Bench Scale Microcosm Study to Determine the Effectiveness of Emulsified Soybean Oil on the Biodegradation of Chlorinated Volatile Organic Compounds

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Submitted to the graduate degree program in Civil & Environmental and Architectural Engineering and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

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Date Defended: April 22, 2016

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Date approved: April 22, 2016

Abstract

The effectiveness of soybean oil on the bioremediation of tetrachloroethylene (PCE) was determined in this bench scale microcosm study. The contaminated site is located at a former dry cleaning facility in Ft. Riley, Kansas, where the PCE leaked into the surrounding groundwater and soil. Permanganate was injected by the Army Corps of Engineers near the contaminated groundwater wells; however, the levels of PCE rebounded in the Area of Contamination 3 (AOC3). A pilot study was performed in AOC2 by the Army Corps, where CAP18, emulsified soybean oil, was injected into the contaminated area to stimulate biodegradation. The study proved successful, with lower concentrations of PCE and degradation intermediates detected during routine groundwater monitoring.

Soil cores were collected from two different depths, "A" and "C" in AOC3, and from one depth in AOC2 where the injection of soybean oil was successful. Microcosms were filled with soil slurries from AOC3 Horizon A and C, AOC2 was used as a positive control, and a composite sample from AOC3 was autoclaved and used as the negative control. The microcosms also contained artificial groundwater, with nutrients added to support microbial growth, and EOS Pro emulsified soybean oil. Two starting concentrations of PCE were used in the microcosms, 50 ppb and 150 ppb. Air was evacuated from the microcosms and they were sealed to create an anaerobic environment. Sacrifice sampling was used for this bench scale study, which means the microcosms were discarded after sampling was performed. The sacrifice sampling was used to avoid the loss of volume from the microcosms during sampling, and the study lasted for 16 weeks, with sampling performed in Weeks 0, 1, 2, 3, 4, 6, 8, 12, and 16.

Analysis by Gas Chromatography – Mass Spectrometry with Solid Phase Microextraction showed erratic results for PCE concentrations over time due to inconsistent partitioning of the

PCE into the organic phase. Degradation intermediates – trichloroethylene (TCE), cis-Dichloroethylene (cis-DCE), trans-Dichloroethylene (trans-DCE), and vinyl chloride (VC) were detected in AOC3-A, and low levels were detected in AOC3-C. The moderate levels of vinyl chloride in AOC3-A indicated there was likely bioremediation occurring in the microcosms.

DNA analysis by qPCR showed that active bacterial communities were present in AOC3-A, AOC3-C, and AOC2. In AOC3-A where bioremediation occurred, the DNA concentration increased from Week 0 to 3, but then decreased some when vinyl chloride was produced from Week 6 onwards. The vinyl chloride was likely toxic to the bacteria, which caused the decrease in bacterial concentration. Terminal electron acceptor concentrations (bicarbonate, nitrate, sulfate, phosphate, ferrous iron) were measured using Inductively Coupled Plasma – Optical Emission Spectra (ICP-OES) and Ion Chromatography. The levels of bicarbonate increased in all microcosms as the organic carbon from the soybean oil was converted to inorganic carbon. The levels of nitrate remained low in all microcosms and was difficult to distinguish from the detection limit, since only a small amount of nitrate was initially present in the artificial groundwater. Sulfate levels decreased for AOC3-A, where bioremediation was occurring, indicating there were sulfate reducing conditions present in the microcosms. Phosphate concentrations in solution decreased rapidly in all microcosms, indicating in was likely the growth limiting nutrient. Detectable ferrous iron concentrations were present in AOC3-A only in later weeks when bioremediation was happening, indicating microbial iron reduction was occurring.

The AOC3-A microcosms had active microbial communities capable of bioremediation at the contaminated site. It is recommended that soybean oil be injected into the depth around the A horizon in AOC3. The terminal electron acceptors native to the site are sufficient to sustain

microbial growth, however, phosphate concentrations should be monitored over time to see if supplemental addition of phosphate is necessary. Bioaugmentation with a strain of *Dehalococcoides*, which is known to degrade PCE is recommended to sustain bioremediation. Vinyl chloride will likely accumulate in the groundwater and soil because it is the rate limiting step in the bioremediation of PCE. The addition of methanol as a carbon source is recommended because it will promote the growth of methanogens which will more easily degrade the vinyl chloride to ethene gas, which is an environmentally acceptable end product.

