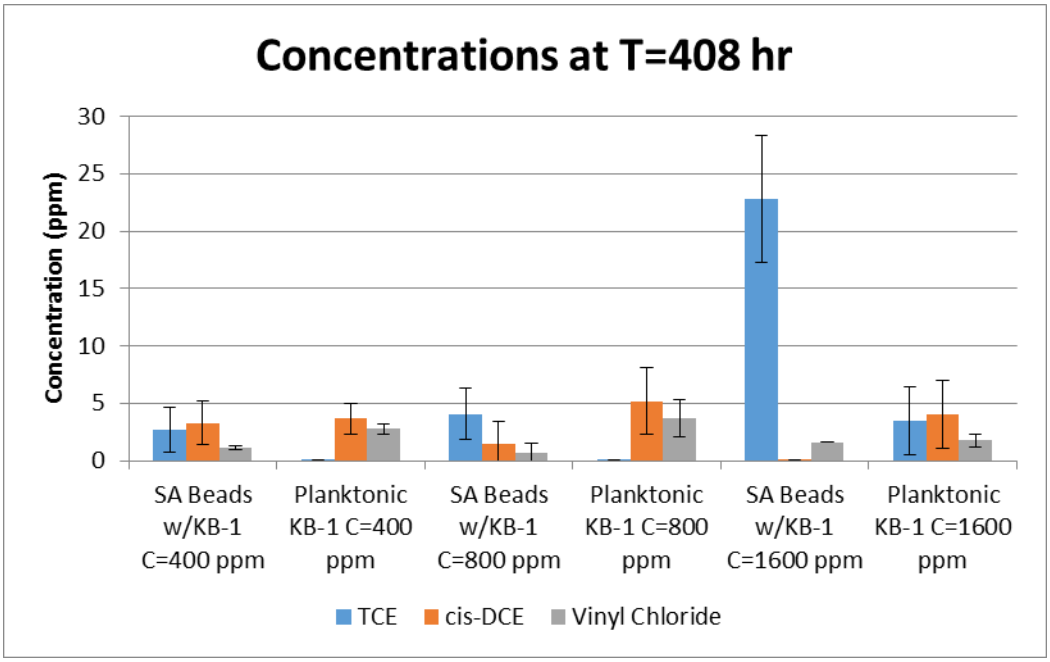

Figure 4.3 Concentrations of TCE, cis-DCE, and Vinyl Chloride at the Post Filtered Media Experiment

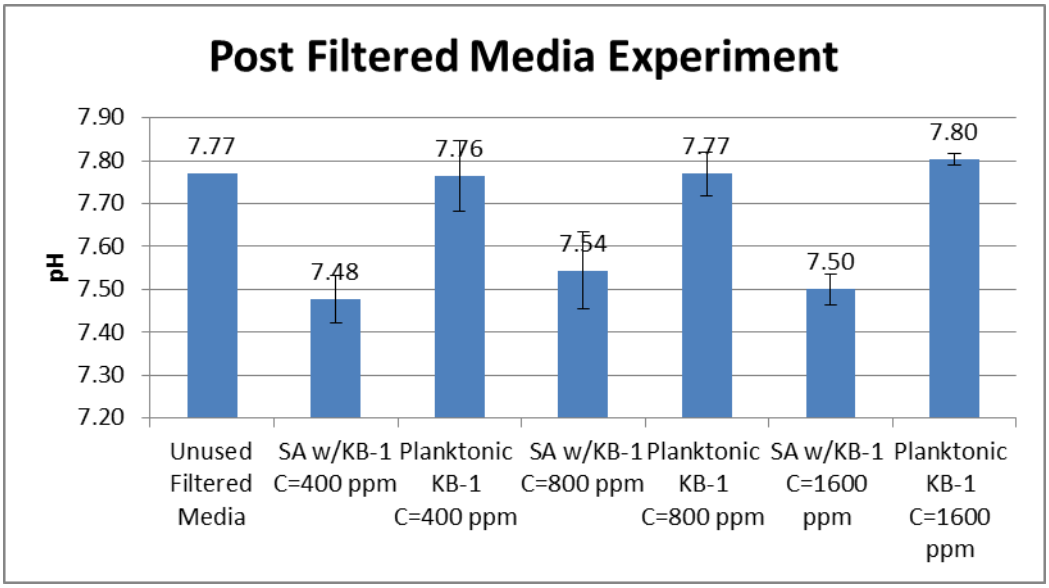

Figure 4.4: pH of Post Filtered Media Experiment

From Figure 4.3, it was observed that nearly all the TCE was removed from the experiment after 408 hours with exception of the sodium alginate (SA) beads with encapsulated KB-1 with a starting concentration of 1600 ppm. Even stranger is the comparison of Figure 4.3 and Figure 4.4. The planktonic KB-1 showed the overall greatest

removal of TCE and from the systems however the pH in those bottles remained unchanged for the duration of the experiment. Then when looking at the SA beads with encapsulated KB-1 results the pH of these bottles dropped a greater amount however the degree of reduction does not appear much different from the planktonic KB-1 with exception of SA beads encapsulating KB-1 with a starting concentration of 1600 ppm. These observed results have little explanation due to the lack of data obtained during the experiment. To better understand what exactly occurred during the experiment, the experiment needs to be redone. The next bioremediation experiment will not have the data loss issue this experiment occurred because the GCMS is set-up properly and the methods will remain unchanged for the duration of the experiment.

Upon evaluation of the results or lack thereof, the final bioremediation experiment was designed. For this experiment all six types of bottles (just media without KB-1, planktonic KB-1 in media, sodium alginate beads without KB-1, sodium alginate beads with KB-1, PVA/SA beads without KB-1, and PVA/SA beads with KB-1) were prepared in triplicate except for the just media bottles which were created in duplicate. The just media bottles were prepared in duplicate to make the number of bottles in the experiment more manageable. Each type of bottle was then run at four different starting concentrations of 100 ppm, 200 ppm, 400 ppm, and 600 ppm of TCE. Thus bringing the total of bottles run during the experiment to 68. To aid KB-1 in the reduction of TCE ethanol was added to the media as a carbon source. 2mL of filter sterilized 95% ethanol was added to 2L of media solution.

Prior to the addition of the ethanol to the media solution, the media solution was checked for contamination. It was unclear if the media solution received from SiREM was contaminated; therefore a test was developed to check for contamination. This test involved autoclaving six anaerobic culture “Balch-type” tubes. The tubes were then divided into three sets of pairs. Each pair of “Balch-type” tubes were filled with media from different media solution bottles to confirm that each bottle of media solution was not contaminated. Then one tube in each of the pairs was dosed with 1mL of KB-1. All of the tubes were then dosed with 2.8mL of a 950 ppm TCE stock solution so the concentration in each tube was 100 ppm

TCE. The tubes were sampled three times, at time zero (when the TCE was introduced), at 12 hours, and at 24 hours. The results showed that the tubes only containing KB-1 reduced TCE to cis-DCE in those 24 hours. The tubes not containing KB-1 did not reduce TCE to cis-DCE therefore; this test successfully showed that the media solution received from SiREM was not contaminated. Due to the media solution not being contaminated, the media did not need to be filter sterilized like the previous experiment.

After the media was deemed sterile and safe to use in the experiment, the experiment could proceed. The bottles not containing KB-1 were prepared first. The just media bottles were filled with 160mL of media solution with ethanol and sealed with two Teflon septa and a crimp. Then the sodium alginate beads were produced using the method described in the formation of polymer beads section in chapter two in 10mL batches. Each 10mL batch of sodium alginate beads were then added to a serum bottle which was then filled with 150mL of media solution with ethanol and sealed with two Teflon septa and a crimp. The PVA/SA beads were produced also using the method described in the formation of polymer beads section in chapter two in 10mL batches. Each 10mL batch of PVA/SA beads were then added to a serum bottle which was then filled with media solution with ethanol and sealed with two Teflon septa and a crimp. Once all the bottles not containing KB-1 were created and sealed, KB-1 was brought into the anaerobic chamber to create the bottles containing KB-1. The bottles containing planktonic KB-1 were constructed by adding 2mL of KB-1 to 158mL of media solution with ethanol and sealed with two Teflon septa and a crimp. The sodium alginate beads encapsulating KB-1 were produced using the procedure found in the encapsulation of anaerobic bacteria section in chapter two in 10mL batches. Each 10mL batch of sodium alginate beads encapsulating KB-1 were then added to a serum bottle which was filled with 150mL of media with ethanol and sealed with two Teflon septa and a crimp. The PVA/SA beads encapsulating KB-1 were formed using the procedure found in the encapsulation of anaerobic bacteria section in chapter two in 10mL batches. Each 10mL batch of PVA/SA beads encapsulating KB-1 were then added to a serum bottle filled with 150mL of media solution with ethanol and sealed with two Teflon septa and a crimp. Once all the bottles containing KB-1 were constructed, the entire set of bottles involved in the

experiment with KB-1, were allowed to sit for 24 hours for KB-1 to recover from the encapsulation process. 24 hours after the encapsulation the bottles containing KB-1 were then dosed with TCE. The TCE used for dosing the bottles was pure TCE. Each bottle was dosed with the appropriate amount of TCE using a microliter syringe. The bottles were shaken to help dissolve the TCE into the solution and time zero samples for the KB-1 bottles were taken. Later the same day, the bottles not containing KB-1 were dosed with TCE in the same manner as the bottles containing KB-1 were dosed with TCE. After the TCE was introduced into all the bottles not containing KB-1, they were shaken to help dissolve the TCE and time zero samples were taken for these bottles.

The bottles containing KB-1 were sampled every 24 hours and the bottles not containing KB-1 were sampled every 48 hours. The samples were then analyzed using a GCMS to evaluate the TCE, cis-DCE, and vinyl chloride concentrations present at that point in time. The bottles containing KB-1 were sampled more often because the reduction reactions were occurring in the bottles with KB-1 and to limit the headspace in the bottles not containing KB-1. The bottles not containing KB-1 needed to have limited growing headspace to prevent the volatilization of TCE present in those bottles. The purpose of the bottles not containing KB-1 was a control for the bottles containing KB-1. In order for the controls to be the most effective, the TCE needs to stay in solution and not volatilize into the headspace. This experiment was then run for nine days (216 hours). The experiment would have been run for a longer period of time however the headspace in the bottles containing KB-1 after nine days and ten samples was approximately 15% of the bottle's volume which was decided to be too large for accurate analysis. A large headspace gives space for TCE, cis-DCE, and vinyl chloride to volatilize and leave the system which then leads to error in the concentrations obtained from the samples analyzed. The concentration data obtained from the GCMS is shown in Figure 4.5-8 for the bottles containing KB-1 and 4.10-12 for the bottles not containing KB-1. In addition to the concentration data obtained from the GCMS, the pH of each bottle was measured to determine if the bioremediation experiment causes acidification to the systems. The pH data can be seen in Figure 4.9 and 4.13. The complete

data set obtained from this experiment, both GCMS concentration data and pH data, can be found in Appendix E.

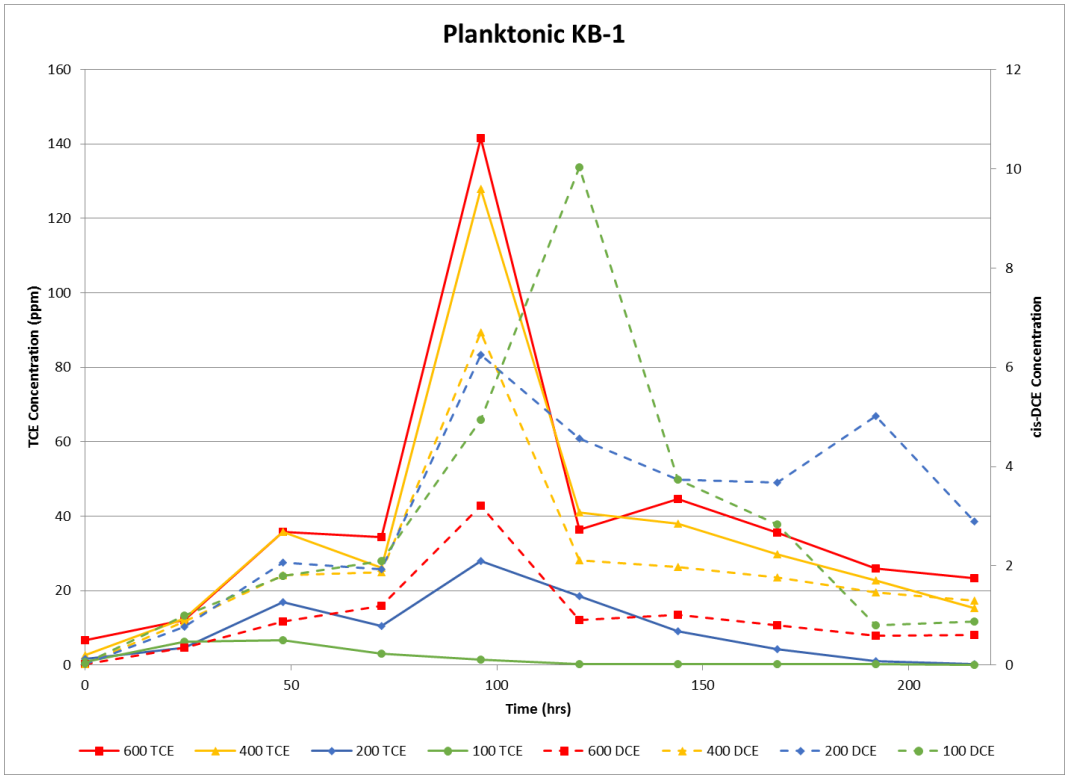

Figure 4.5: Time vs. Concentration of TCE and cis-DCE of Planktonic KB-1

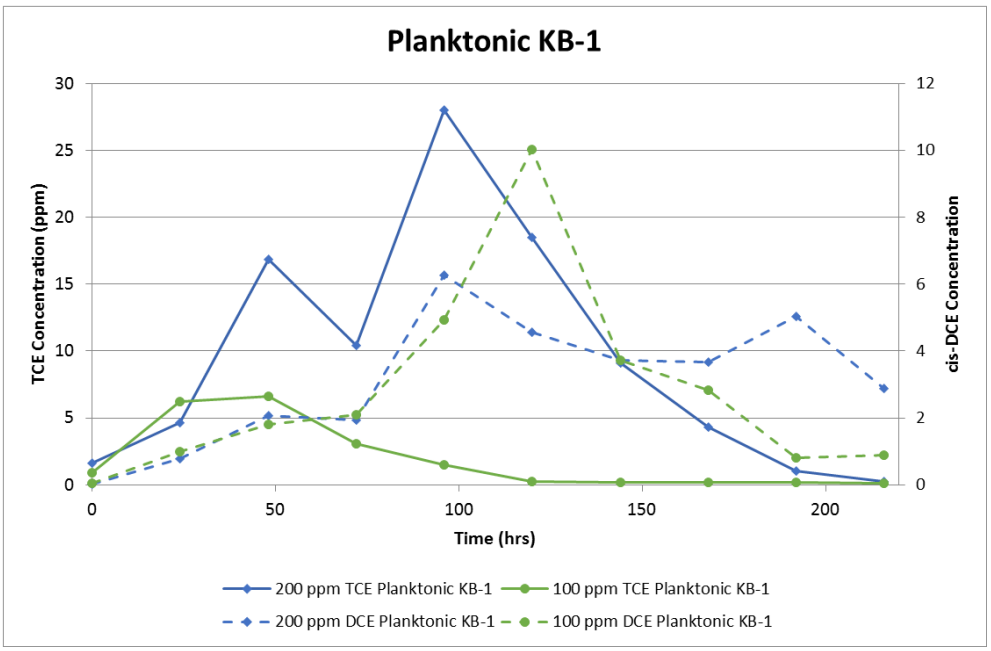

Figure 4.6: Time vs. Concentration of TCE and cis-DCE of 100 ppm and 200 ppm Starting Concentration of Planktonic KB-1

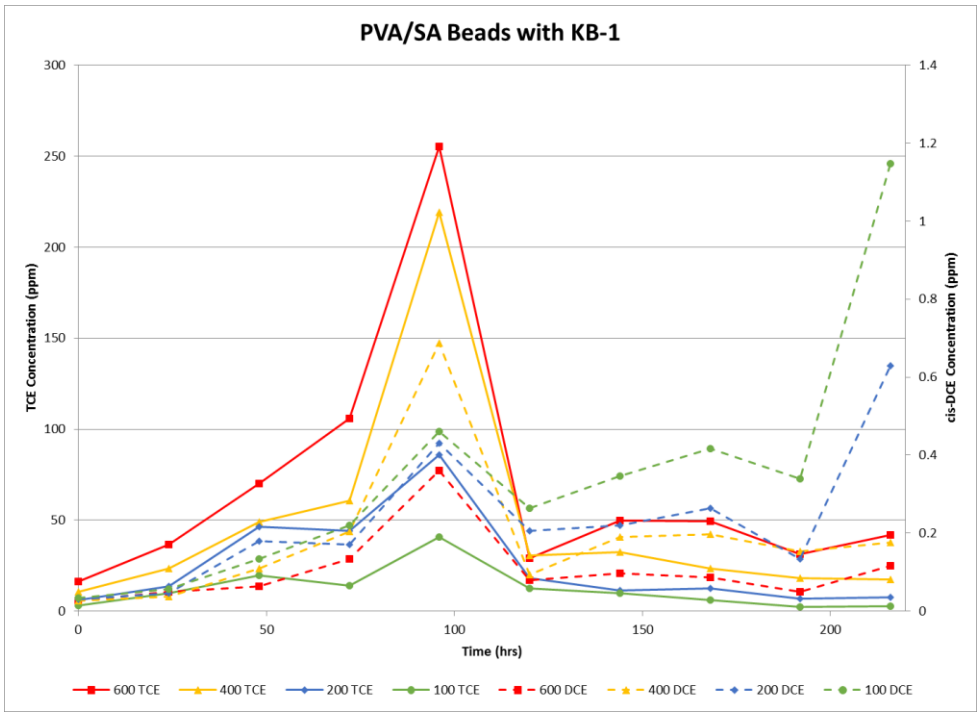

Figure 4.7: Time vs. Concentration of TCE and cis-DCE of PVA/SA Beads with Encapsulated KB-1

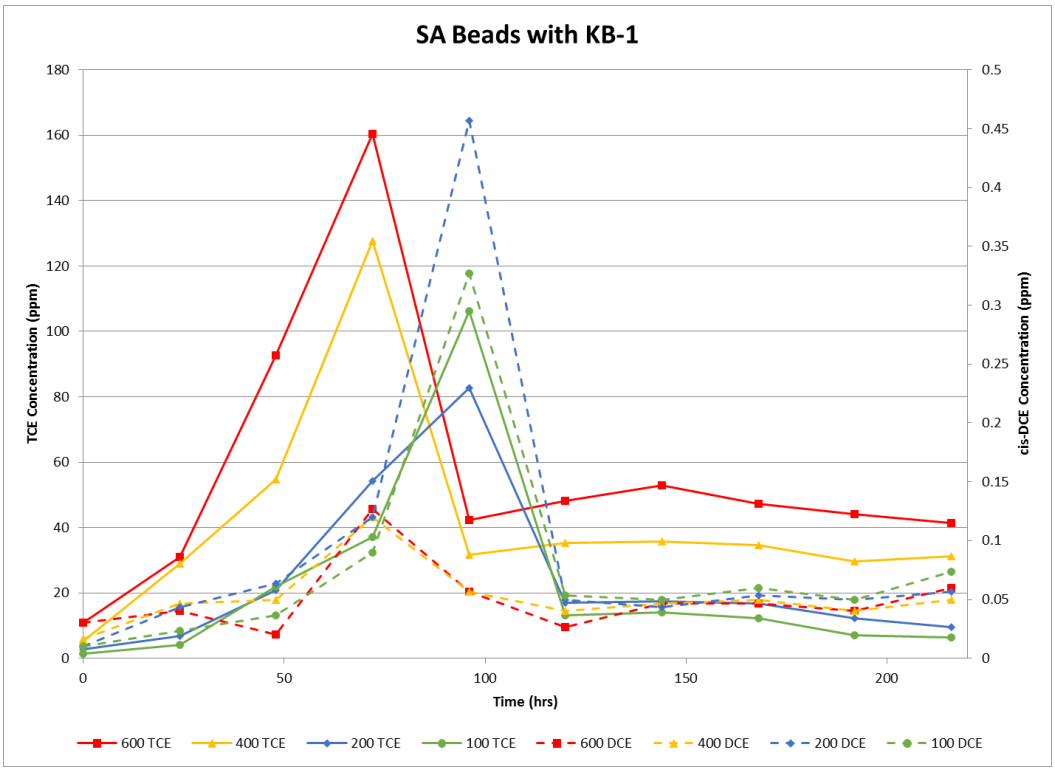

Figure 4.8: Time vs. Concentration of TCE and cis-DCE of SA Beads with Encapsulated KB-1

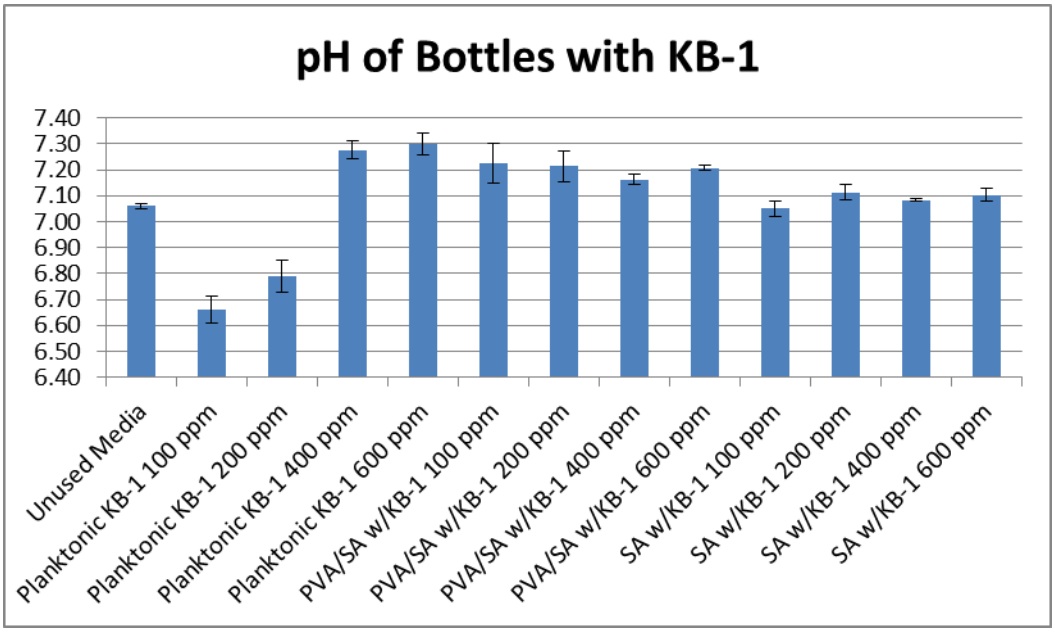

Figure 4.9: pH of Unused Media Compared to Planktonic KB-1, PVA/SA Beads with Encapsulated KB-1, and SA Beads with Encapsulated KB-1

As seen in the figures, the TCE did undergo remediation in some of the bottles. It is known that the TCE underwent remediation in some of the bottles because of the production of cis-DCE was present. The concentration of TCE increases in the beginning of the experiment because pure TCE was used for the experiments. The pure TCE was added to the bottles at time zero but had not yet dissolved into the systems. The pure TCE then dissolved into the bottles over the course of the first 100 hours of the experiments. This can be seen in Figures 4.5-8, as the slow increase in TCE concentration leading to the spike in concentration around 100 hours. This increase was also observed in the bottles not containing KB-1 which is seen in Figures 4.10-12. This shows that the increase in TCE is indeed the pure TCE dissolving into the systems and not instrument error. Due to this slow increase and then immediate decrease of TCE concentration, it makes incredibly difficult to determine the reaction rates of experiments. It is possible that the planktonic KB-1 with a starting concentration of 100 ppm and planktonic KB-1 with a starting concentration of 200 ppm completed their bioremediation of TCE as seen in Figure 4.6. However, it is not confirmed because vinyl chloride was never observed. However, vinyl chloride was not observed in any of the samples analyzed throughout the experiment. It was possible that the vinyl chloride vaporized and escaped from the bottles during the course of the experiment or it was possible that the vinyl chloride escaped the samples during the filtering step of obtaining the samples. Either of the options was entirely possible because of the highly volatile nature of vinyl chloride. The pH of the planktonic KB-1 with starting concentrations of 100 ppm and 200 ppm also confirms that these bottles completed their bioremediation because the pH has dramatically decreased compared to the unused media and the other bottles containing KB-1 in the experiment as shown in Figure 4.9. The pH of all the other bottles in the experiment that contained KB-1 increased in pH when compared to the unused media as seen in Figure 4.9. The explanation for the increase in pH of these bottles is unknown. There is not enough information of the metabolic pathways and the products formed for KB-1. It is possible that some of the intermediates formed, cis-DCE and vinyl chloride, during the bioremediation are basic thus causing the pH to increase. However, the intermediates are not known so this is just a vague idea. The bioremediation of the TCE

contained in the bottles containing PVA/SA beads with encapsulated KB-1 and SA beads with encapsulated KB-1 may also have been completed without a strong appearance of cis-DCE or vinyl chloride. This could have been possible if the entire bioremediation reduction reactions occurred inside the beads. Since KB-1 was encapsulated inside the beads, it is possible that during the reduction, the less chlorinated forms were consumed inside the bead by KB-1 prior to the release of these less chlorinated forms, cis-DCE and vinyl chloride. This explains the decrease in TCE during the course of the experiments without the production of cis-DCE or vinyl chloride. This also makes it incredibly difficult if not impossible to determine the reaction rates of the experiments involving the PVA/SA beads and SA beads encapsulating KB-1. The controls, the bottles not containing KB-1, had the purpose of observing the effect TCE had the beads not containing KB-1. The data of the bottles not containing KB-1 is shown Figures 4.10-13.

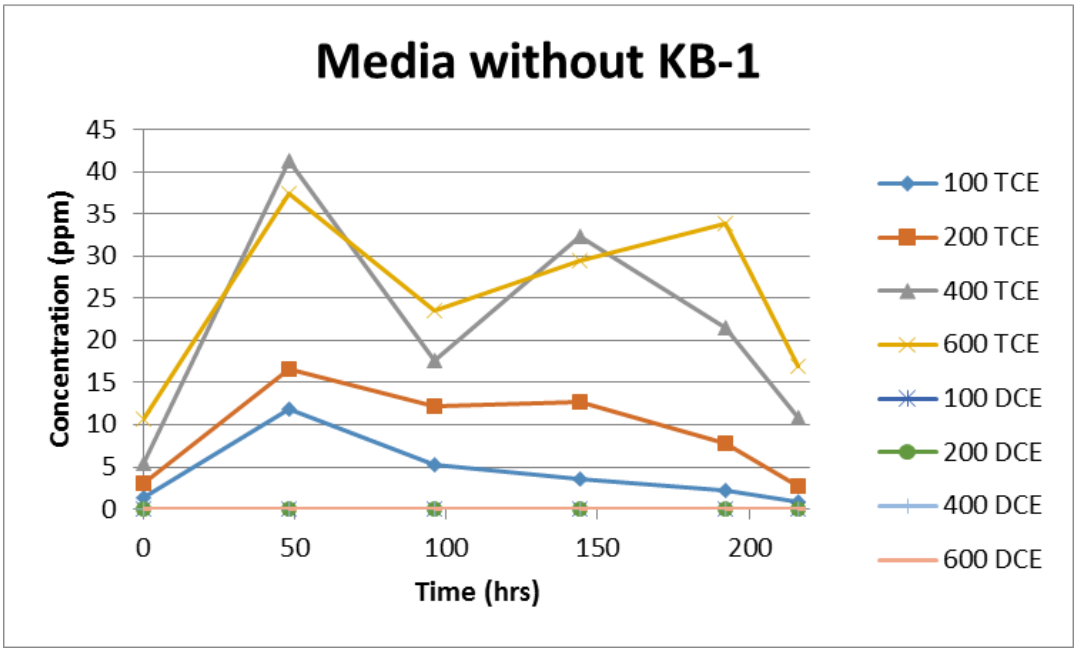

Figure 4.10: Time vs. Concentration of Media without KB-1

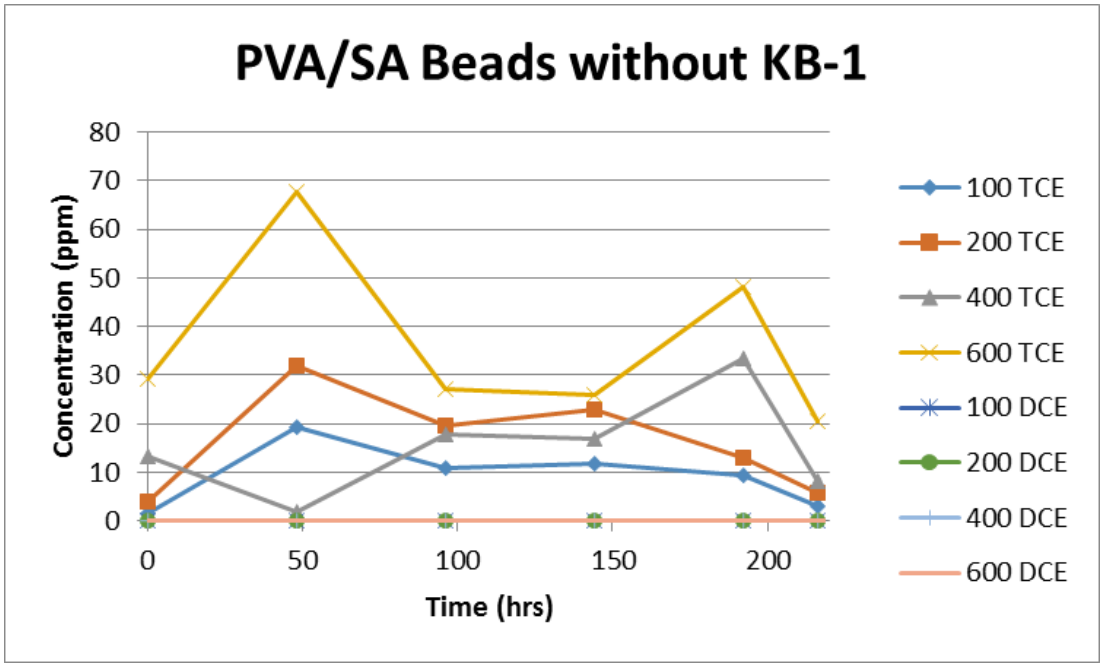

Figure 4.11: Time vs. Concentration of PVA/SA Beads without KB-1

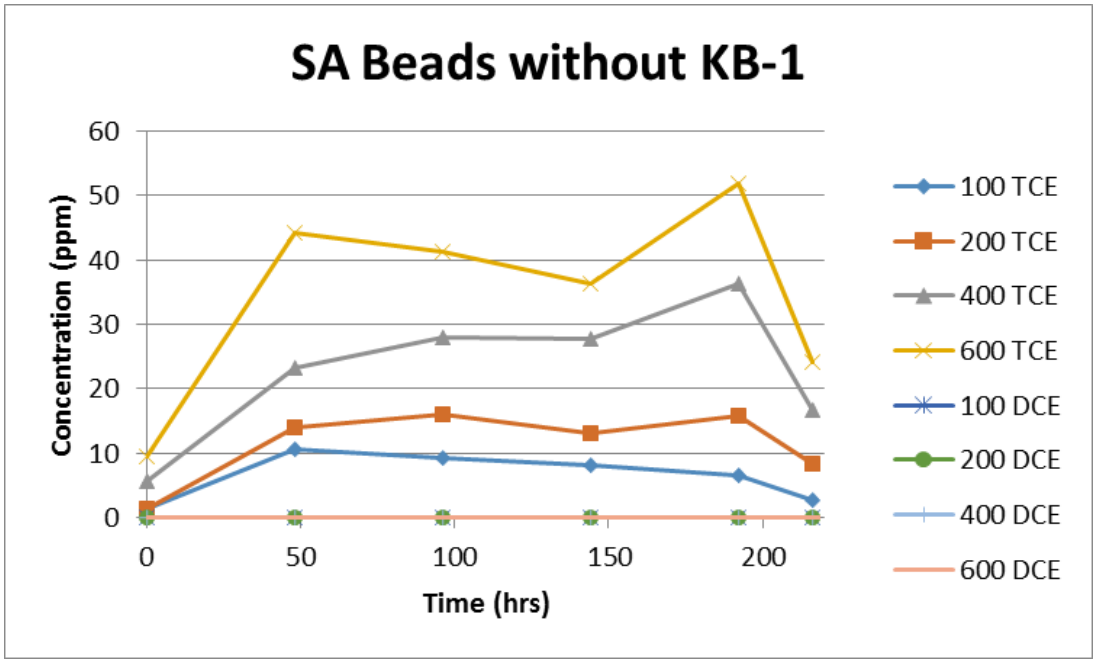

Figure 4.12: Time vs. Concentration of SA Beads without KB-1

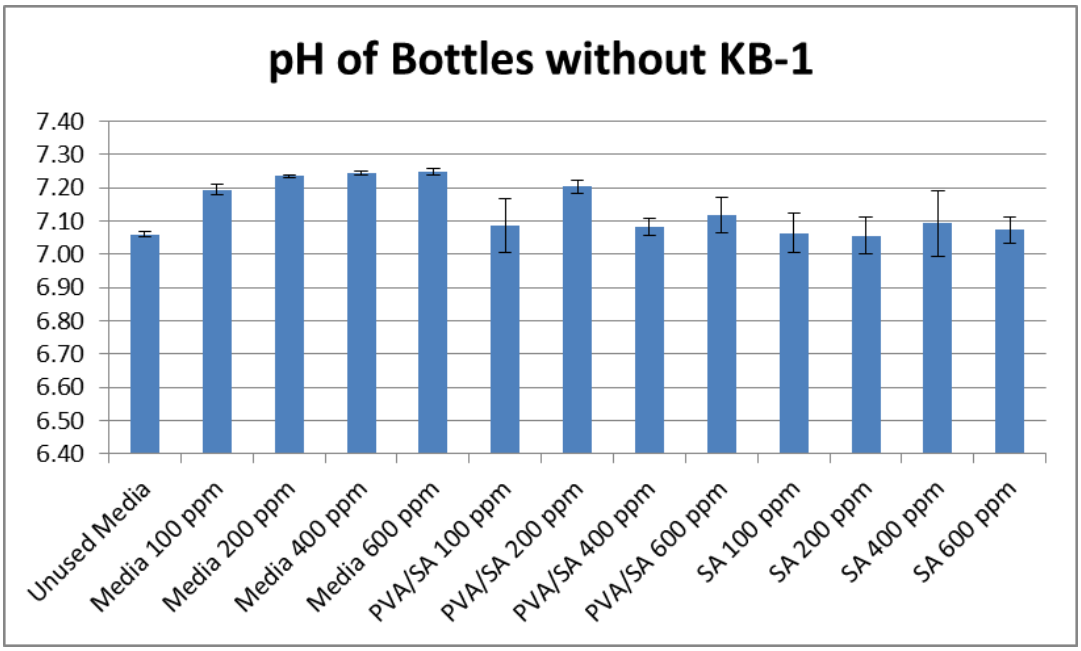

Figure 4.13: pH of Unused Media Compared to Media, PVA/SA Beads, and SA Beads without KB-1

As seen in the extensive data above, there were several inconsistencies in the data, the first being seen in Figures 4.10-12 in the bottle sets not containing KB-1. These sets of bottles were intended to be the controls for the experiments. The concentration in the bottles not containing KB-1, especially the bottles of just media were expected to maintain a constant concentration of TCE and not produce any less chlorinated forms of TCE. These bottles did not produce any cis-DCE or vinyl chloride as seen in Figures 4.10-13, which is desired because it shows the bottles, were not contaminated with KB-1. However, it remains unexplained for why the concentration did not remain constant. A few possible explanations for the concentration not remaining constant were that the TCE was escaping from the bottles, the TCE is drawn to the Teflon septa, or the beads were absorbing the TCE. None of these can be ruled out or determined as the main cause for the decrease in TCE concentration of the control bottles without KB-1. The pH data obtained from the bottles not containing KB-1 indicate that the systems became slightly more neutral, higher in pH, at the end of the experiment. The only explanation for this is that the addition of the TCE to the media forces the pH to rise.

Due to the inconsistencies of the data from the controls, it is impossible to accurately determine the reaction rates of the reduction reactions occurring. There is not enough information of the beads or what is occurring inside the beads to determine the reaction rates or kinetics. There is much future work to be done in the bioremediation experiments in order to accurately determine the reaction rate and kinetics which will be discussed in the following chapter.

CHAPTER 5: CONCLUSIONS AND FUTURE WORK

The experiments over the course of this thesis were extensive and some more successful than others. The quantification of diffusion experiments were highly successful however they were extremely limited and some future work could be done. The bioremediation experiments on the other hand were not very successful and much future work needs to be completed to better understand the reduction reaction kinetics of the bioremediation of TCE.

CONCLUSIONS AND FUTURE WORK FOR QUANTIFICATION OF DIFFUSION

In conclusion, the quantification of diffusion experiments that were completed were accomplished very successfully. The diffusion coefficient for TCE through sodium alginate membrane was determined to be $1.880 \pm 0.6610 \times 10^{-7}$ cm^2 s^{-1} at 20°C. The diffusion coefficient for TCE through PVA/SA membrane was determined to be $1.923 \pm 0.5089 \times 10^{-8}$ cm^2 s^{-1} at 20°C.

However, the diffusion coefficient for TCE through sodium alginate and PVA/SA membranes does need to be further studied. The diffusion needs to be quantified over a greater range of temperatures. The experiments will be run same as those ran in chapter 3, utilizing the same method; however, they will be conducted at various concentrations and various temperatures to understand the temperature dependence on the diffusion coefficient. This is highly important to understand in order to comprehend the mass transfer of the bio-beads at different temperatures. It is highly unlikely that the bio-beads will be implemented in the environment at the temperature of 20°C and therefore, the parameters for developing the bio-beads will need to be modified for an environment for a different temperature. Once the temperature dependence on the diffusion coefficients has been determined for the PVA/SA and sodium alginate polymers, the bio-beads can be theoretically implemented in any environment to remediate TCE.

After the temperature dependence on the diffusion coefficient has been determined, the diffusion coefficient for sodium alginate and PVA/SA with KB-1 should be determined.

This needs to be determined in order to observe if the KB-1 has an effect on the diffusion of TCE. Since the bio-beads contain KB-1, quantifying the diffusion coefficient will provide an even more in depth understanding of the mass transfer occurring in the bio-beads. Completing these experiments at a variety of temperatures will also provide knowledge of the effect of temperature on KB-1 in the bio-beads.

In addition to the further quantification of TCE through sodium alginate and PVA/SA, polymers that were initially removed from the experiments will be revisited. The chitosan mixture will be revaluated to see if it can be modified to withstand the tyndallization process. One method of modification is to add more chitosan polymer powder to the molten polymer solution. This would make the molten polymer solution more viscous thus increasing its probability of surviving the tyndallization process. If the chitosan molten polymer solution can be modified to survive the tyndallization process then the polymer is fit for the bioremediation experiments and the diffusion coefficient for chitosan should be determined at a wide range of temperature in order to understand the mass transfer of TCE through the polymer. The pectin molten polymer solution could also be reevaluated for the experiments the same way the chitosan molten polymer solution should be revaluated. The pectin could be tested as a copolymer solution similar to that of the PVA/SA copolymer solution. This could be a highly effective solution for the pectin polymer because it is so unstable on its own. All of these suggestions for future work could help with the development of the bio-beads and increase the success of the bioremediation of TCE by encapsulating KB-1 in biodegradable polymers.

CONCLUSIONS AND FUTURE WORK FOR BIOREMEDIATION OF TCE

Due to the lack of success of the bioremediation experiments conducted in this thesis, there is little to conclude and much future work needs to be done. It is clear that bioremediation does occur with KB-1 in the media solution. However, it is unclear if the remediation reduction reactions are completed by KB-1. It is unclear because cis-DCE is not formed in the stoichiometric ratio that it should have been or if vinyl chloride was produced or not. To better understand the bioremediation reactions, the final bioremediation

experiment should be re-run for a longer period of time. Had the experiment been run longer, it is possible that the reduction products of TCE could have been observed and the effect of the reduction on the pH of the system could have also been further observed.

If running the experiment for a longer period of time does not show more reduction from the current results, then the set-up of the experiments needs to be modified. One possible modification could be sacrificial sampling. The experiments would most likely be run in “Balch-type” tubes rather than in serum bottles. The ratio would be modified so that it would match the current ratio. This would reduce the headspace issues and the volatilization issues with the current experimental set-up. If this modification does not rectify the lack of the bioremediation occurring, then the ratio of KB-1 to molten polymer solution will be studied to determine if the ratio of the bio-beads needs to be modified. The modification of the ratio will likely improve the bioremediation of TCE because an optimum will be found thus making the reduction reaction more efficient. Once the bio-beads have been optimized then the reduction reaction kinetics of the bioremediation can be determined.

Then using the reaction kinetics of the bioremediation the bio-beads can be implemented in a more realistic real world experimental set-up. This set-up could involve many modifications. One modification could be the changing the solution the bio-beads are placed in. Instead of using the media solution SiREM provided for the experiments in this thesis, a simulated groundwater solution would be used instead. A simulated groundwater solution would make the experimental set-up closer to that of a real world environment. Another modification could be the implementation of a permeable reactive barrier of the bio-beads. The TCE contaminated simulated groundwater solution is then pumped through the permeable reactive barrier and the bioremediation is then analyzed by sampling the water after the permeable reactive barrier. The pH will also be analyzed to determine if acidification does indeed occur to the degree of harming the aquifer. The experimental apparatus will be similar that of the one shown in Figure 1.6. This experimental set-up is the closest to a real world test for the bioremediation of TCE by use of bio-beads. The real world test will determine whether or not the bio-beads will be an effective technology for environmental clean-up of TCE in contaminated groundwater aquifers.

This future work could help the research and development of more effective bio-bead technology. This would then lead to an economically feasible and environmentally friendly solution to the extensive TCE contamination problem across the United States.