To my family who have always supported me in my education

Acknowledgements

I would like to thank:

Dr. Belinda S.M. Sturm, my advisor, for providing guidance and support throughout my graduate studies.

Dr. Jennifer Roberts and Dr. Stephen Randtke, for being part of my committee and their guidance in my research and study.

The CEAE department and the Ross E. McKinney scholarship for providing funding for my graduate studies.

The Army Corps of Engineers for funding this project under agreement number W912DQ-14-02-0005.

Dr. Ray Carter, for helping me in developing the analytical methods and his guidance to operate the analytical instruments.

Devinda Hiripitiyage for his help with the DNA analysis on this project.

Adam Yoerg for the assistance with field collection of samples and field analyses of iron and phosphate.

Masato Ueshima for analyzing the inorganic aqueous chemistry with IC and ICP-OES.

All my fellow lab group students, for all their advice and support.

My family, for supporting and encouraging me during my graduate studies at KU.

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Abbreviations

AOC	Area of concern (1, 2, or 3)
CVOC	Chlorinated volatile organic carbon
DCE	cis- and trans-dichloroethylene
DCF	Dry cleaning facility
DO	Dissolved oxygen
EOS	Soybean oil emulsion
ft.	feet
GC/MS	Gas chromatography with mass selective detector
IC	Ion chromatography
ICP-OES	Inductively coupled plasma with optical emission spectroscopy
m	meters
MDL	Method detection limit
mg/L	milligram per liter
mL	milliliters
mM	millimolar
mm	millimeters
$NaMnO_4$	Sodium permanganate
ORP	Oxidation reduction potential
PCE	Perchloroethylene
ppb	Parts per billion
ppm	Parts per million
qPCR	Quantitative Polymerase Chain Reaction
SPME	Solid Phase Microextraction
TCE	Trichloroethylene
µg/L	micrograms per liter
VC	Vinyl chloride

Chapter 1: Introduction

1.1 Purpose of Study

The purpose of this microcosm study is to determine whether *in situ* biodegradation of perchloroethylene (PCE) is possible at a contaminated site at Ft. Riley with biostimulation using a soybean oil emulsion as an organic substrate for the native microorganisms in the site soil.

1.2 Site History

The contaminated site at Fort Riley is southwest of decommissioned laundry and dry cleaning facilities that were in operation from 1915 to 2002. The contaminated site is located in close proximity to the Kansas River as pictured in Figure 1.

Figure 1: Contaminated site at Fort Riley. The areas of concern are located off of Custer Road between the road and the railroad. The site is located closely to the Kansas River (Google Maps, 2016).

From 1944 to 1966 a petroleum distillate mixture, a Stoddard solvent, was used for dry cleaning operations. Stoddard solvent is a petroleum distillate mixture of $C_7 - C_{12}$ hydrocarbons,

From 1966 until the dry cleaning facilities were closed, a PCE based solvent was used as a dry cleaning agent. PCE infiltrated the groundwater and soil due to accidental spills that most likely reached the dry cleaning facility (DCF) building floor drains. The contaminants were also released into the ground through leaky sanitary sewer lines used for the facilities' wastewater disposal (United States EPA Region 7 Kansas City, 2010).

The contaminated site at Fort Riley contained three areas of concern (AOC1, AOC2 and AOC3) which are shown in Figure 2 and Figure 3. In AOC3, a pilot study was performed with the injection of sodium permanganate in January and February 2006. 7,400 pounds of sodium permanganate solution ($NaMnO_4$) was injected at 23 sites into the vadose zone near groundwater monitoring well DCF02-42 as shown in Figure 4. The location of the groundwater monitoring wells is included in Figure 2 and Figure 3. The purpose of the permanganate injection was to oxidize tetrachloroethylene (PCE) as well as trichloroethylene (TCE), cis and trans-dichloroethylene (DCE) to chlorine, carbon dioxide and manganese dioxide. The reaction of PCE with permanganate proceeds by the following reaction: $C_2Cl_4 + 2MnO_4^- \rightarrow 4Cl^- + 4CO_2 + 2MnO_2$ (s) (H. Zhang & Schwartz, 2000). Gates et al. found that permanganate treatment was more effective at oxidizing PCE and TCE than Fenton's reagent, with permanganate consistently degrading up to 90% of PCE and TCE (Gates-Anderson, Siegrist, & Cline, 2001).