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APPENDIX A: ELECTROCHEMICAL DIFFUSION DATA

COPPER ELECTRODE DATA

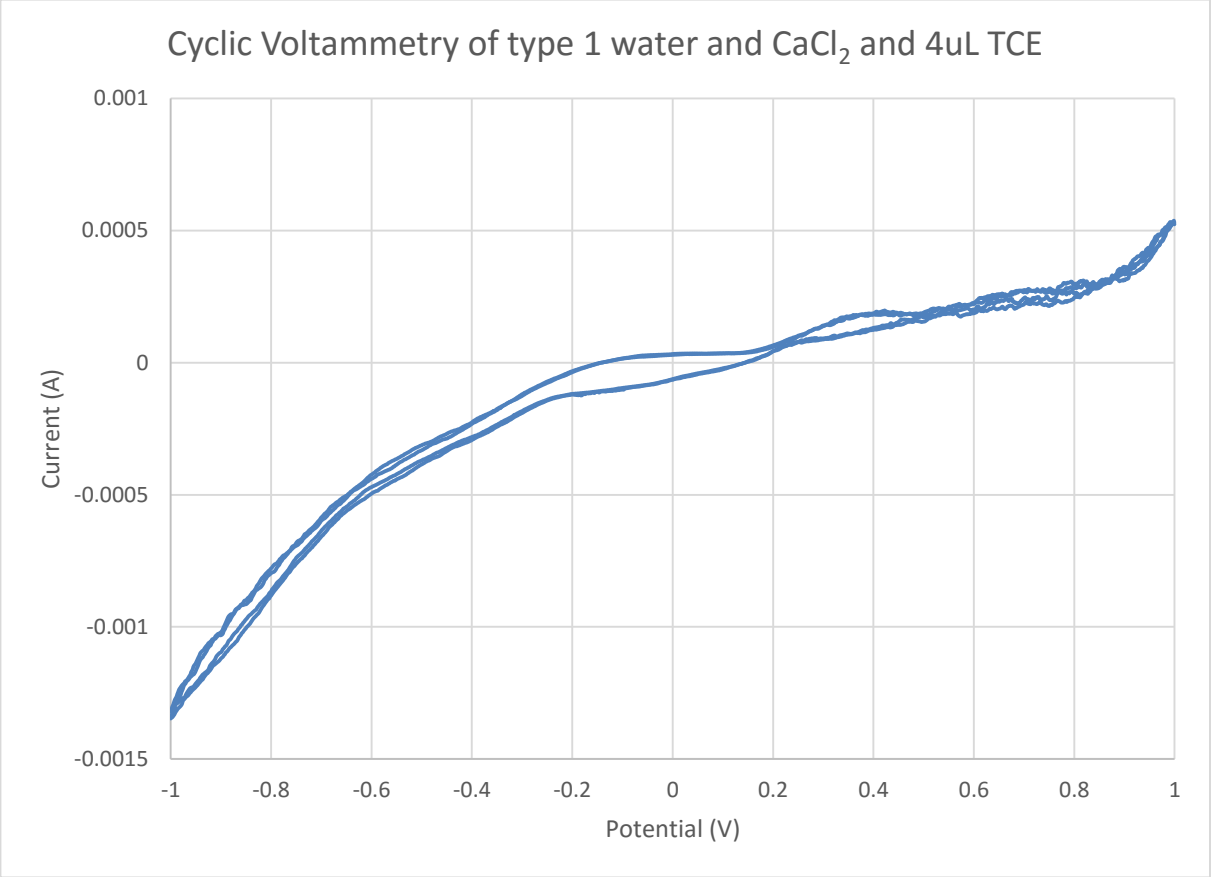

Figure A.1: First Cyclic Voltammetry of type 1 water and $CaCl_2$ and 4uL of TCE in the diffusion cell

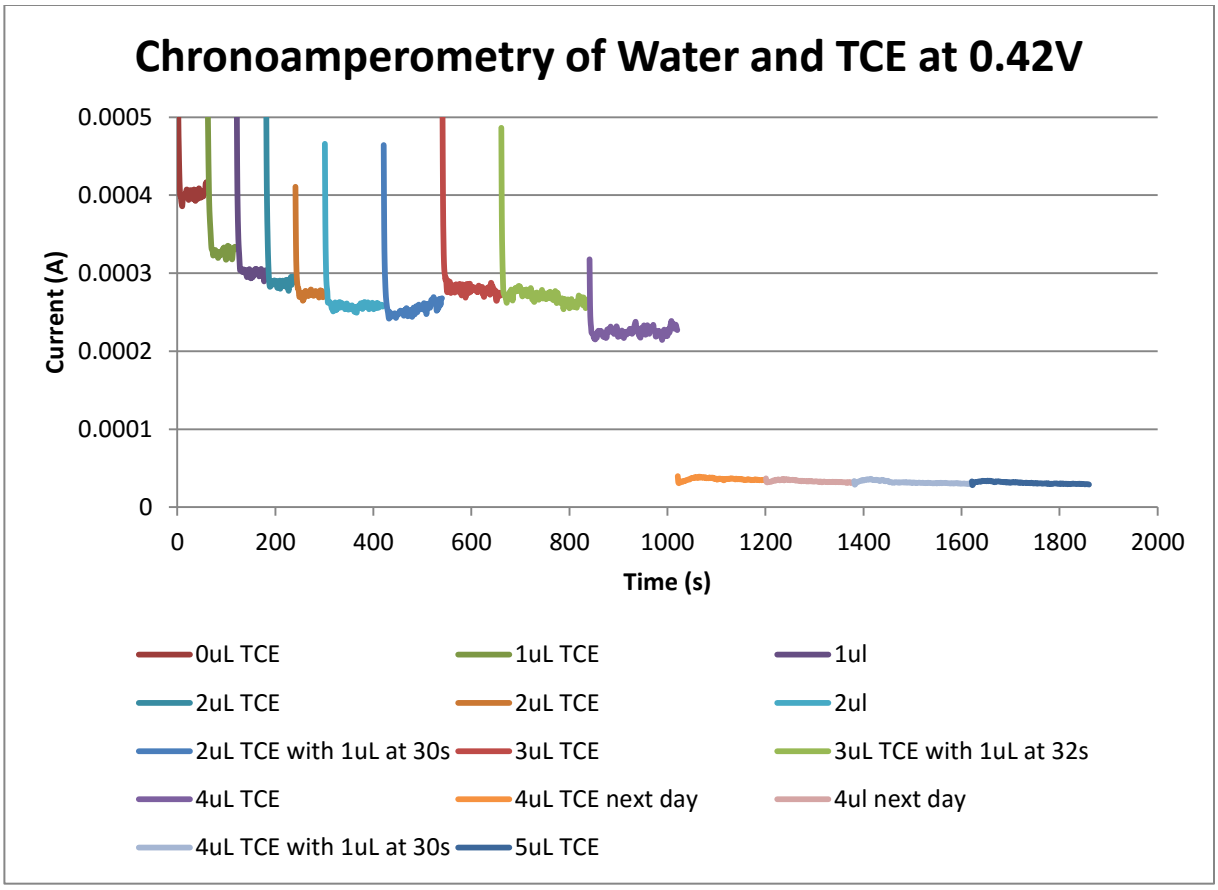

Figure A.2: First Chronoamperometry of type 1 water and $CaCl_2$ at 0.42V

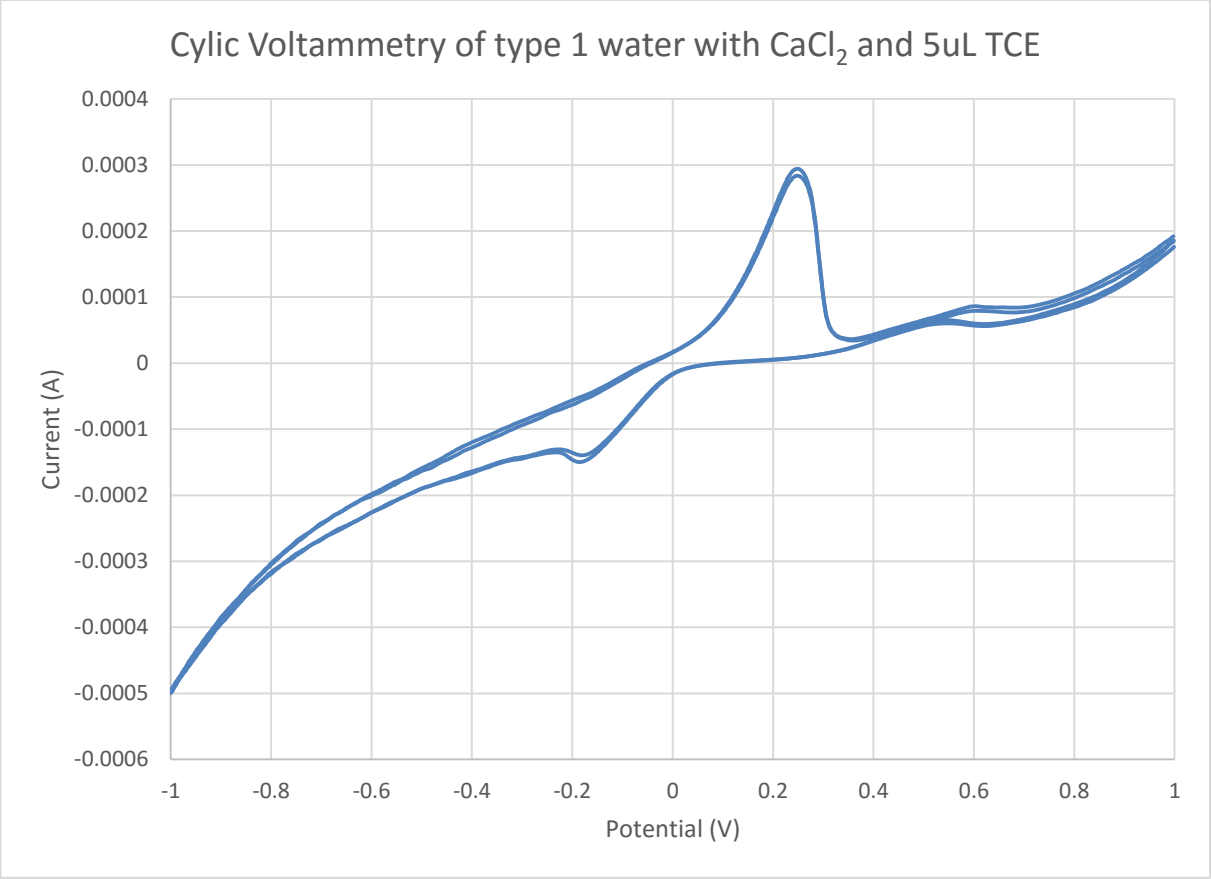

Figure A.3: Second Cyclic Voltammetry of type 1 water with $CaCl_2$ and 5uL TCE

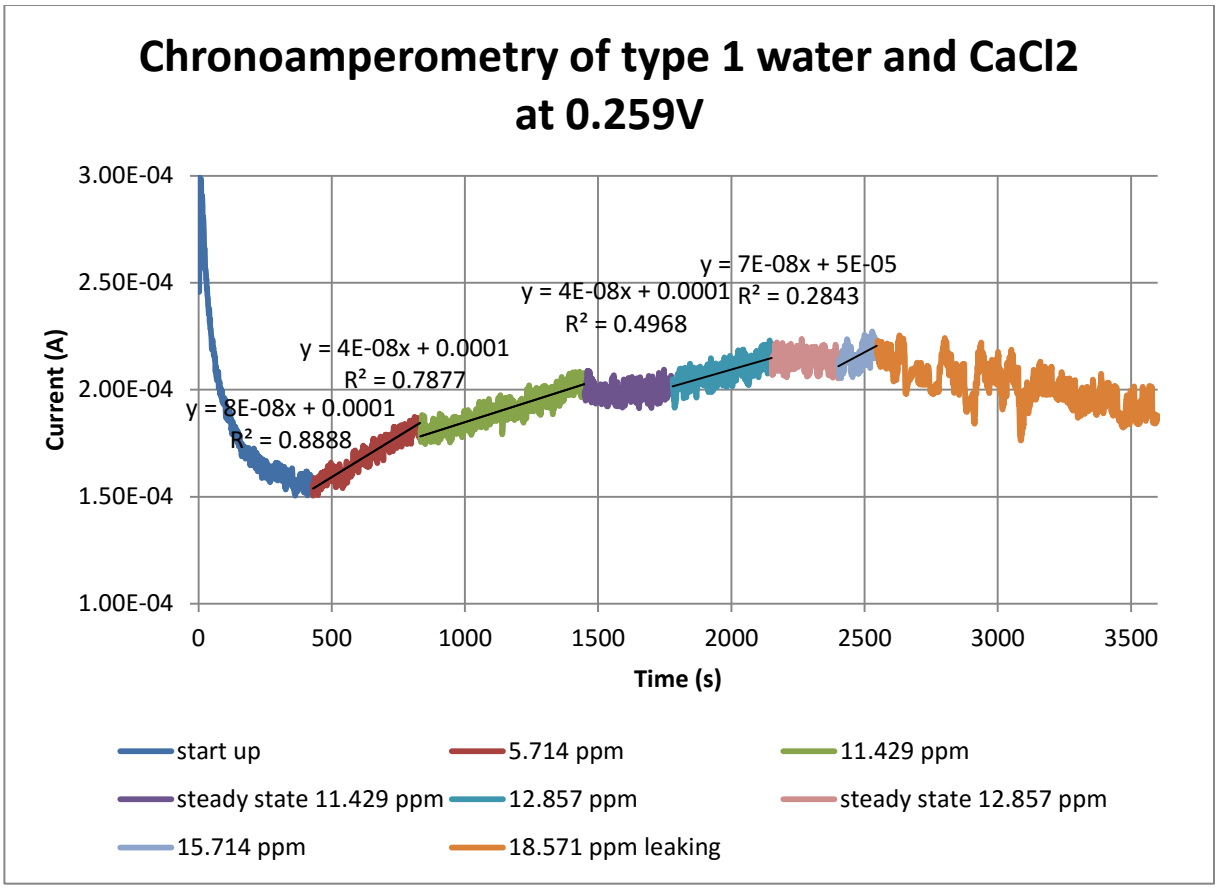

Figure A.4: Second Chronoamperometry of type 1 water and $CaCl_2$ at 0.259V

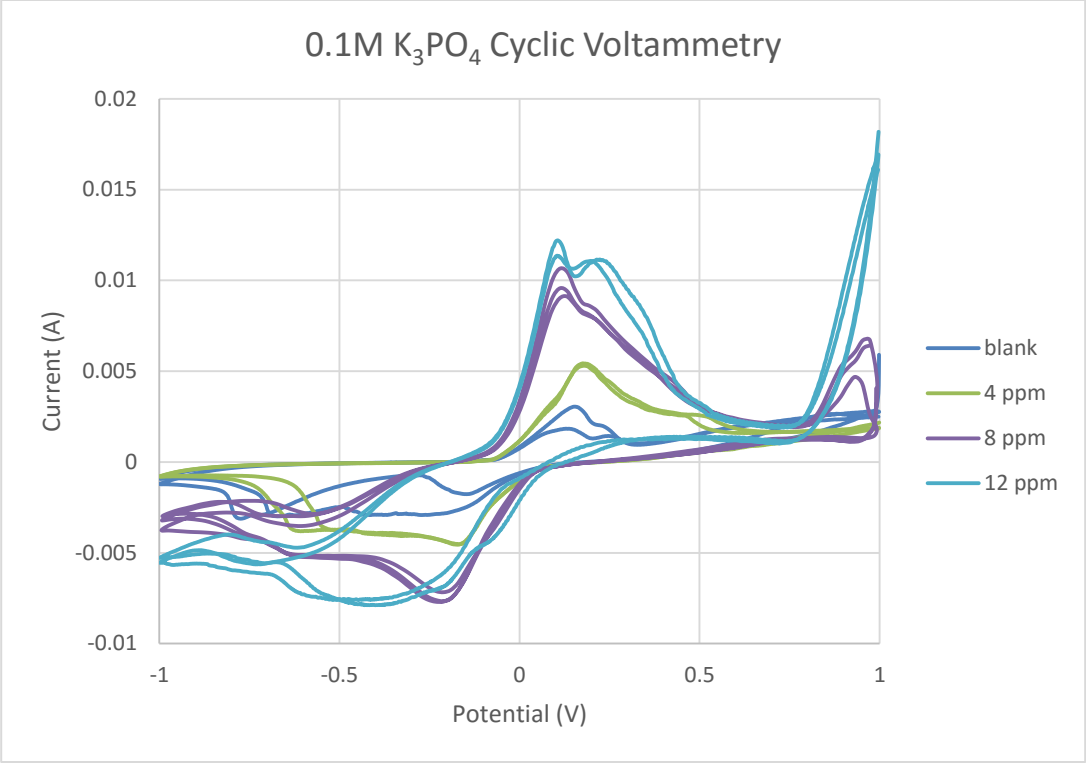

Figure A.5: Cyclic Voltammetry using 0.1M K_3PO_4

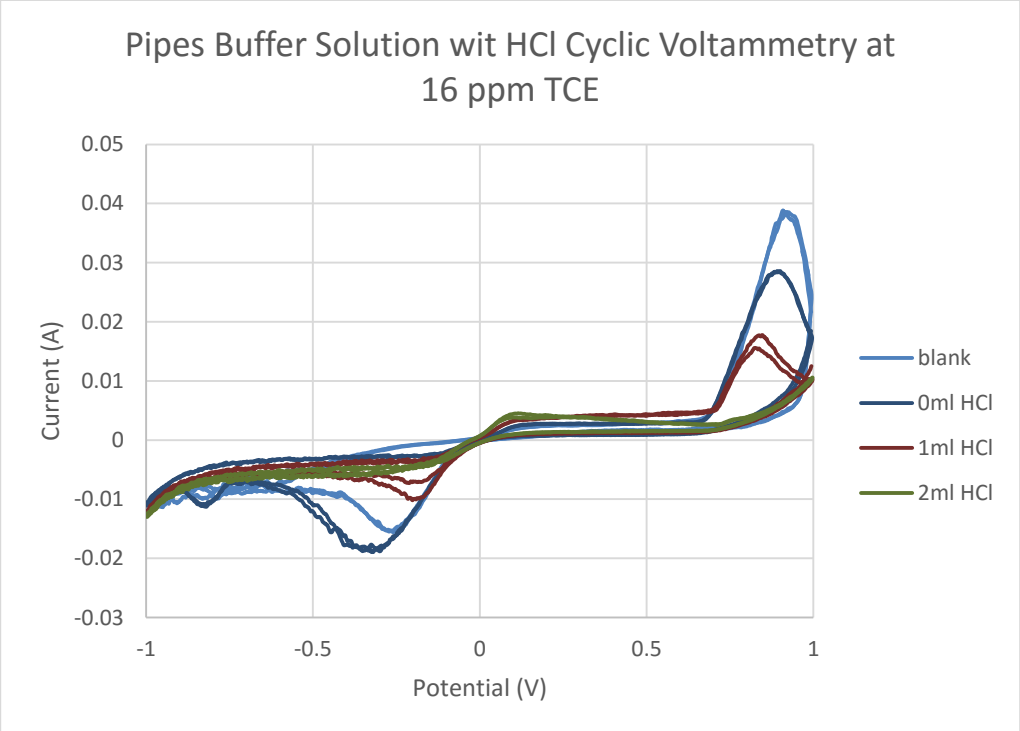

Figure A.6: Cyclic Voltammetry using Pipes Buffer Solution

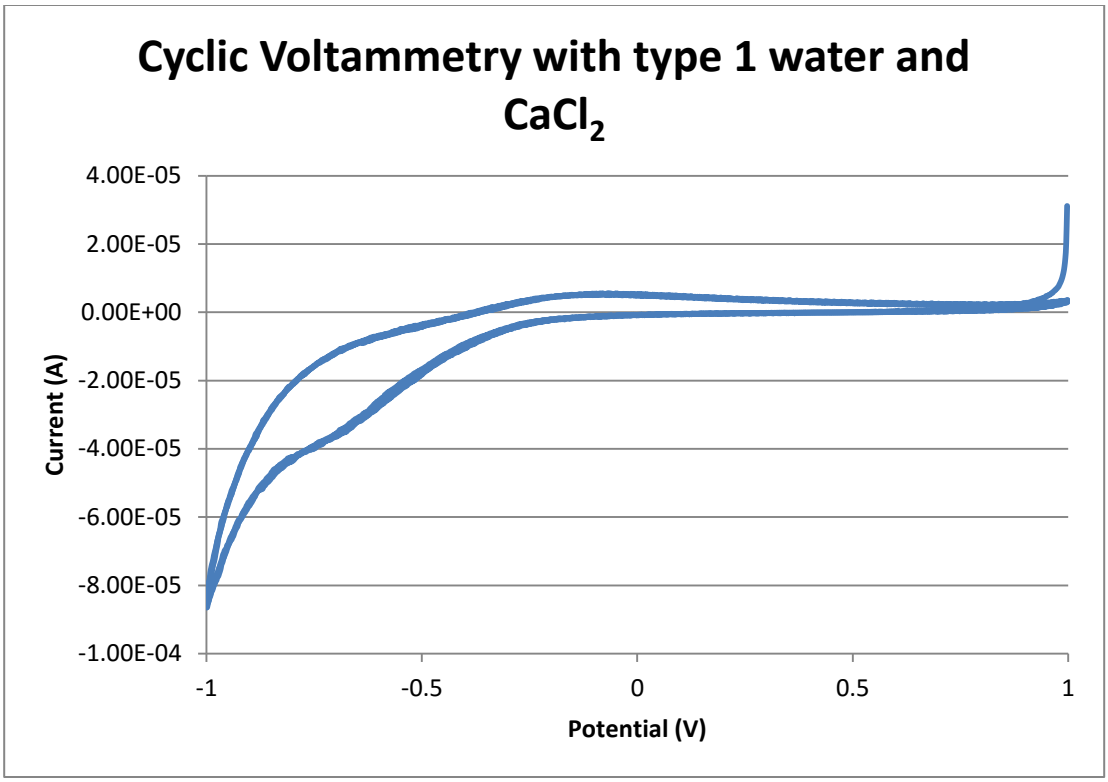

Figure A.7: Cyclic Voltammetry with type 1 water with $CaCl_2$ using the Titanium Electrode

APPENDIX B: GCMS CONCENTRATION DIFFUSION DATA

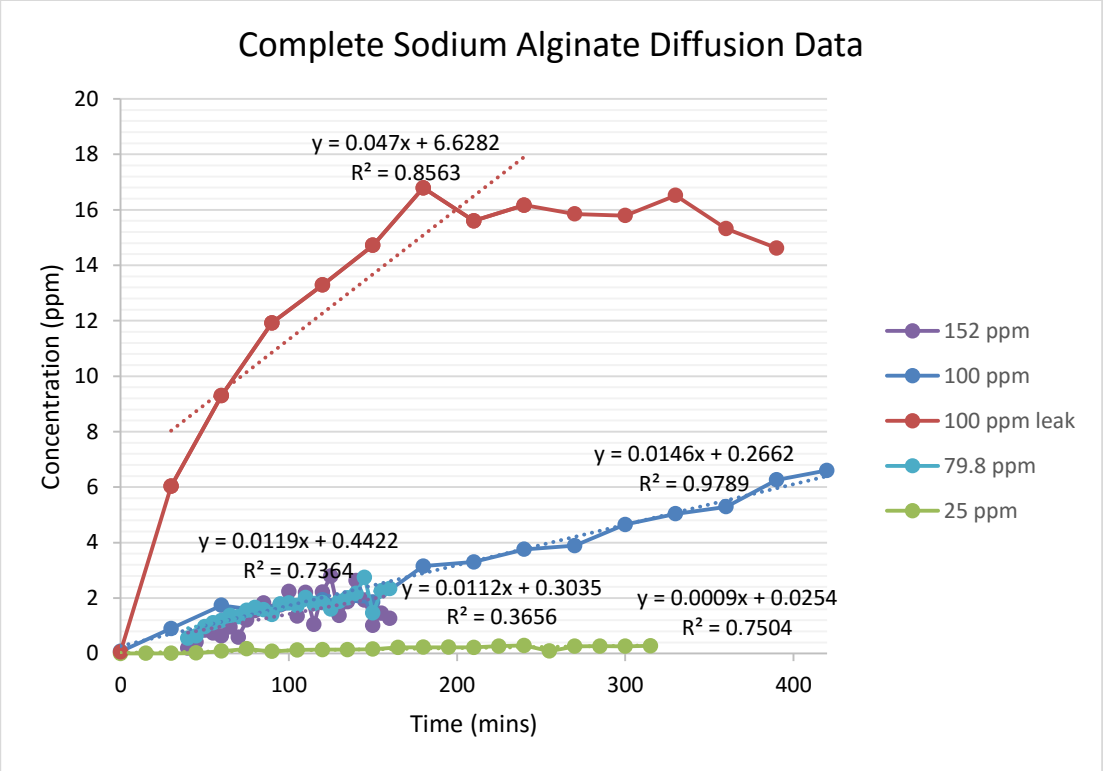

Figure B.1: Complete Sodium Alginate Diffusion Data

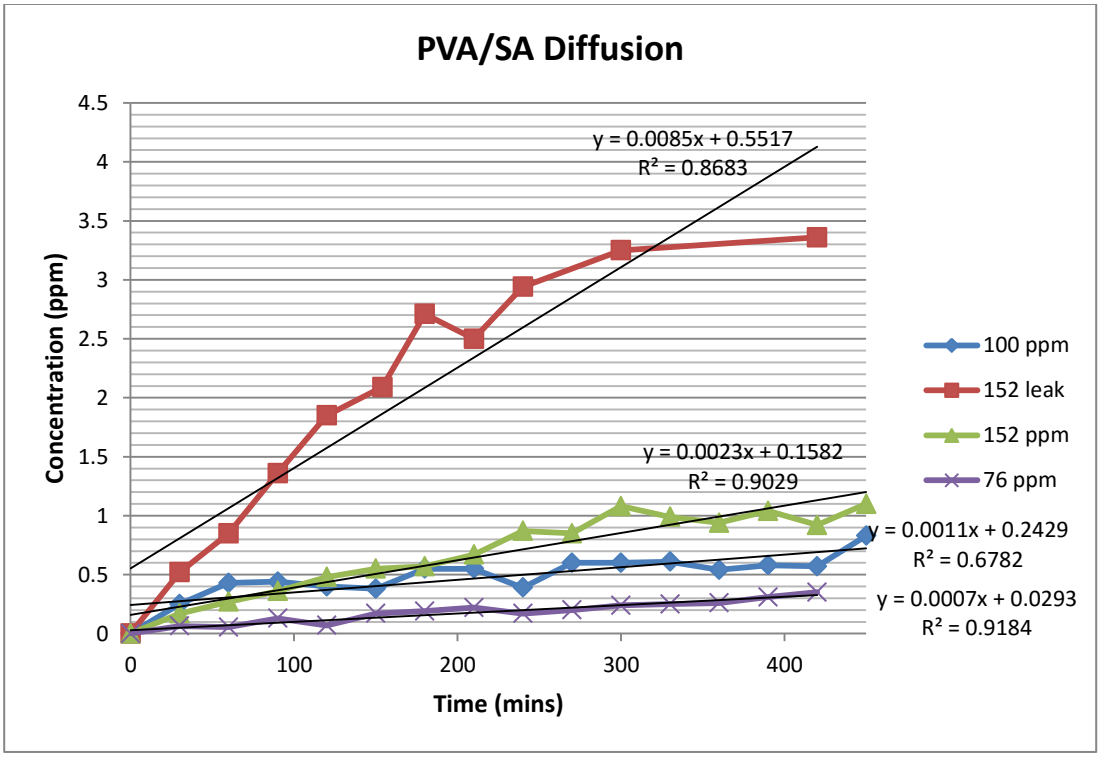

Figure B.2: Complete PVA/SA Diffusion Data

Sample Diffusion Calculation

$ppm := \frac{mg}{L}$

$Dab\_water := 8.16 \cdot 10^{-6} \frac{cm^2}{s}$

$error100 := 0.00125 \frac{ppm}{min}$

$m100 := 0.0146 \frac{ppm}{min}$

$Cao100 := 100 ppm$

$t100 := 0.435in - 0.183in = 6.401 \times 10^{-3} m$

$m152 := 0.0112 \frac{ppm}{min}$

$error152 := 0.0064 \frac{ppm}{min}$

$Cao152 := 152 ppm$

$t152 := 0.435in - 0.160in = 6.985 \times 10^{-3} m$

$error79 := 0.00275 \frac{ppm}{min}$

$m79 := 0.0119 \frac{ppm}{min}$

$Cao79 := 79.8 ppm$

$t79 := 0.435in - 0.160in = 6.985 \times 10^{-3} m$

$r := \frac{1.206in}{2} = 0.015 m$

$Area := 3.14159 r^2 = 7.37 \times 10^{-4} m^2$

$Kp := 1 cm^{-3}$

$De\_100plus := \frac{[(m100 + error100) \cdot t100]}{(Area \cdot Cao100)} = 2.294 \times 10^{-7} \cdot \frac{1}{cm \cdot s}$

$De\_100minus := \frac{[(m100 - error100) \cdot t100]}{(Area \cdot Cao100)} = 1.932 \times 10^{-7} \cdot \frac{1}{cm \cdot s}$

$De\_100 := \frac{[(m100) \cdot t100]}{(Area \cdot Cao100)} = 2.113 \times 10^{-7} \cdot \frac{1}{cm \cdot s}$

$$Dab\_100plus := \frac{De\_100plus}{Kp} = 2.294\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$Dab\_100 := \frac{De\_100}{Kp} = 2.113\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$Dab\_100minus := \frac{De\_100minus}{Kp} = 1.932\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$\frac{[(Dab\_100plus - Dab\_100) + (Dab\_100 - Dab\_100minus)]}{2} = 1.809\times 10^{-8}\cdot\frac{cm^2}{s}$$

$$De\_152plus := \frac{[(m152 + error152)\cdot t152]}{(Area\cdot Cao152)} = 1.829\times 10^{-7}\cdot\frac{1}{cm\cdot s}$$

$$De\_152minus := \frac{[(m152 - error152)\cdot t152]}{(Area\cdot Cao152)} = 4.988\times 10^{-8}\cdot\frac{1}{cm\cdot s}$$

$$De\_152 := \frac{[(m152)\cdot t152]}{(Area\cdot Cao152)} = 1.164\times 10^{-7}\cdot\frac{1}{cm\cdot s}$$

$$Dab\_152plus := \frac{De\_152plus}{Kp} = 1.829\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$Dab\_152 := \frac{De\_152}{Kp} = 1.164\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$Dab\_152minus := \frac{De\_152minus}{Kp} = 4.988\times 10^{-8}\cdot\frac{cm^2}{s}$$

$$\frac{[(Dab\_152plus - Dab\_152) + (Dab\_152 - Dab\_152minus)]}{2} = 6.651\times 10^{-8}\cdot\frac{cm^2}{s}$$

$$De\_79plus := \frac{[(m79 + error79)\cdot t79]}{(Area\cdot Cao79)} = 2.9\times 10^{-7}\cdot\frac{1}{cm\cdot s}$$

$$De\_79minus := \frac{[(m79 - error79)\cdot t79]}{(Area\cdot Cao79)} = 1.811\times 10^{-7}\cdot\frac{1}{cm\cdot s}$$

$$De\_79 := \frac{[(m79)\cdot t79]}{(Area\cdot Cao79)} = 2.356\times 10^{-7}\cdot\frac{1}{cm\cdot s}$$

$$Dab\_79plus := \frac{De\_79plus}{Kp} = 2.9\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$Dab\_79 := \frac{De\_79}{Kp} = 2.356\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$Dab\_79minus := \frac{De\_79minus}{Kp} = 1.811\times 10^{-7}\cdot\frac{cm^2}{s}$$

$$\frac{[(Dab\_79plus - Dab\_79) + (Dab\_79 - Dab\_79minus)]}{2} = 5.444\times 10^{-8}\cdot\frac{cm^2}{s}$$