In April 2006, 21,755 pound of potassium permanganate was injected in the saturated zone near groundwater monitoring wells DCF02-42 and DCF96-05. The groundwater contaminant monitoring of well DCF02-42 showed that the PCE concentrations decreased from 60 ppb in November 2005 to below 10 ppb in May 2012. In well DCF06-25 the concentration of PCE declined from 60 ppb in May 2005 to 10 ppb in May 2007; however, the PCE concentration

had increased to about 40 ppb by May 2013. The PCE concentrations in wells DCF02-44a and DCF02-44c also showed a rebound in PCE concentration and exceeded 20 ppb in May 2013.

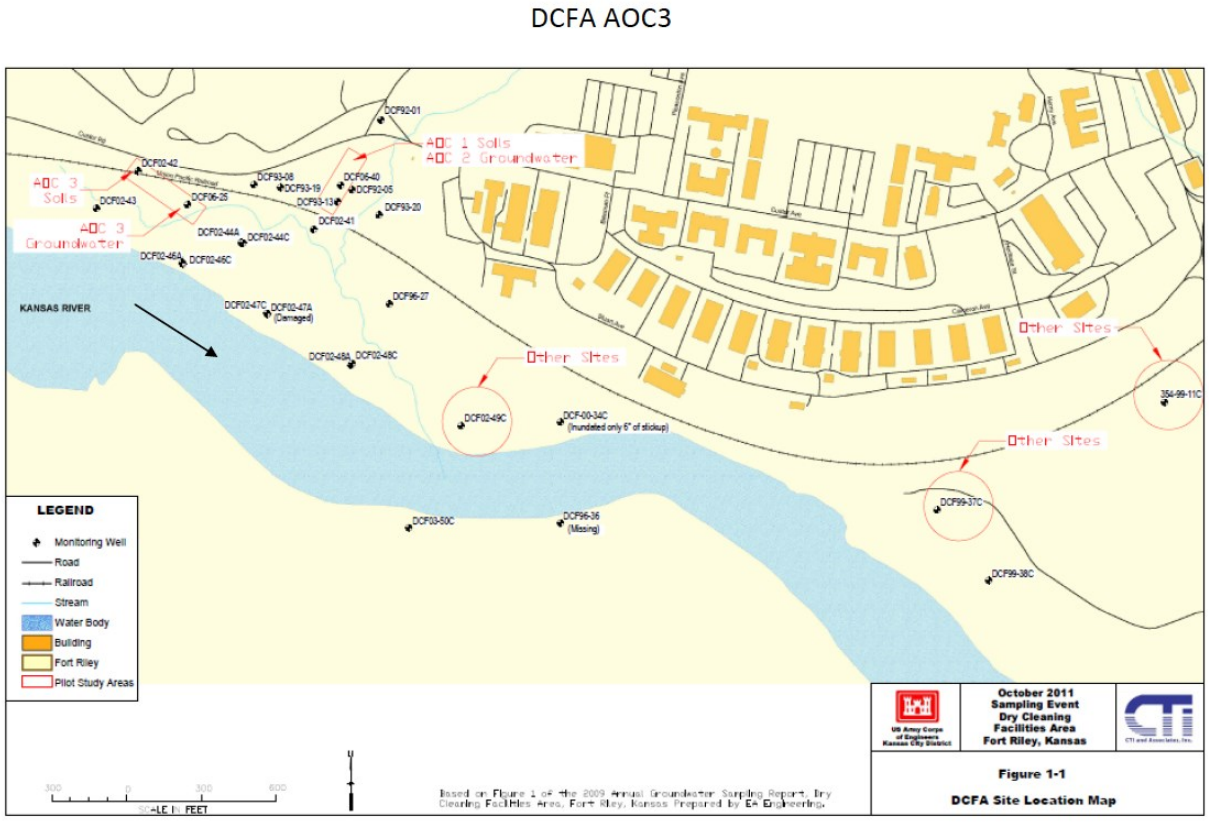


Figure 2: Monitoring well locations and Areas of Concern (AOC) at Ft. Riley, Kansas (US Army Corps of Engineers, 2014)

In April 2006, a pilot study was performed with CAP18 (a non-emulsified vegetable oil product), which was injected into AOC2 to provide a carbon source and stimulate biodegradation of the chlorinated hydrocarbons (PCE and degradants). About 8,200 pounds of CAP18 vegetable oil were injected into 72 points within AOC2 in the area around groundwater monitoring wells DCF06-40 and DCF93-13. In February 2010, 2,500 pounds of CAP18 were injected at 10 points near monitoring wells DCF06-40 and DCF93-13 to further enhance biodegradation of PCE. Historical groundwater monitoring data showed that PCE and TCE concentrations decreased significantly after the injection of CAP18 into AOC2. The PCE concentrations decreased

dramatically near well DCF06-40, going from 78.1 ppb in March 2006 to below the detection limit in April 2013.