APPENDIX C: PRELIMINARY BIOREMEDIATION EXPERIMENT DATA

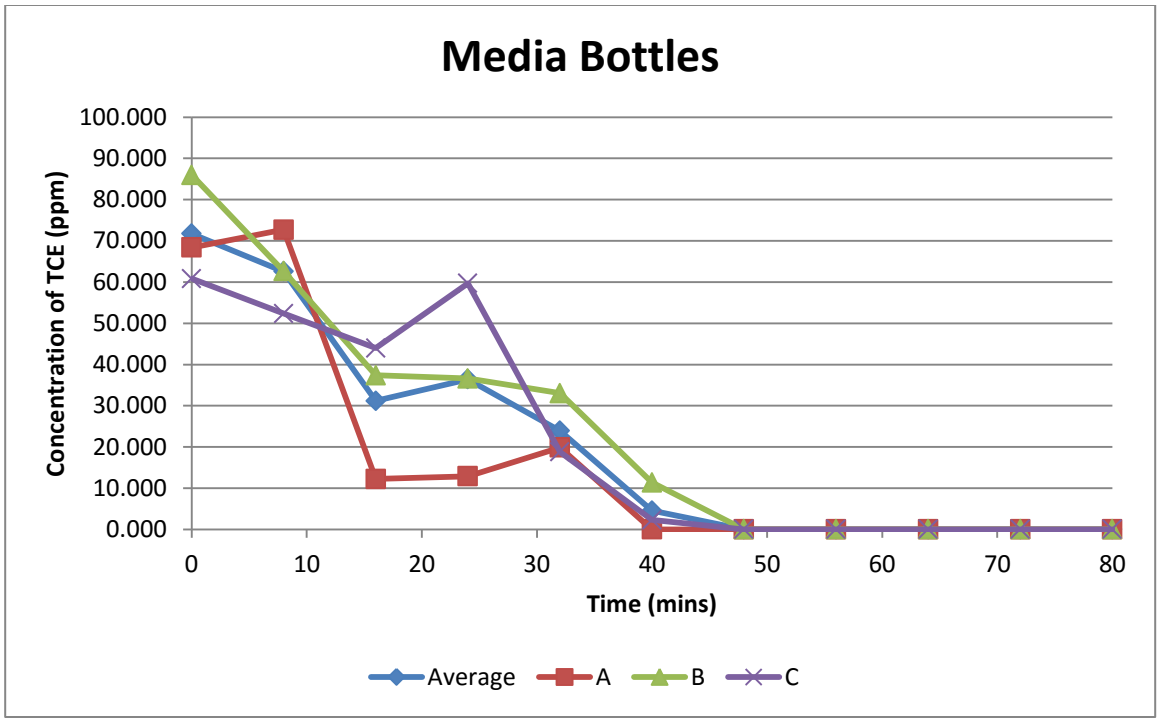

Figure C.1: Time vs. TCE Concentration Data of Just Media Bottles

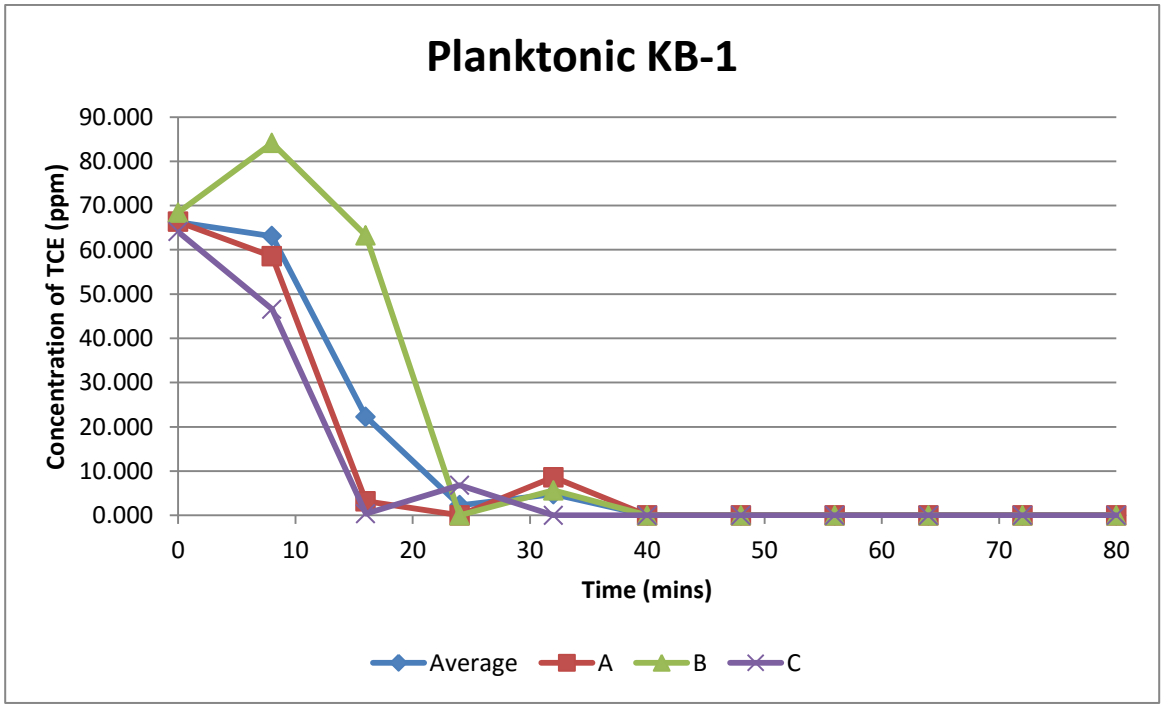

Figure C.2: Time vs. TCE Concentration of Planktonic KB-1 Bottles

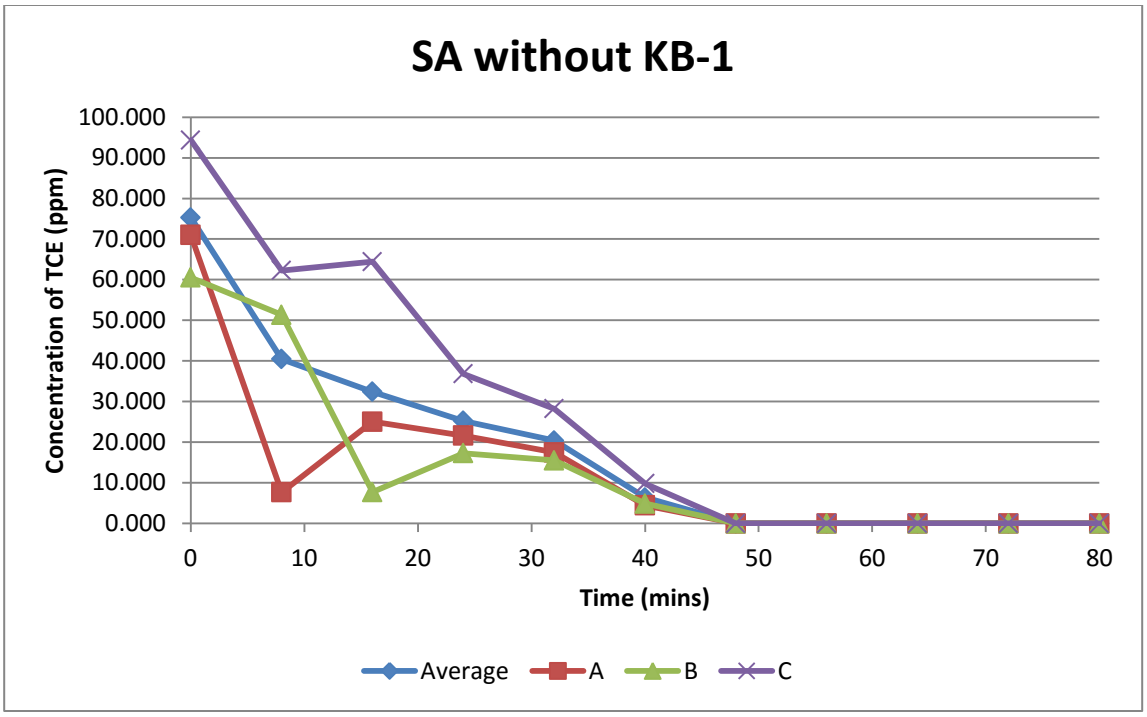

Figure C.3: Time vs. TCE Concentration of SA beads without KB-1 Bottles

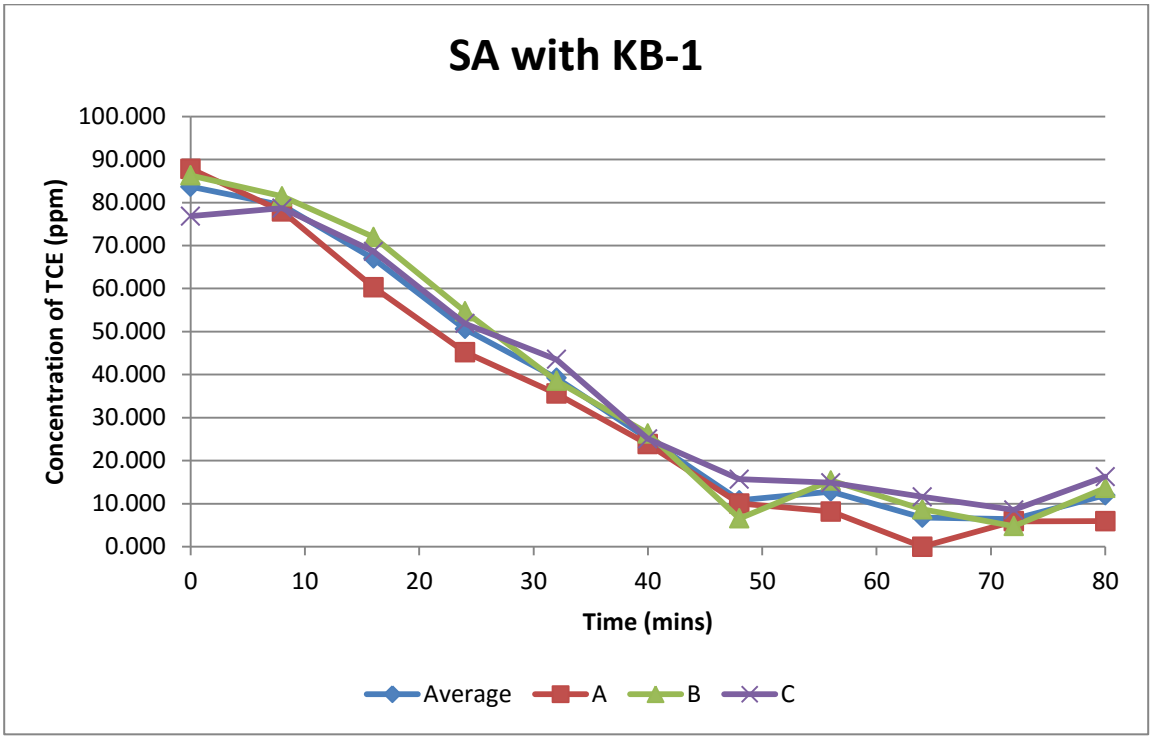

Figure C.4: Time vs. TCE Concentration of SA beads with KB-1

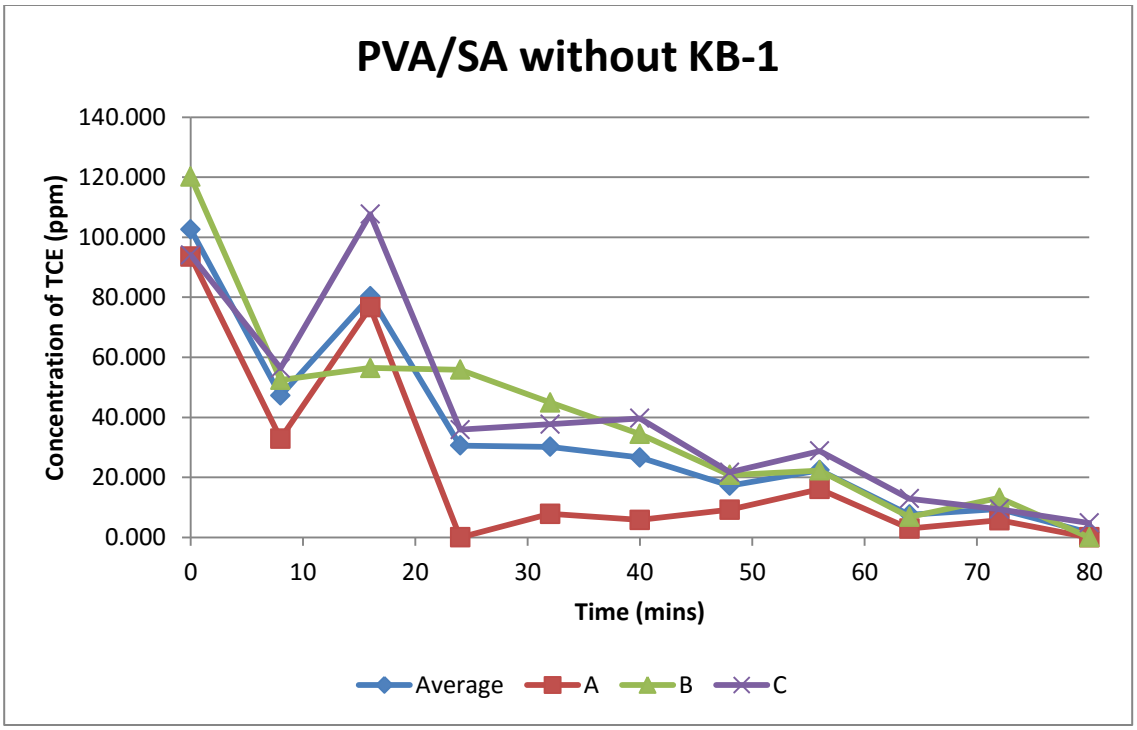

Figure C.5: Time vs. TCE Concentration of PVA/SA Beads without KB-1

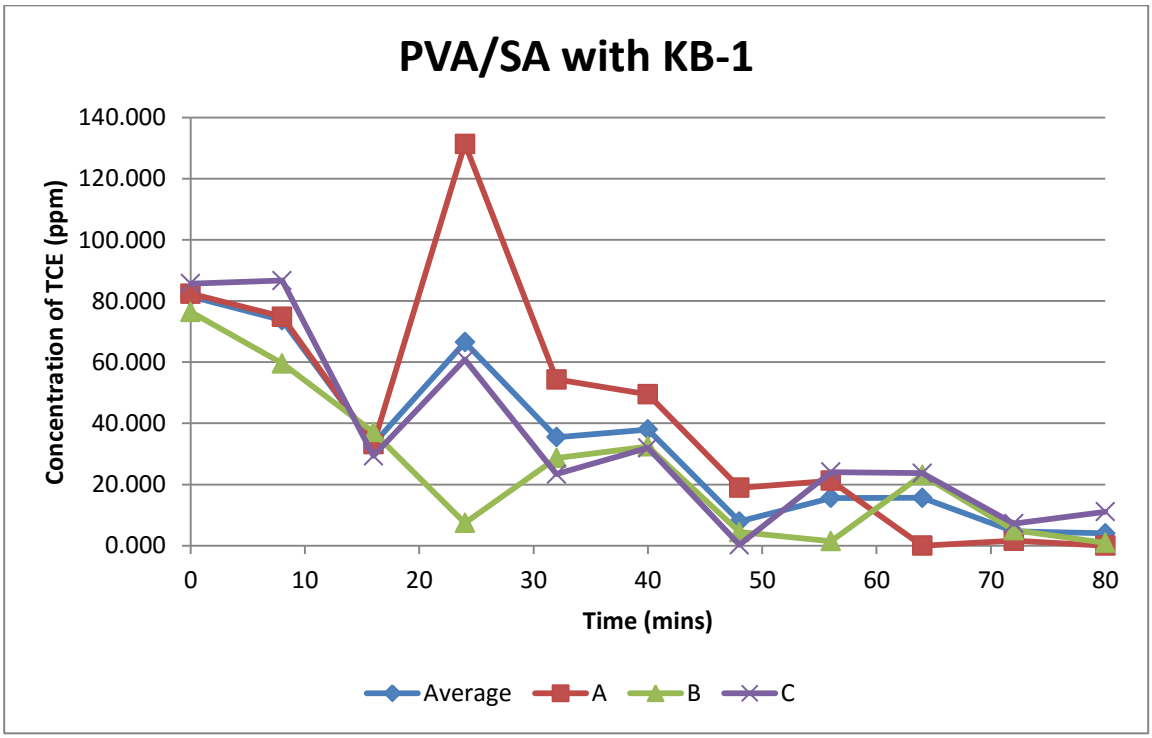

Figure C.6: Time vs. Concentration of PVA/SA Beads with KB-1

sample	pH
Media A	7.70
Media B	7.81
Media C	8.06
Bugs A	8.08
Bugs B	7.93
Bugs C	7.60
SA A	7.47
SA B	7.50
SA C	7.54
SA bugs A	7.39
SA bugs B	7.25
SA bugs C	7.24
PVA A	7.48
PVA B	7.47
PVA C	7.42
PVA bugs A	7.62
PVA bugs B	7.54
PVA bugs C	7.63
Unused Media	7.96

Table C.1: pH measurements obtained at the end of the experiment

APPENDIX D: FILTERED MEDIA BIOREMEDIATION EXPERIMENT DATA

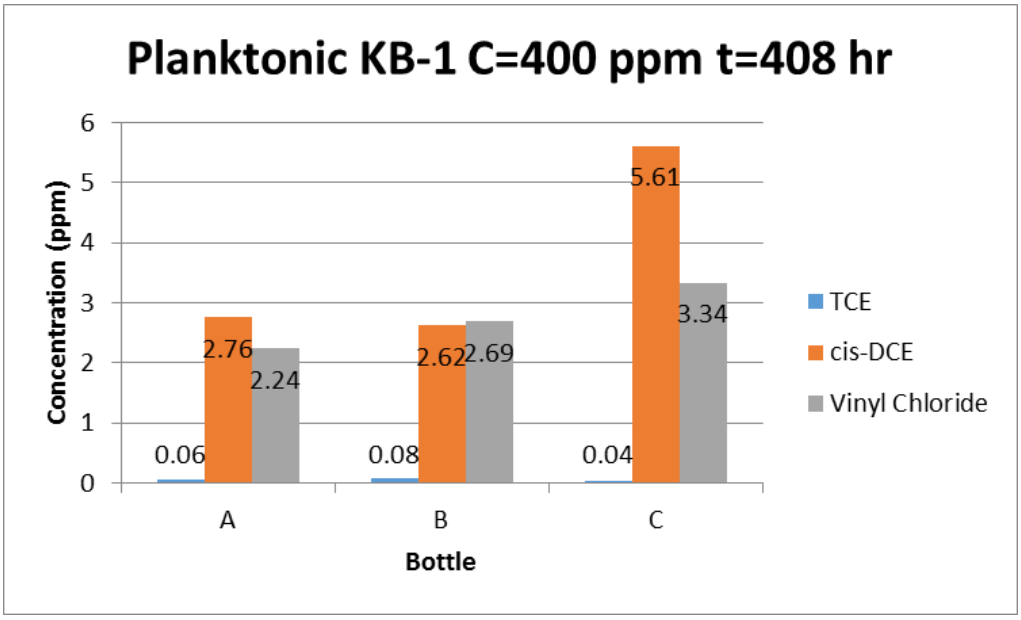

Figure D.1: Planktonic KB-1 starting concentration of 400 ppm TCE concentrations after 408 hours

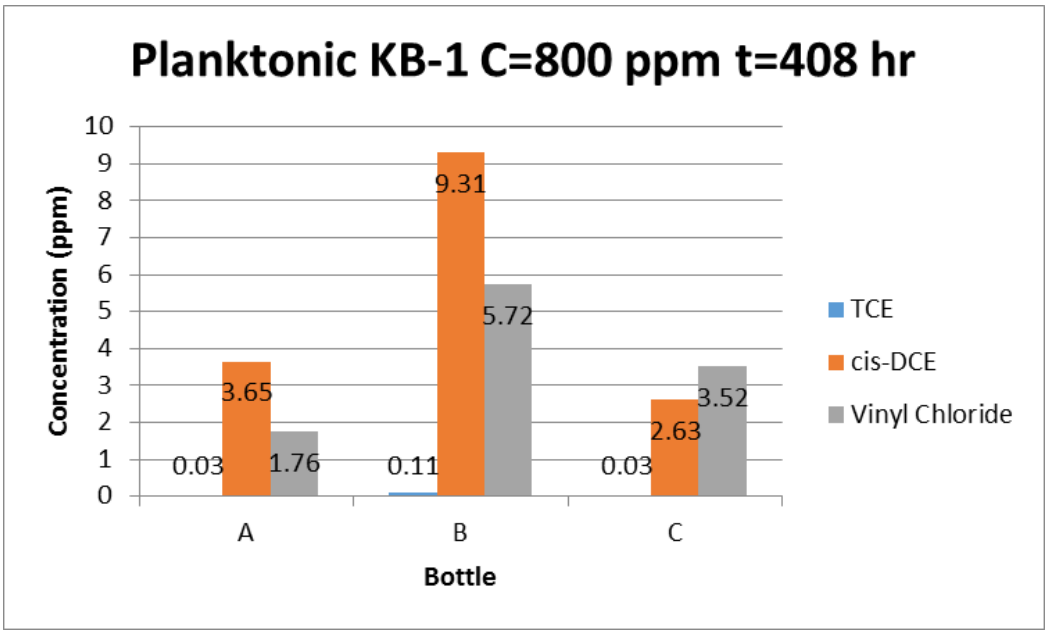

Figure D.2: Planktonic KB-1 starting concentration of 800 ppm TCE concentrations after 408 hours

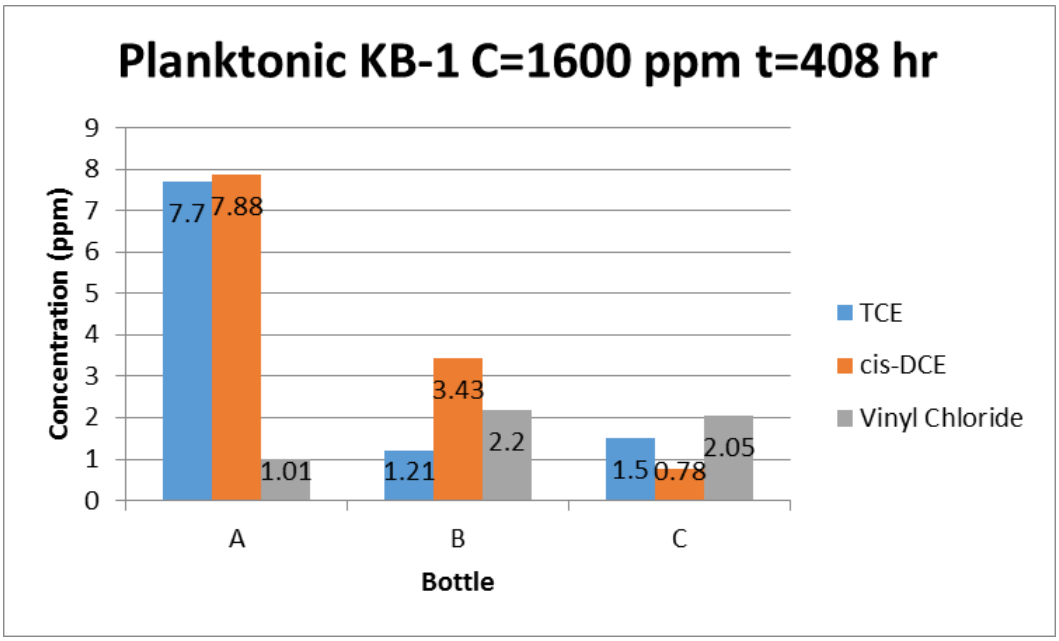

Figure D.3: Planktonic KB-1 starting concentration of 1600 ppm, concentrations after 408 hours

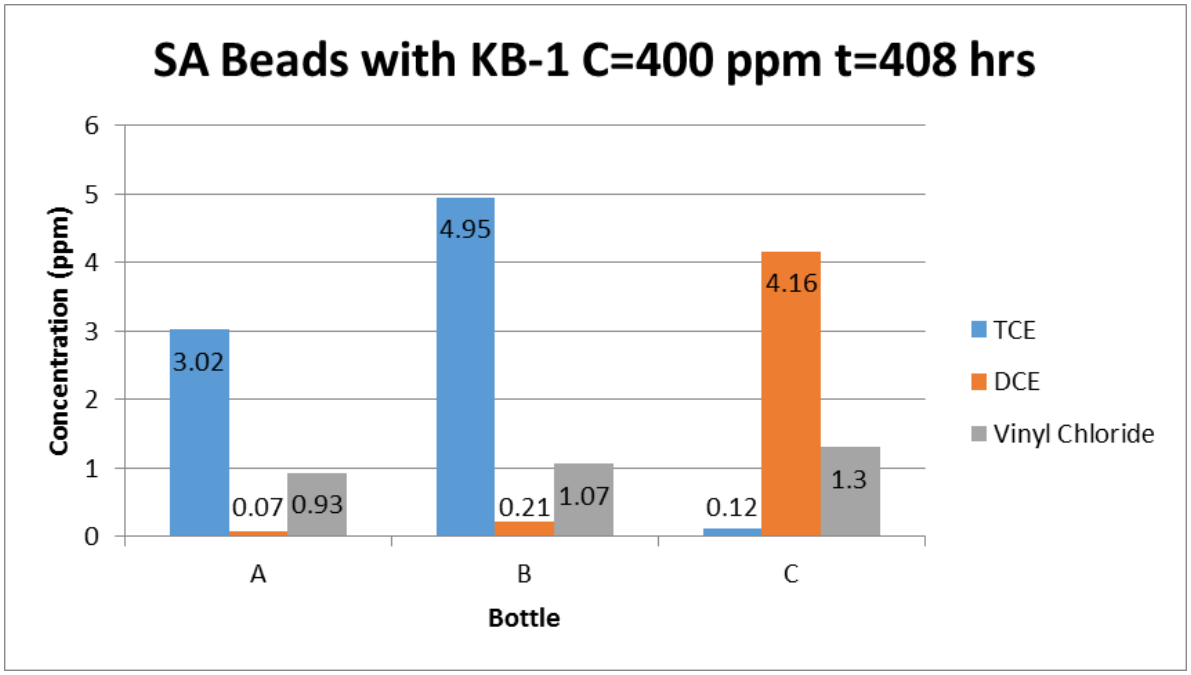

Figure D.4: SA Beads with KB-1 starting concentration of 400 ppm, concentrations after 408 hours

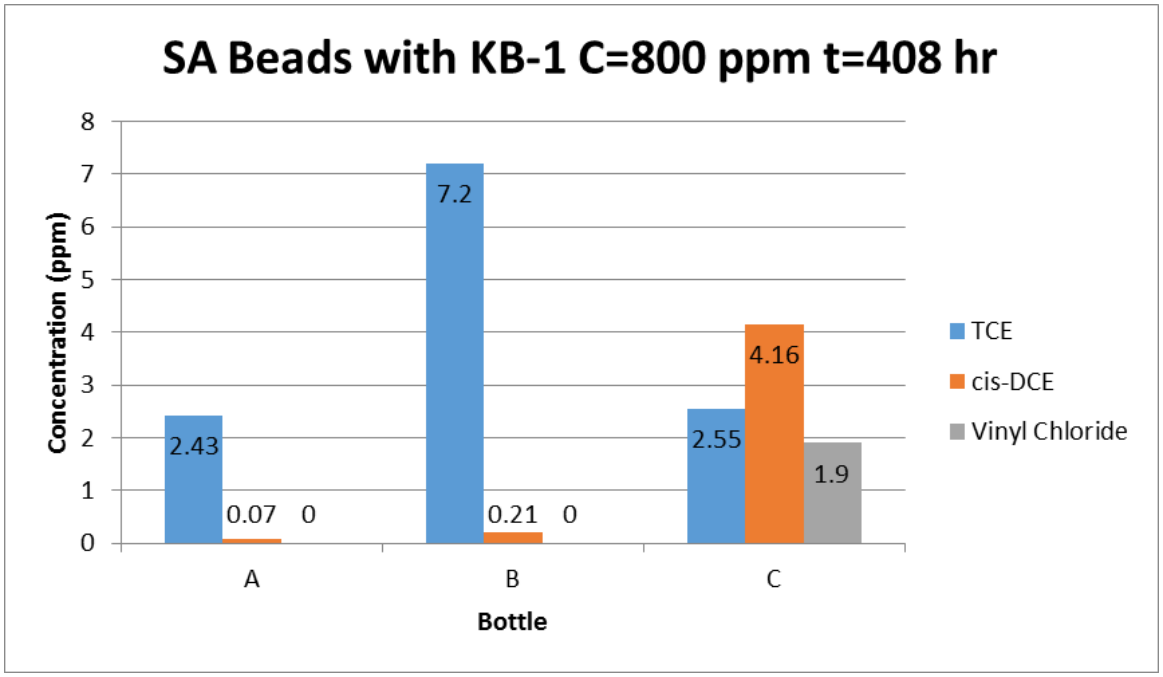

Figure D.5: SA Beads with KB-1 starting concentration of 800 ppm, concentrations after 408 hours

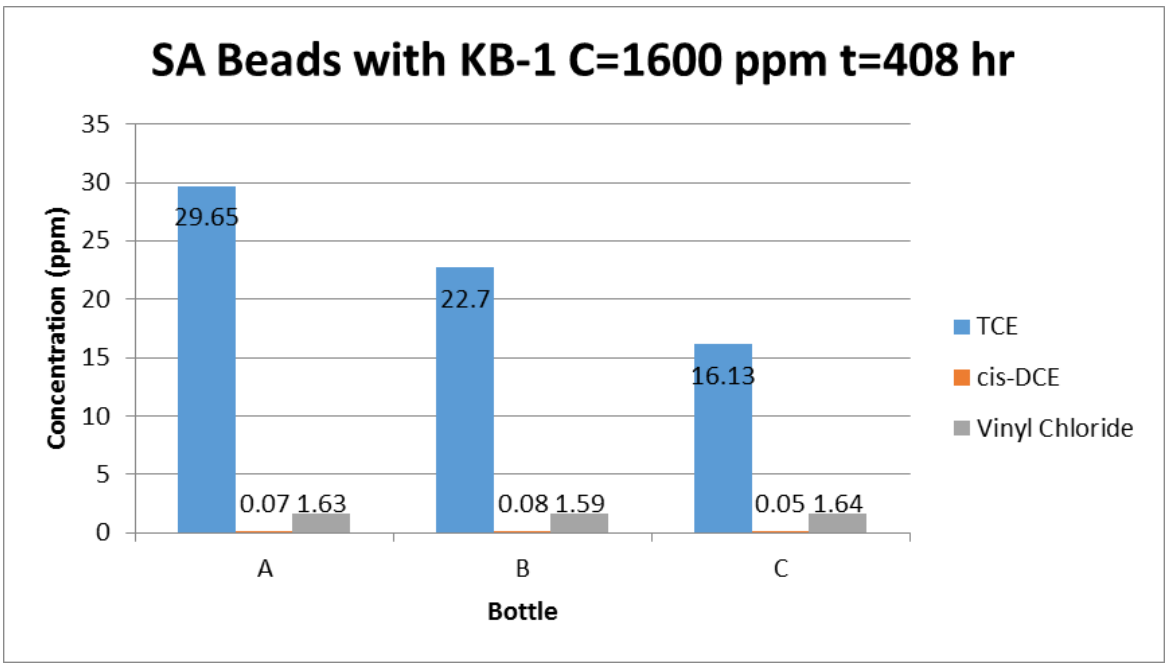

Figure D.6: SA Beads with KB-1 starting concentration of 800 ppm, concentrations after 408 hours

sample	pH
400 Bugs A	7.88
400 Bugs B	7.71
400 Bugs C	7.70
400 Alginate A	7.40
400 Alginate B	7.51
400 Alginate C	7.52
800 Bugs A	7.82
800 Bugs B	7.70
800 Bugs C	7.79
800 Alginate A	7.58
800 Alginate B	7.42
800 Alginate C	7.63
1600 Bugs A	7.82
1600 Bugs B	7.79
1600 Bugs C	7.80
1600 Alginate A	7.53
1600 Alginate B	7.52
1600 Alginate C	7.45

Table D.1: pH measurements obtained after the experiment

APPENDIX E: EXTENDED BIOREMEDIATION EXPERIMENT DATA

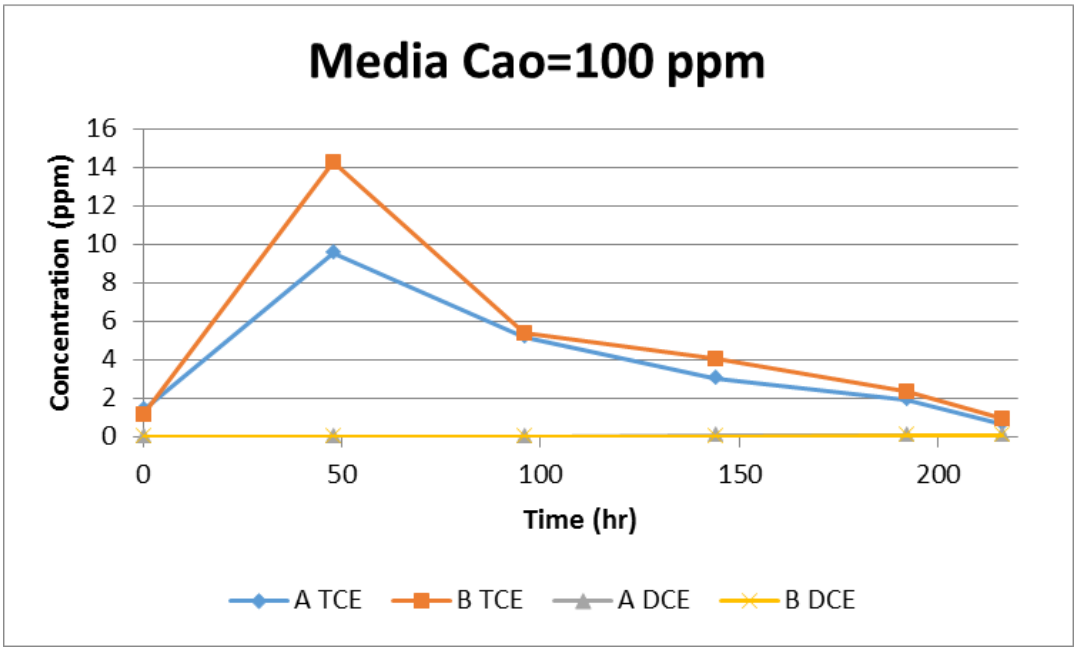

Figure E.1 Time vs. Concentration of TCE and DCE of Just Media with a Starting Concentration of 100 ppm.

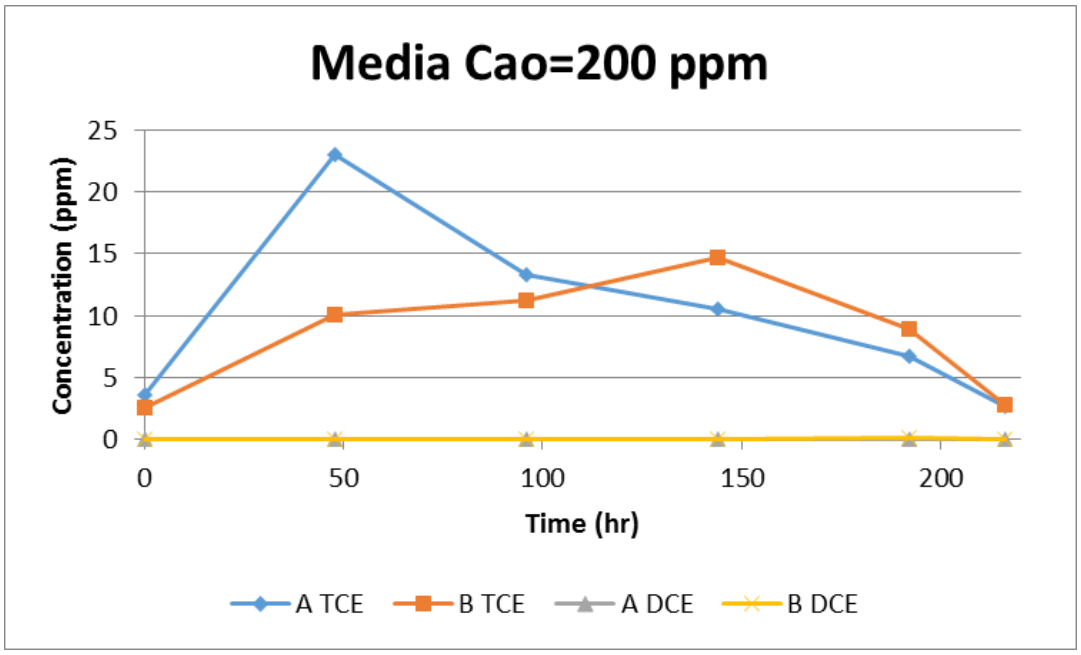

Figure E.2: Time vs. Concentration of TCE and DCE of Just Media with a Starting Concentration of 200 ppm

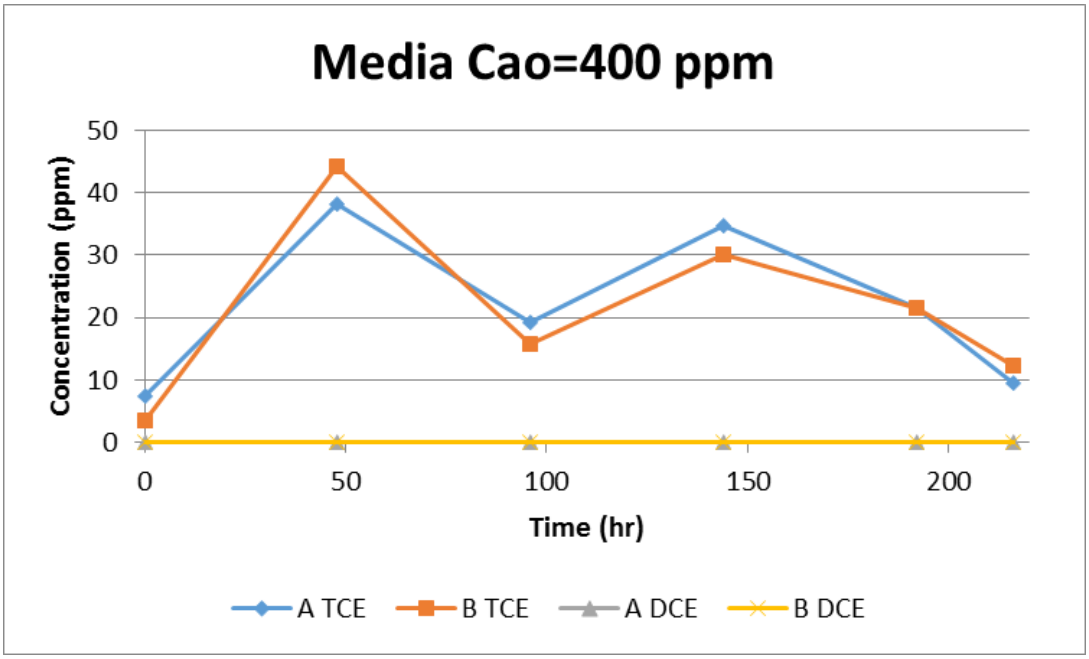

Figure E.3: Time vs. Concentration of TCE and DCE of Just Media with a Starting Concentration of 400 ppm

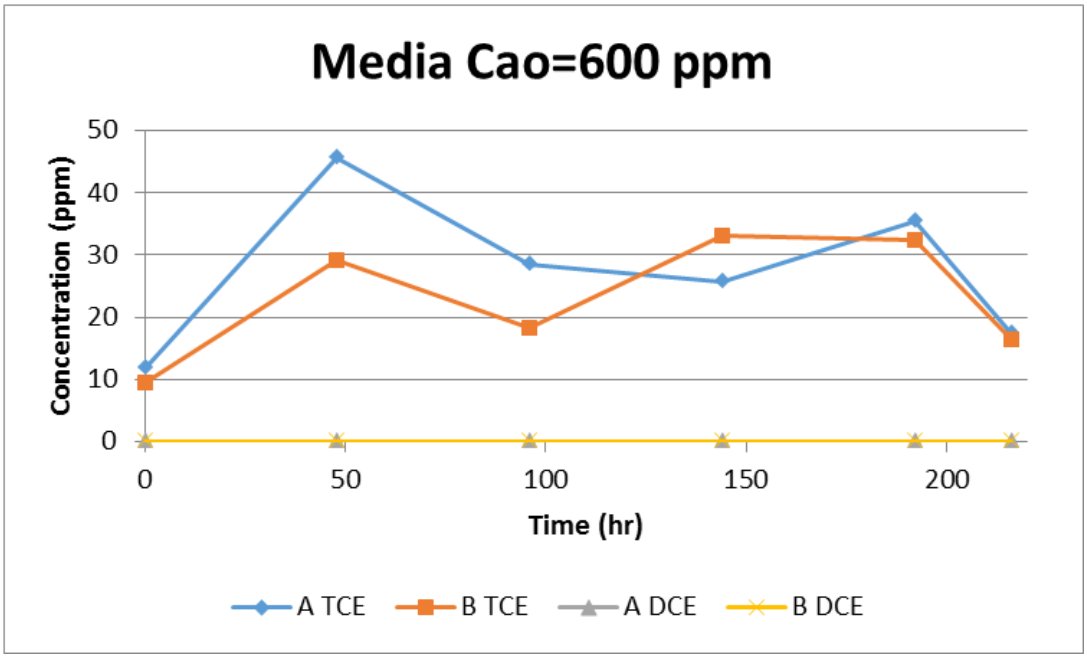

Figure E.4: Time vs. Concentration of TCE and DCE of Just Media with a Starting Concentration of 600 ppm

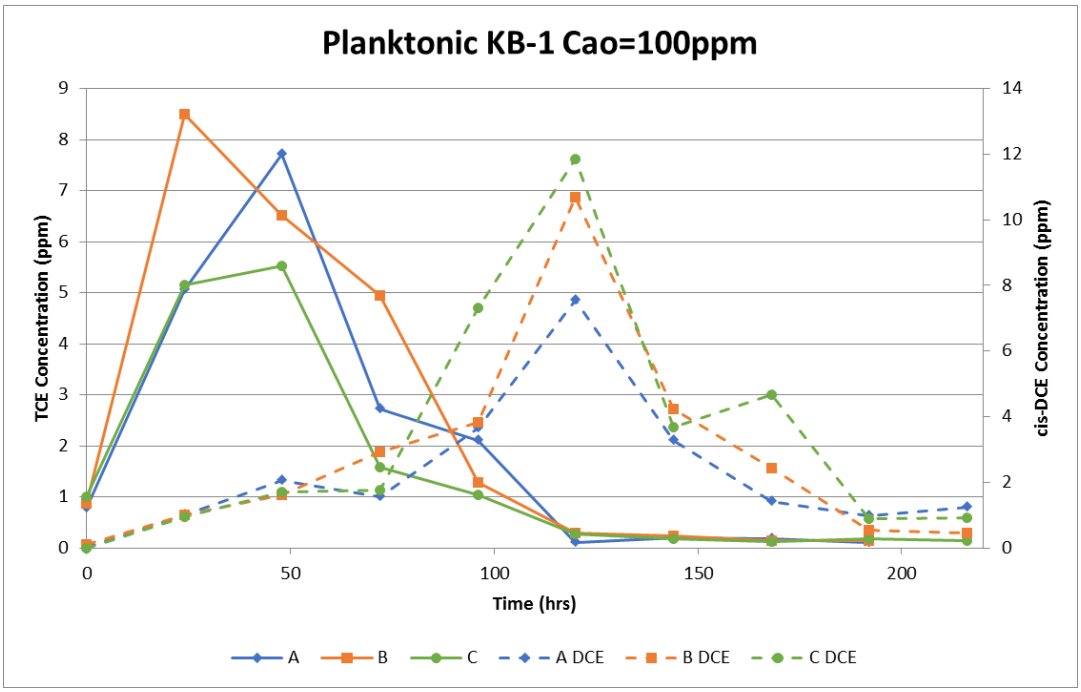

Figure E.5: Time vs. Concentration of TCE and DCE of Planktonic KB-1 with a Starting Concentration of 100 ppm

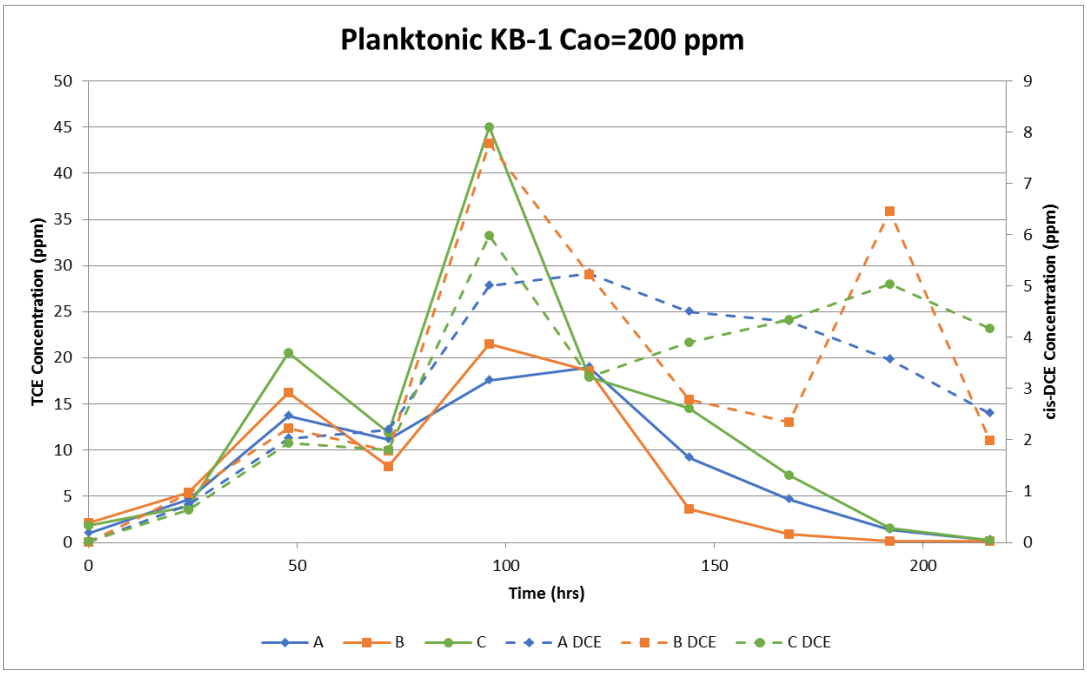

Figure E.6: Time vs. Concentration of TCE and DCE of Planktonic KB-1 with a Starting Concentration of 200 ppm

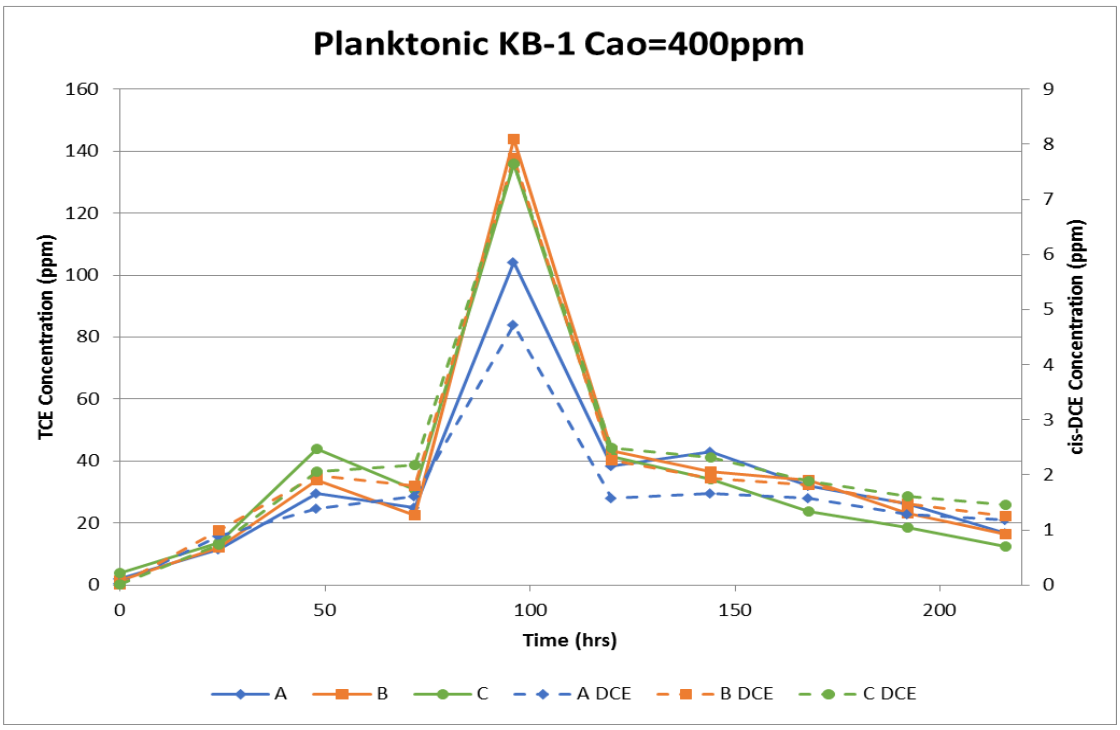

Figure E.7: Time vs. Concentration of TCE and DCE of Planktonic KB-1 with a Starting Concentration of 400 ppm

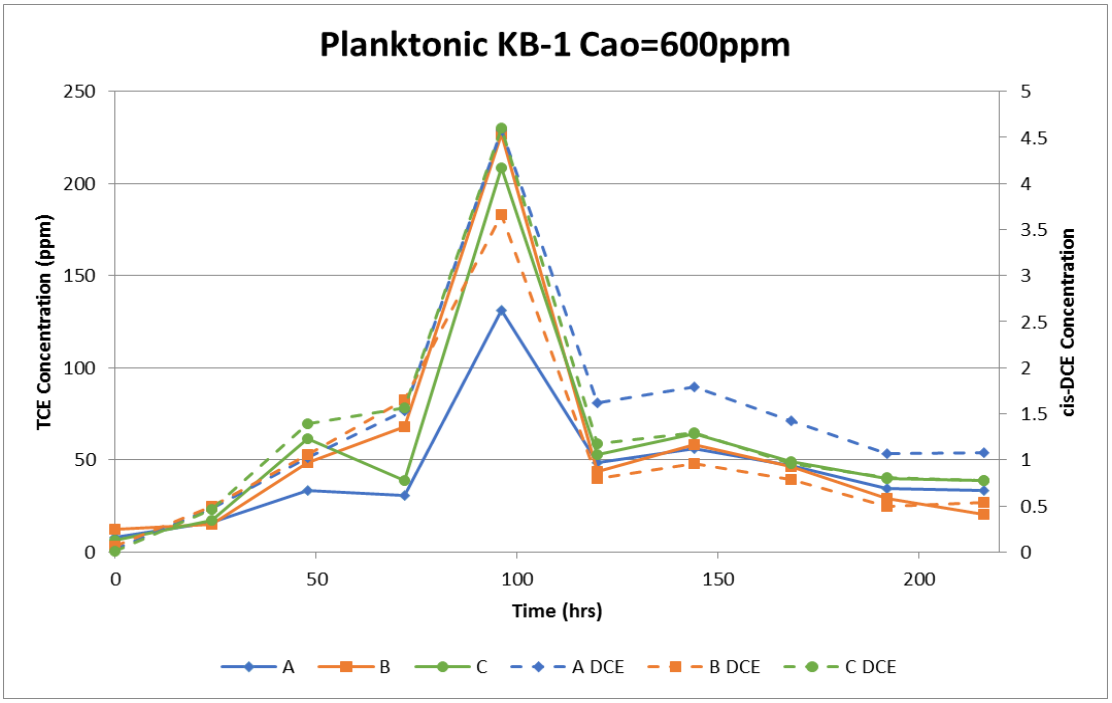

Figure E.8: Time vs. Concentration of TCE and DCE of Planktonic KB-1 with a Starting Concentration of 600 ppm

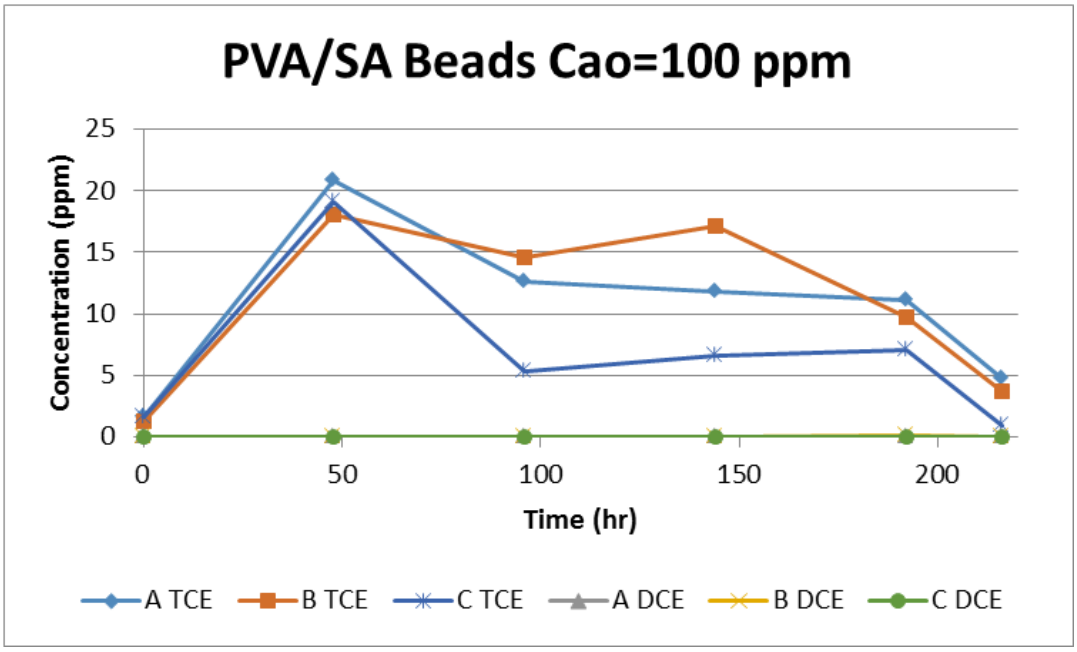

Figure E.9: Time vs. Concentration of TCE and DCE of PVA/SA Beads with a Starting Concentration of 100 ppm