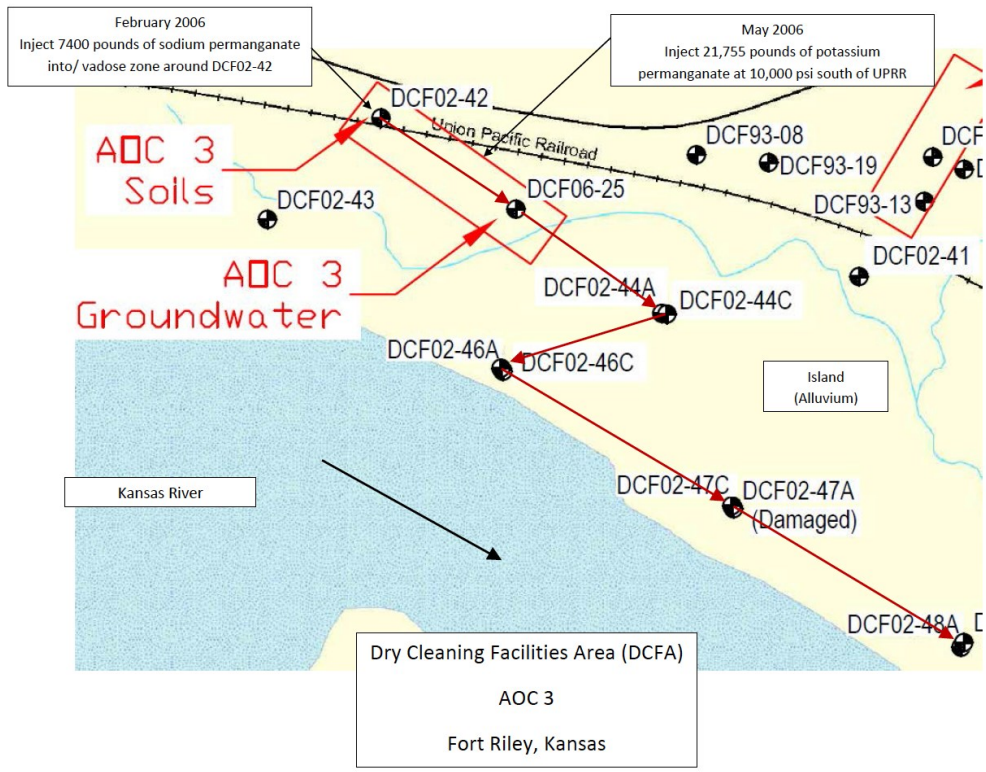


Figure 3: Monitoring well locations and Areas of Concern (AOC) at Ft. Riley, Kansas (US Army Corps of Engineers, 2014)

The purpose of this investigation was to determine, through a bench-scale microcosm study, if native bacteria in AOC3-Can bioremediate PCE. Previous microcosm studies have used soybean oil as an organic substrate to stimulate PCE degradation (Long & Borden, 2006) (Borden, 2007a). PCE is biodegraded to ethene by a series of steps that release chloride ions, called reductive dechlorination. This process requires an electron donor, which is usually an organic substrate, and PCE acts as the electron acceptor. Several studies have been performed showing the potential of edible oils, such as soybean oil, to act as the electron donor to stimulate anaerobic bioremediation of PCE (Long et al., 2006). Researchers have reported having