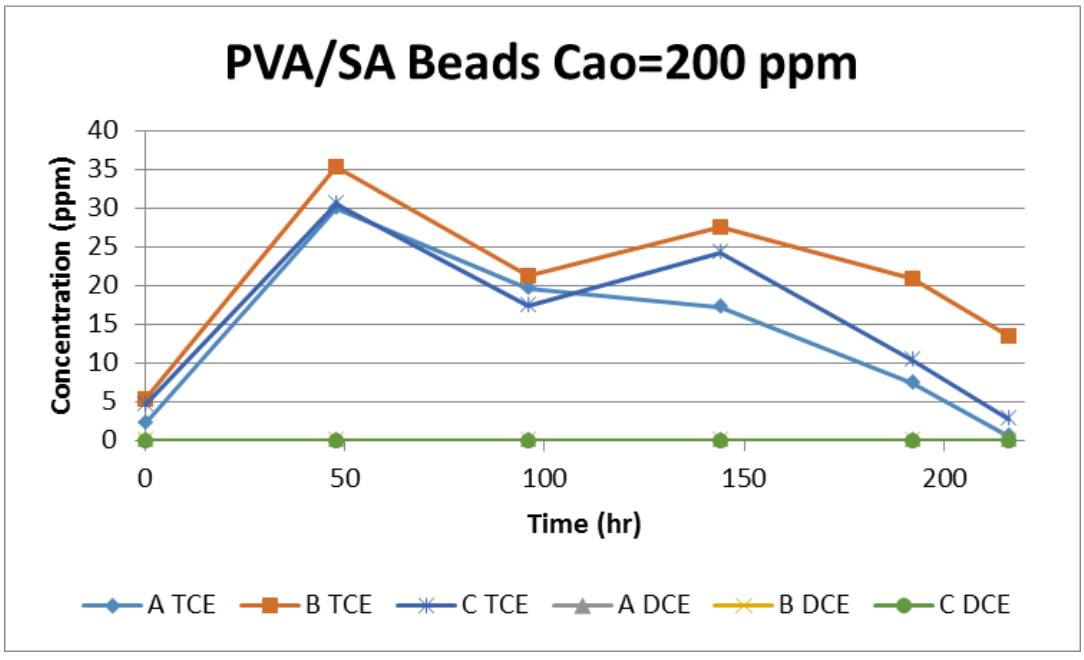

Figure E.10: Time vs. Concentration of TCE and DCE of PVA/SA Beads with a Starting Concentration of 200 ppm

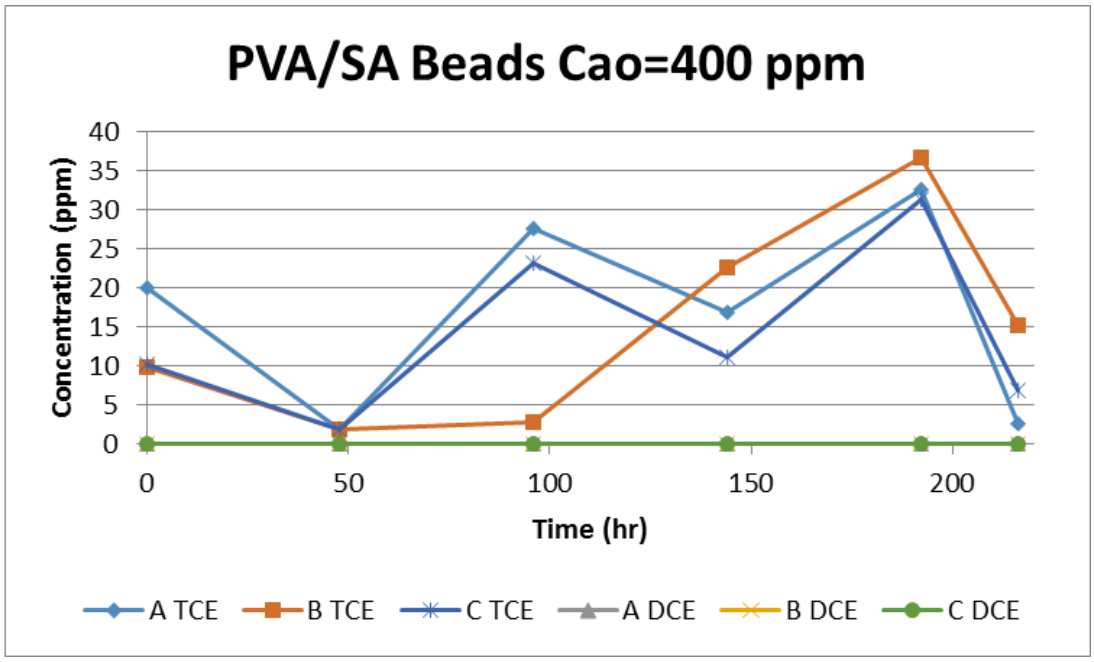

Figure E.11: Time vs. Concentration of TCE and DCE of PVA/SA Beads with a Starting Concentration of 400 ppm

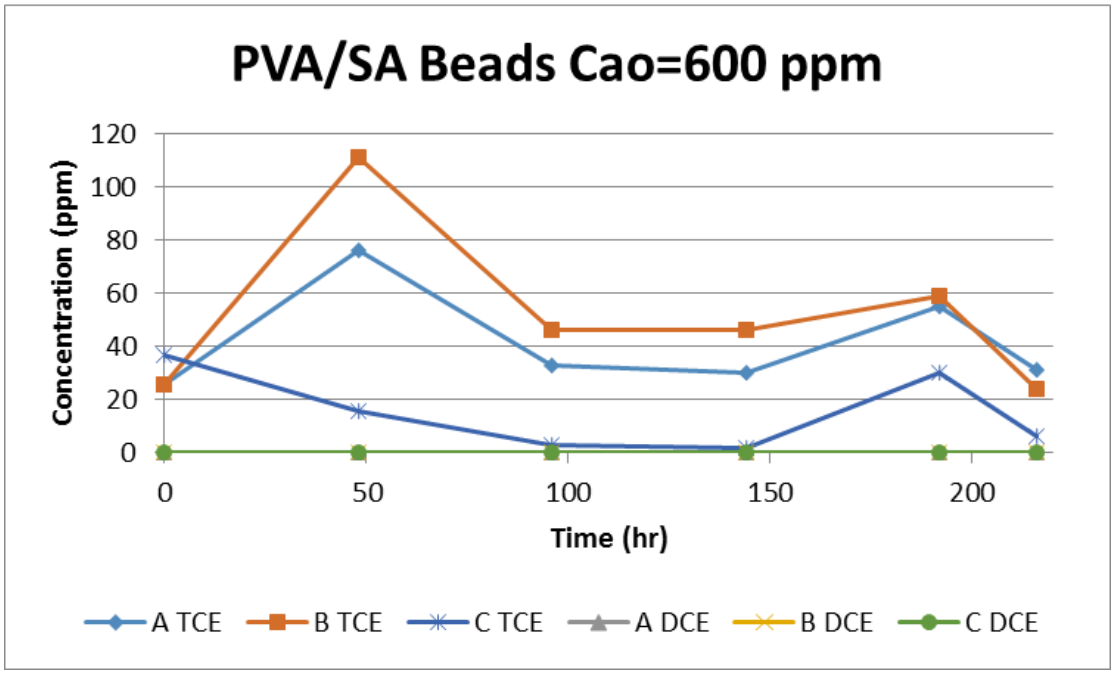

Figure E.12: Time vs. Concentration of TCE and DCE of PVA/SA Beads with a Starting Concentration of 600 ppm

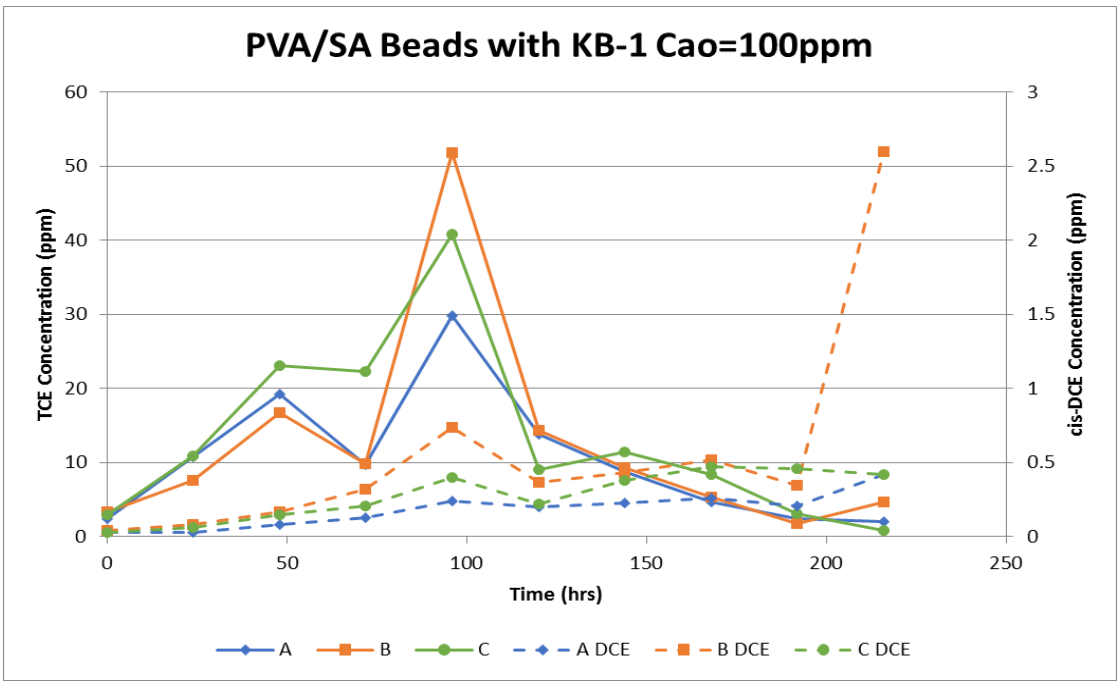

Figure E.13: Time vs. Concentration of TCE and DCE of PVA/SA Beads with KB-1 with a Starting Concentration of 100 ppm

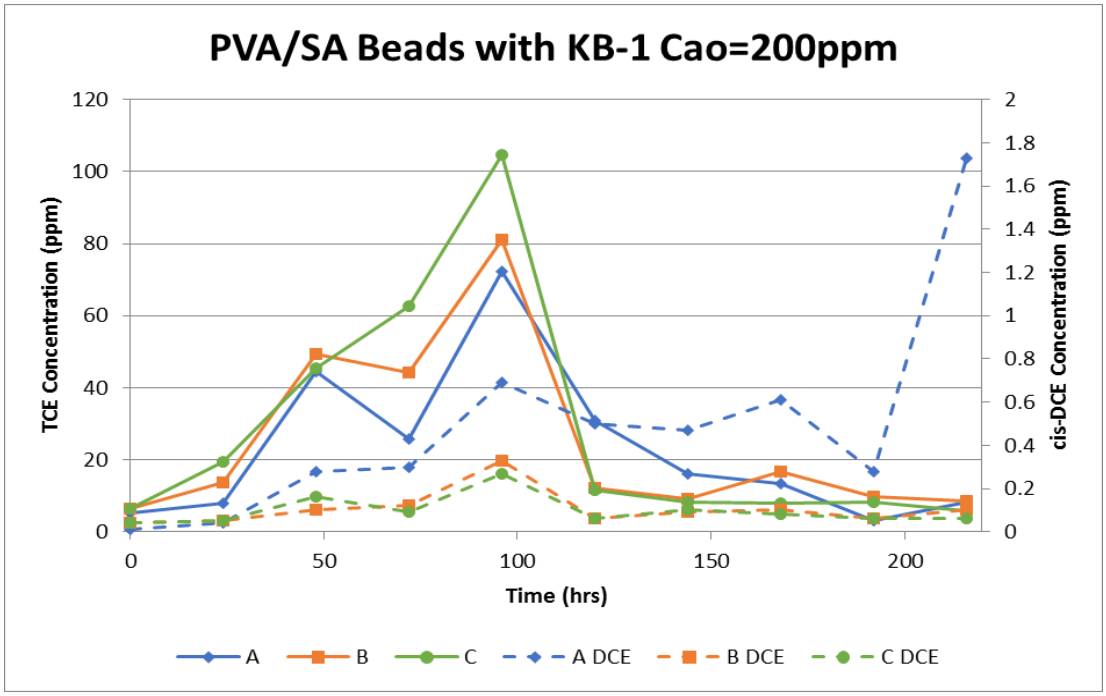

Figure E.14: Time vs. Concentration of TCE and DCE of PVA/SA Beads with KB-1 with a Starting Concentration of 200 ppm

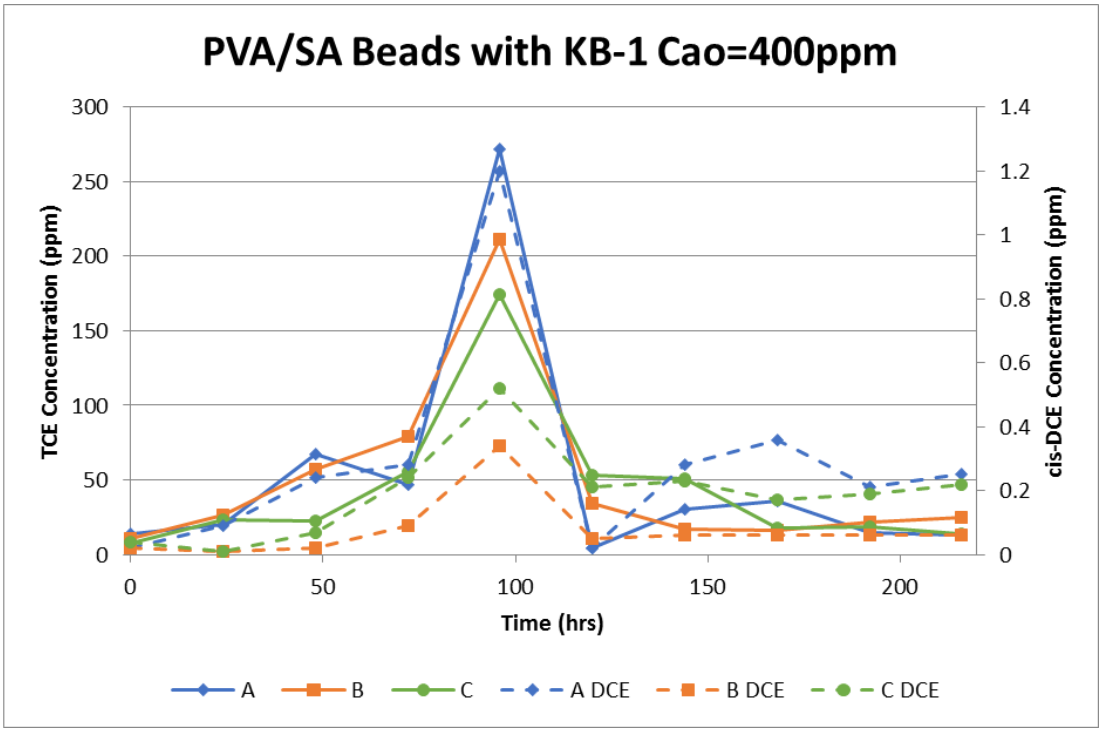

Figure E.15: Time vs. Concentration of TCE and DCE of PVA/SA Beads with KB-1 with a Starting Concentration of 400 ppm

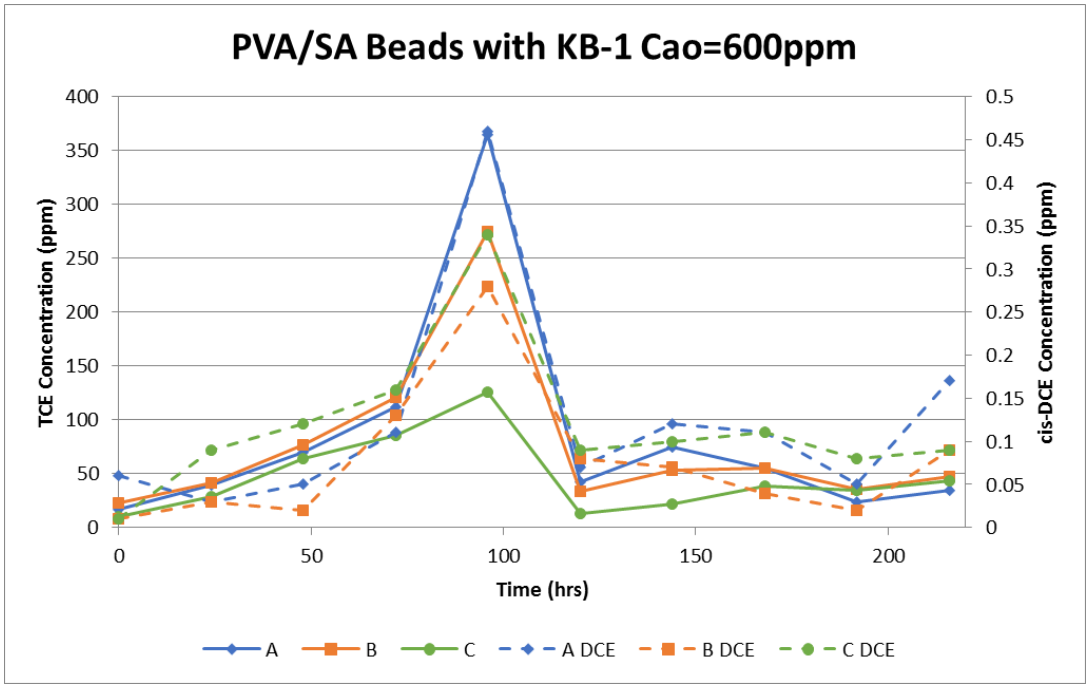

Figure E.16: Time vs. Concentration of TCE and DCE of PVA/SA Beads with KB-1 and a Starting Concentration of 600 ppm

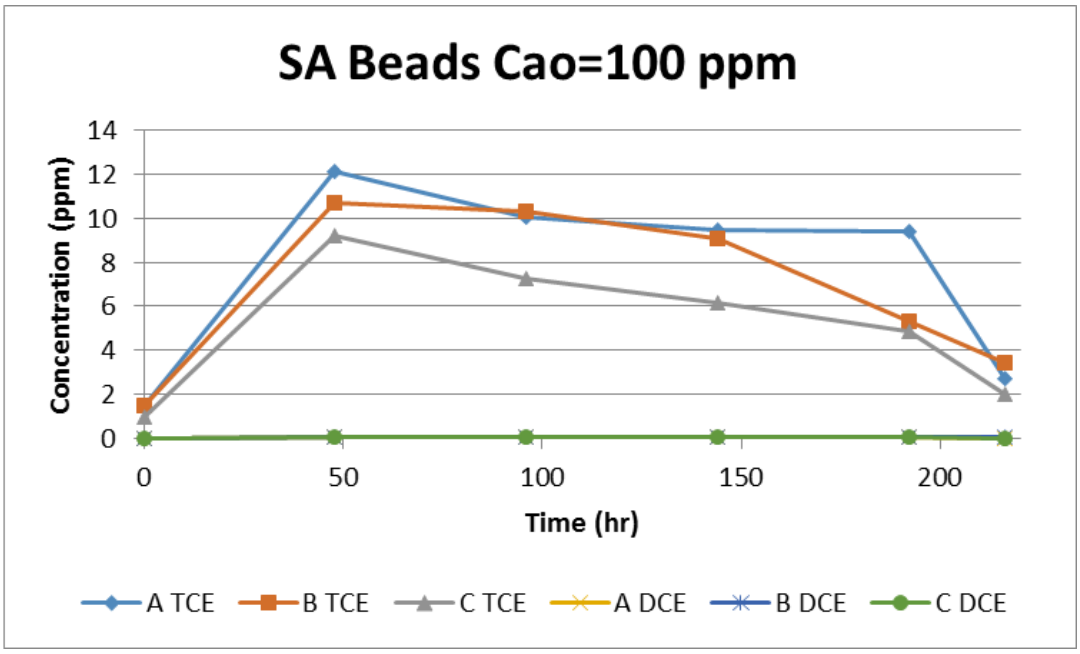

Figure E.17: Time vs. Concentration of TCE and DCE of SA Beads with a Starting Concentration of 100 ppm