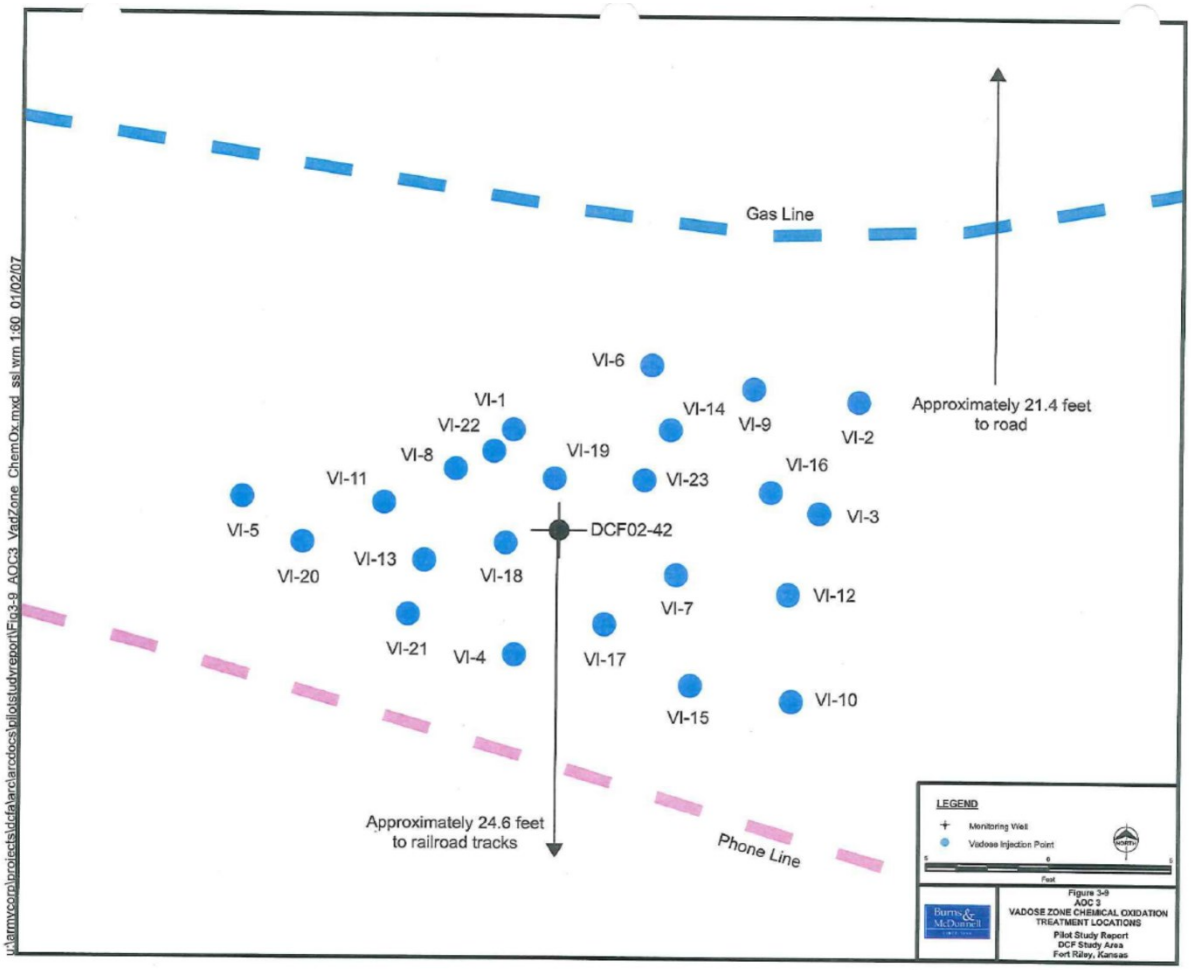


Figure 4: Injection locations of permanganate in AOC3 (US Army Corps of Engineers, 2014)

problems with the distribution of neat soybean oil into soil because of permeability loss (Borden, 2007b). Emulsified soybean oils, which can be obtained from remediation companies such as EOS (Raleigh, North Carolina), contain small oil droplets that can be transported better through sediment pores. The EOS emulsion is prepared "using high energy mixing with nonionic surfactants to generate an emulsion with small uniform droplets that have a negative surface charge to enhance oil droplet distribution in the aquifer" (Borden, 2007b). After it is injected into the soil, emulsified oil is able to work for a long period of time to continue degrading PCE. A study by Borden et al showed that soybean oil was effective in the degradation of PCE for over 2

years (Borden, 2007a). This literature was taken into consideration when designing the microcosm study.

Since bioremediation was the goal for AOC3, there was a concern that the previous permanganate treatment in this area had negatively affected the indigenous microcosms. Permanganate has disinfection properties and can oxidize bacteria (Cleasby, Baumann, & Black, 1964), so it is likely that microbes capable of bioremediation were inactivated during the permanganate treatment. The permanganate was injected into several locations near monitoring well DCF02-42 at depths ranging from 5 to 32 feet below the surface of the ground. The injection sites are shown in Figure 4, and the depths and amounts of each injection are given in Appendix A: Permanganate Injections. Most of the injections in AOC3 were at depths ranging from 14 to 26 ft below the ground surface.