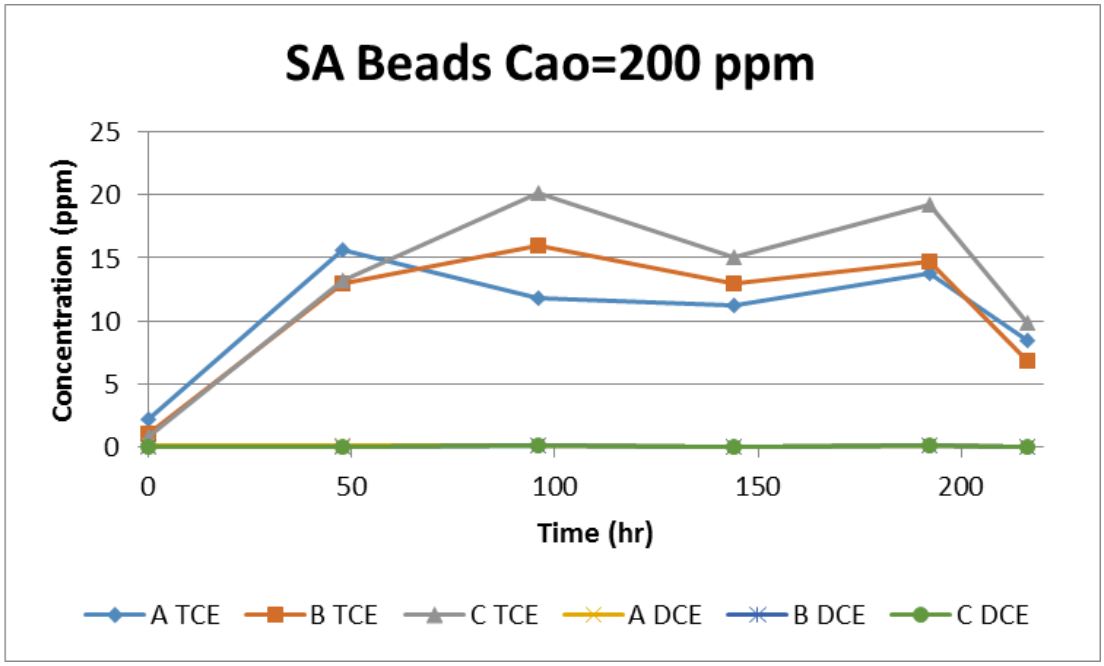

Figure E.18: Time vs. Concentration of TCE and DCE of SA Beads with a Starting Concentration of 200 ppm

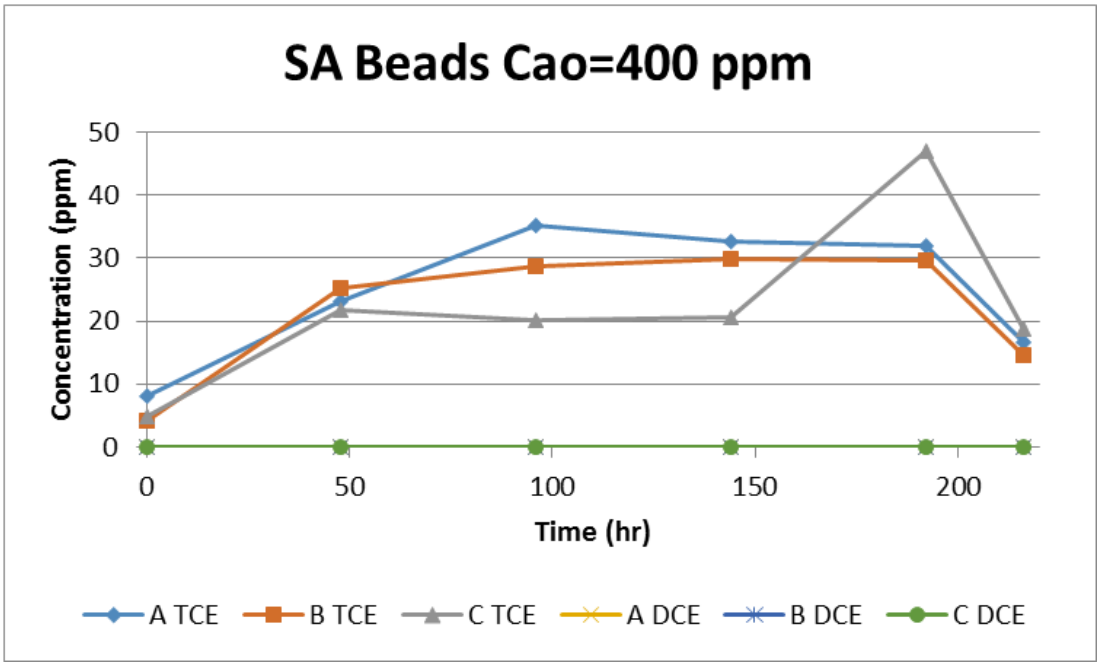

Figure E.19: Time vs. Concentration of TCE and DCE of SA Beads with a Starting Concentration of 400 ppm

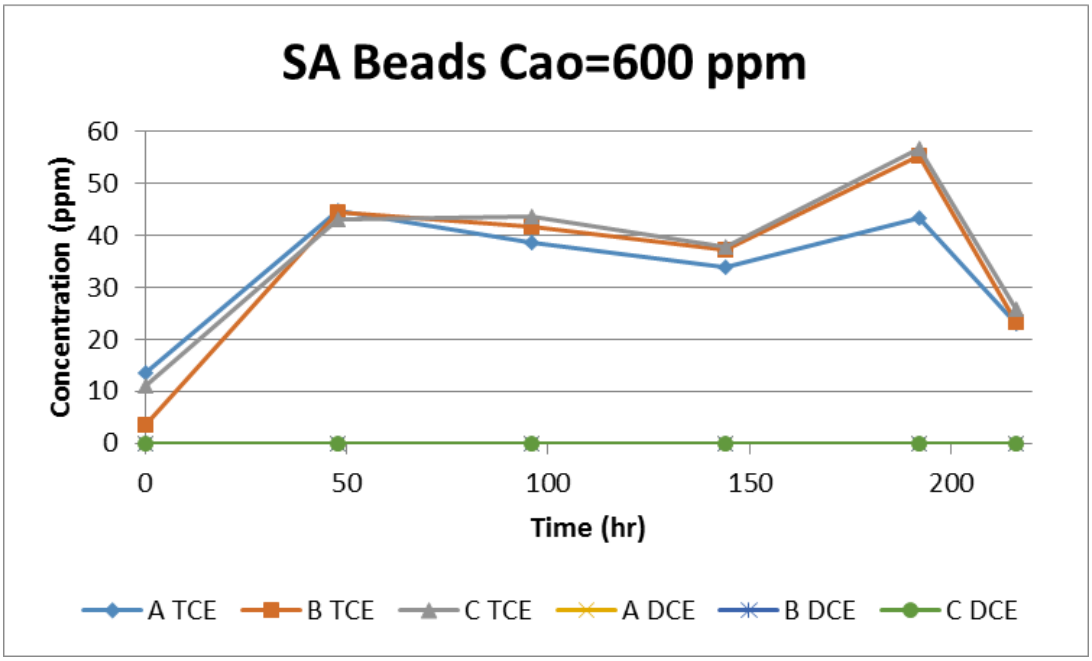

Figure E.20: Time vs. Concentration of TCE and DCE of SA Beads with a Starting Concentration of 600 ppm

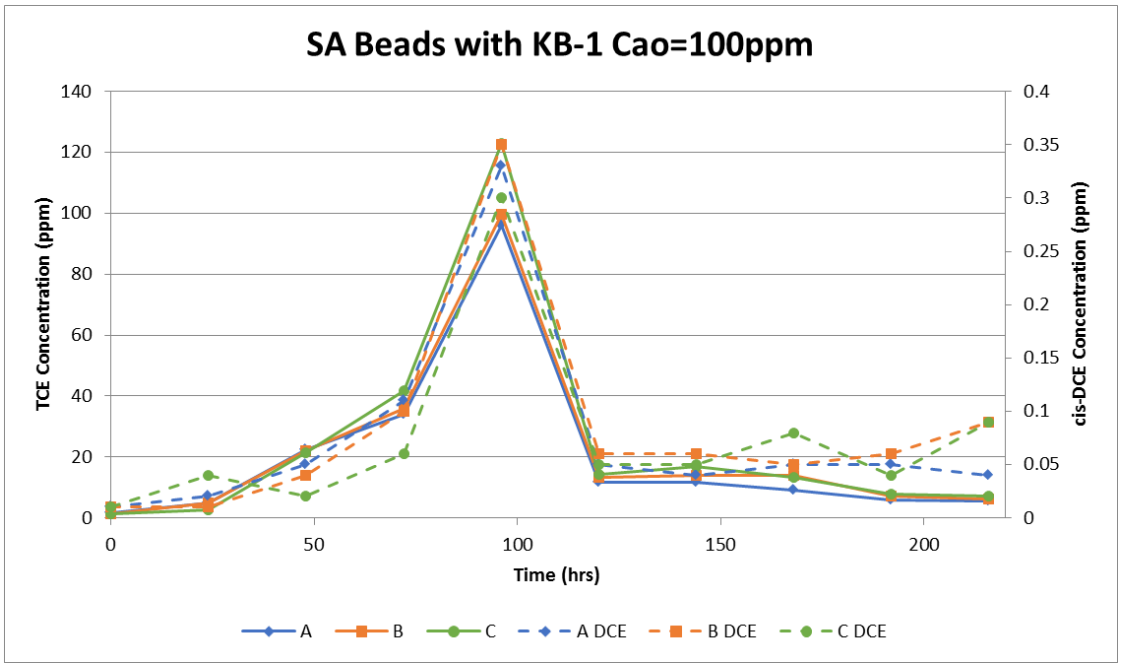

Figure E.21: Time vs. Concentration of TCE and DCE of SA Beads with KB-1 with a Starting Concentration of 100 ppm

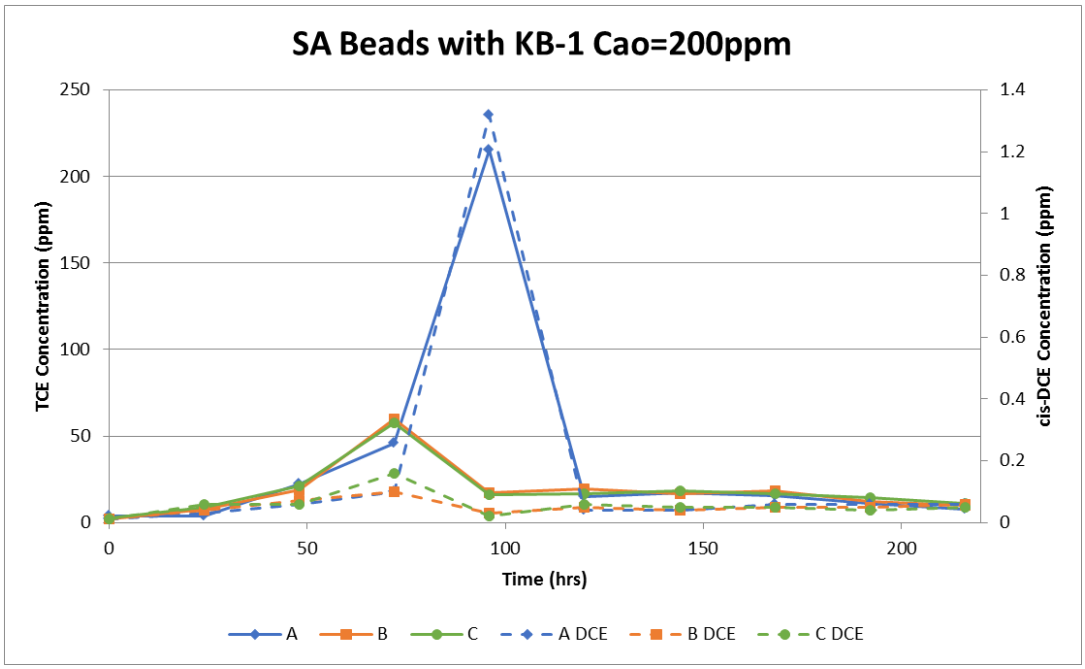

Figure E.22: Time vs. Concentration of TCE and DCE of SA Beads with KB-1 with a Starting Concentration of 200 ppm

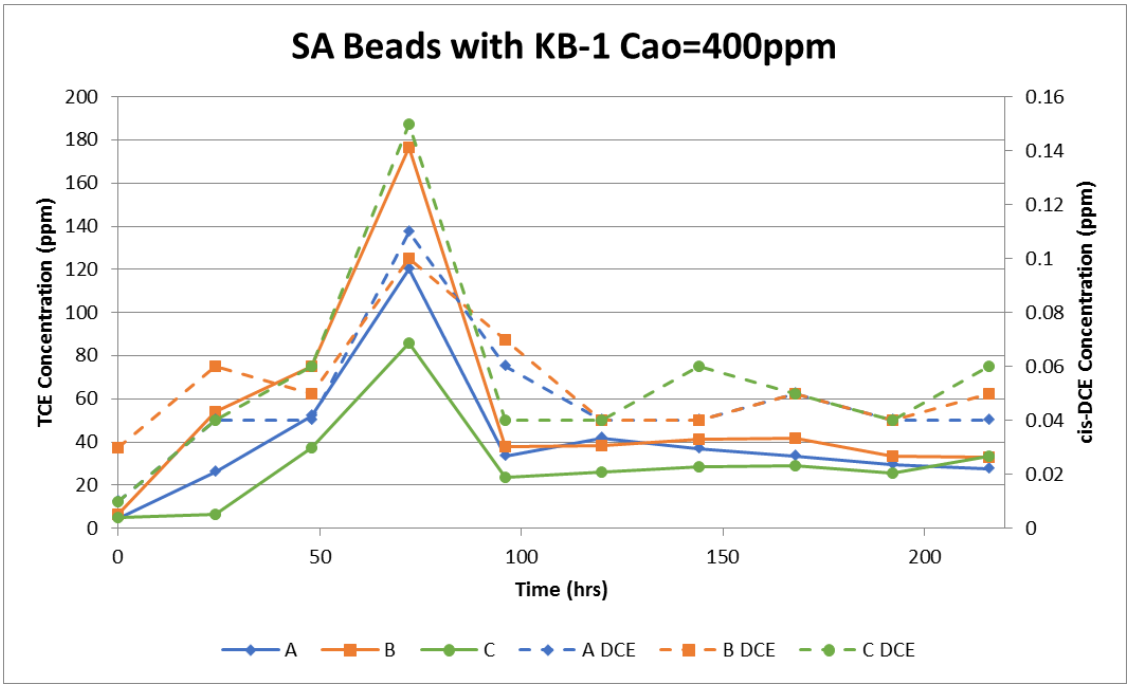

Figure E.23: Time vs. Concentration of TCE and DCE of SA Beads with KB-1 with a Starting Concentration of 400 ppm

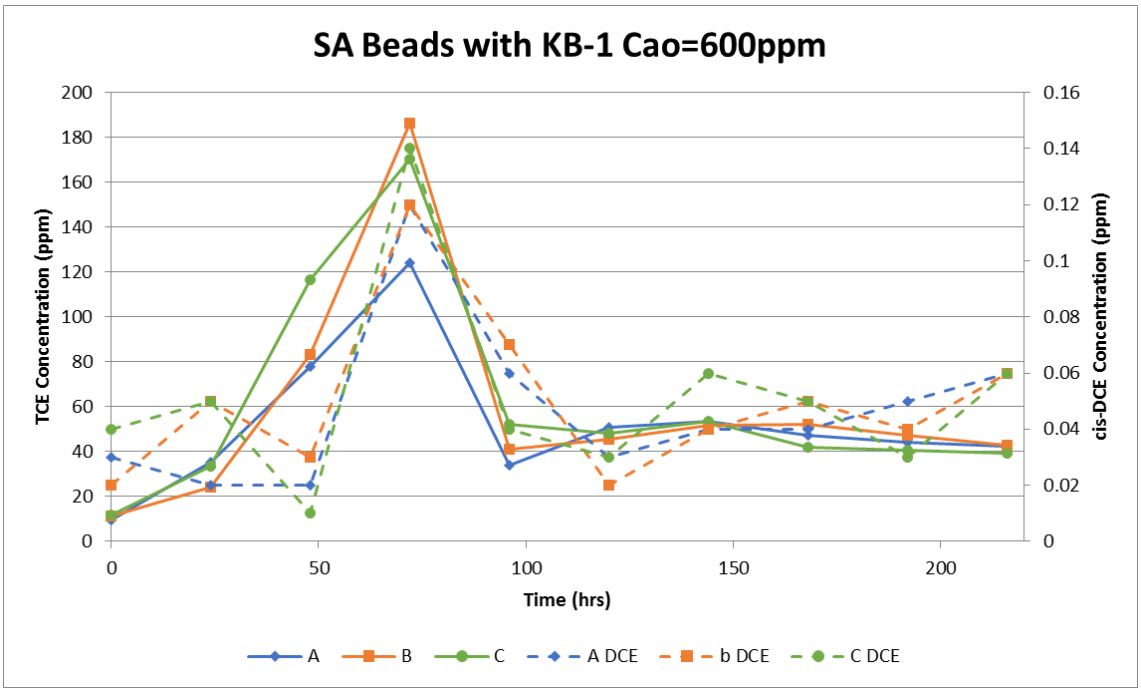

Figure E.24: Time vs. Concentration of TCE and DCE of SA Beads with KB-1 with a Starting Concentration of 600 ppm

sample	pH	Average	SD
media 100 A	7.18	7.195	0.015
media 100 B	7.21		
media 200 A	7.24	7.235	0.005
media 200 B	7.23		
media 400 A	7.24	7.245	0.005
media 400 B	7.25		
media 600 A	7.24	7.250	0.010
media 600 B	7.26		

Table E.1: pH of just media bottles after the experiment

sample	pH	Average	SD
PVA/SA 100 A	7.04	7.087	0.081
PVA/SA 100 B	7.02		
PVA/SA 100 C	7.20		
PVA/SA 200 A	7.19	7.203	0.019
PVA/SA 200 B	7.19		
PVA/SA 200 C	7.23		
PVA/SA 400 A	7.09	7.083	0.025
PVA/SA 400 B	7.05		
PVA/SA 400 C	7.11		
PVA/SA 600 A	7.13	7.120	0.054
PVA/SA 600 B	7.05		
PVA/SA 600 C	7.18		

Table E.2: pH of PVA/SA Bead Bottles after the experiment

sample	pH	Average	SD
SA 100 A	7.10	7.063	0.059
SA 100 B	7.11		
SA 100 C	6.98		
SA 200 A	6.98	7.057	0.054
SA 200 B	7.10		
SA 200 C	7.09		
SA 400 A	6.98	7.093	0.098
SA 400 B	7.22		
SA 400 C	7.08		
SA 600 A	7.04	7.073	0.040
SA 600 B	7.13		
SA 600 C	7.05		

Table E.3: pH of SA Bead Bottles after the experiment

sample	pH	Average	SD
Planktonic KB-1 100 A	6.73	6.660	0.051
Planktonic KB-1 100 B	6.64		
Planktonic KB-1 100 C	6.61		
Planktonic KB-1 200 A	6.72	6.790	0.062
Planktonic KB-1 200 B	6.78		
Planktonic KB-1 200 C	6.87		
Planktonic KB-1 400 A	7.32	7.277	0.033
Planktonic KB-1 400 B	7.27		
Planktonic KB-1 400 C	7.24		
Planktonic KB-1 600 A	7.24	7.300	0.043
Planktonic KB-1 600 B	7.34		
Planktonic KB-1 600 C	7.32		

Table E.4: pH of Planktonic KB-1 Bottles after the experiment

sample	pH	Average	SD
PVA/SA w/KB-1 100 A	7.13	7.227	0.078
PVA/SA w/KB-1 100 B	7.23		
PVA/SA w/KB-1 100 C	7.32		
PVA/SA w/KB-1 200 A	7.13	7.213	0.060
PVA/SA w/KB-1 200 B	7.27		
PVA/SA w/KB-1 200 C	7.24		
PVA/SA w/KB-1 400 A	7.19	7.163	0.019
PVA/SA w/KB-1 400 B	7.15		
PVA/SA w/KB-1 400 C	7.15		
PVA/SA w/KB-1 600 A	7.22	7.207	0.009
PVA/SA w/KB-1 600 B	7.20		
PVA/SA w/KB-1 600C	7.20		

Table E.5: pH of PVA/SA Beads with KB-1 Bottles after the experiment

sample	pH	Average	SD
SA w/KB-1 100 A	7.08	7.050	0.029
SA w/KB-1 100 B	7.01		
SA w/KB-1 100 C	7.06		
SA w/KB-1 200 A	7.13	7.113	0.031
SA w/KB-1 200 B	7.14		
SA w/KB-1 200 C	7.07		
SA w/KB-1 400 A	7.08	7.083	0.005
SA w/KB-1 400 B	7.08		
SA w/KB-1 400 C	7.09		
SA w/KB-1 600 A	7.07	7.103	0.025
SA w/KB-1 600 B	7.13		
SA w/KB-1 600 C	7.11		

Table E.6: pH of SA Beads with KB-1 after the experiment

Sample	pH	Average	SD
Unused Media A	7.05	7.060	0.008
Unused Media B	7.06		
Unused Media C	7.07		

Table E.7: pH of Unused Media with Ethanol