The permanganate injections were performed at two different screen depths, referred to as the "A" and "C" horizons. Table 1 shows the depths to the groundwater monitoring well screens, including the "A" and "C" horizons for the wells in AOC2 and AOC3. The depths to "A" and "C" horizons were not given for wells DCF02-42 and DCF06-25, but can be estimated from the well nearby, DCF02-44. Sediment cores were collected for the microcosm experiment from AOC2 and AOC3. The sediment in AOC3 was collected to 28 ft. at DCF02-42 as bedrock was reached at this depth. For the microcosm study, the "A" horizon soil is defined as the sediment collected from the 3-4 meter range (5-12 ft.), and the last core from 8- 8.6 m (26-28 ft.) was used for the "C" horizon. The permanganate treatments were injected in both the shallow depths (5-12 ft.) and deep depths (20-26 ft.).

Table 1: Groundwater elevations in April 2014, Ft. Riley (US Army Corps of Engineers, 2014)

Well ID	Top of Casing Elevation	Bottom of Screen Elevation	Depth to Water (TOC)	W.L. Elevation April 12, 2004
DCF92-01	1092.04	1043.69	41.81	1050.23
DCF92-05	1082.73	1041.79	32.18	1050.55
DCF93-08	1086.49	1045.79	NM	NM
DCF93-13	1082.86	1042.73	35.58	1047.28
DCF93-19	1087.54	1026.8	43.52	1044.02
DCF93-20	1088.98	1032.37	43.92	1045.06
DCF96-27	1060.81	1027.91	22.4	1038.41
DCF96-36	1060.3	1010.28	NM	NM
DCF99-37C	1065.16	1015.16	27.23	1037.93
DCF99-38C	1064.17	1016.67	26.32	1037.85
DCF00-34C	1050.13	1010.63	11.3	1038.83
DCF02-41	1060.15	1026.7	19.15	1041
DCF02-42	1072.27	1039.1	NM	NM
DCF02-43	1058.5	1016.5	19.6	1038.9
DCF02-44A	1061.15	1029.8	22.29	1038.86
DCF02-44C	1061.01	1018.7	22.34	1038.67
DCF02-46A	1067.4	1031.4	28.63	1038.77
DCF02-46C	1067.09	1011.7	28.36	1038.73
DCF02-47A	1063.17	1029.6	NM	NM
DCF02-47C	1062.86	1010.5	24.21	1038.65
DCF02-48A	1059.49	1029	NM	NM
DCF02948C	1059.43	1009	21.14	1038.29
DCF02-49C	1051.87	1010.3	13.89	1037.98
DCF03-50C	1061.87	1031.5	23.61	1038.26
DCF06-25	1060.92	1030.8	22.06	1038.86
DCF06-40	1087.9	NA	39.99	1047.91
354-99-11C	1058.8	1016.3	21.03	1037.77

1.3 Bioremediation Study

The purpose of this study is to determine the effectiveness of emulsified soybean oil at stimulating the biodegradation of two different doses of PCE. A bench-scale microcosm study was performed for the "A" and "C" horizons of AOC3. Sediments were sampled near monitoring well DCF02-42 from "A" and "C" horizons in AOC3, and near well DCF06-40 from AOC2. Soil from Area of Concern 2 (AOC2) was used as a positive control in the microcosm study. The concentration of PCE and its degradants: TCE, cis-DCE, trans-DCE, and Vinyl Chloride (VC) were measured with gas chromatography/mass spectrometry (GC/MS) to determine if bioremediation was occurring in the microcosms. DNA concentrations in the soil samples and microcosms were also measured to monitor the presence of microorganisms during the study.

Chapter 2: Experimental Methods

2.1 Bench Scale Microcosm Study Experimental Design

2.1.a. Microcosm Study Design

The microcosm study was performed with sediment from the two different sediment depths, "A" and "C", with two different initial concentrations of PCE, 50 ppb and 150 ppb. Sediment samples were homogenized by passing the wet soil through a sterilized sieve. A positive and negative control were included in the experimental matrix (see Table 2) The composite sample from AOC2 was a considered a positive control, since this area had shown successful bioremediation of PCE after emulsified oil injection (CAP 18®) in 2010. The negative control contained soil composited from AOC3 that was autoclaved twice to inactivate any microbes in the soil. The negative control was also included to determine any losses due to the microcosm matrix (losses due to sorption). Each microcosm contained sediment, artificial groundwater (AGW), soybean oil emulsion (EOS®), and a spike of PCE. The artificial groundwater recipe is shown in Table 3. Replicate microcosms were created for each of the treatments to make it possible to perform sacrifice sampling, which ensures that the volume of each microcosm and its contents does not change with time. Nine replicates of each experimental treatment (including duplicates for AOC3) were made, one for each sampling time listed in Table 2. A total of 72 microcosms were made, with 1 extra made for each treatment in case of breakage (total of 9 extra). After each microcosm was sampled, it was discarded. This sampling scheme is referred to as sacrifice sampling because each microcosm is "sacrificed" at the time it is sampled, leaving the remaining microcosms undisturbed.

Table 2: Experimental matrix for microcosm study

	Test Treatments				Positive Control	Negative Control
Sampling Time (week)	AOC3 Horizon A (150 ppb PCE)	AOC3 Horizon C (150 ppb PCE)	AOC3 Horizon A (50 ppb PCE)	AOC3 Horizon C (50 ppb PCE)	AOC2 Composite (150 ppb PCE)	AOC3 Autoclaved (150 ppb PCE)
	Performed in Duplicate		No duplicates		No duplicates	No duplicates
0	40 mL SS + 75 mL AGW + 6 mL EOS + 150 ppb PCE	40 mL SS + 75 mL AGW + 6 mL EOS + 150 ppb PCE	40 mL SS + 75 mL AGW + 6 mL EOS + 50 ppb PCE	40 mL SS + 75 mL AGW + 6 mL EOS + 50 ppb PCE	40 mL SS + 75 mL AGW + 6 mL EOS + 150 ppb PCE	40 mL SS + 75 mL AGW + 6 mL EOS + 150 ppb PCE
1						
2						
3						
4						
6						
8						
12						
16						

SS: Soil suspension (consists of 12g soil and 40mL water)
AGW: Artificial groundwater
PCE: Tetrachloroethylene Stock Solution diluted to indicated concentration
EOS: EOS© Pro soybean oil emulsion from EOS Remediation

Each microcosm was made in a 125 mL serum vial with 40 mL of soil suspension (12 grams of soil in 40 mL of water), 75 mL of artificial groundwater, and 6 mL of EOS Pro® Soybean Emulsion (www.eosremediation.com), and a spike of PCE. The EOS soybean oil was from EOS Remediation and contained approximately 60% soybean oil, 24% water, 2% yeast extract, 10% emulsifier, and 4% lactic acid/sodium lactate. The product information is included in Appendix B: EOS Product Information. The artificial groundwater recipe, shown in Table 3, was based on a recipe from Ferris et al. (Ferris, Phoenix, Fujita, & Smith, 2004) and modified to match the Ft. Riley site chemistry at AOC3. The artificial groundwater supplies terminal electron acceptors to the microbes in the sediment. The microcosms were made anaerobic by purging the headspace with nitrogen gas and sealing each serum bottle with a rubber stopper and aluminum

crimp. During the experiment, the samples were placed on a MaxQ orbital shaker (Thermo Scientific) and mixed continuously at 150 rpm and 20°C.

Table 3: Artificial groundwater recipe

	Concentration (mM)	Concentration (mg/L)
KNO_3	0.04	4.1
$MgSO_4$	1.40	134.5
$CaCl_2$	1.75	194.2
$NaNO_3$	0.044	3.74
$NaHCO_3$	4.00	400.3
$KHCO_3$	0.0623	6.24
PO_4*	0.008	0.76

* phosphate added as KH_2PO_4

2.1.b. Collecting Soil Cores

The Kansas Geological Survey used a 2008 USEECO Roto-Sonic Drill to collect continuous sediment cores from AOC2 and AOC3. The drill is able to take sample cores to a depth of 350 feet using casing with a 63 mm or 100 mm diameter and an AquaLok sampler. The AquaLok sampler takes samples in the saturated zone (below the water table). Surface casing usually does not need to be used with shallow samples (above 30 meters). The Roto-Sonic drill is mounted on a 6 by 6 tandem axle truck. A smaller vehicle accompanies the Roto-Sonic drill to provide fuel and water. To prevent contamination of the sediment cores, liners were used to contain the sediment. The drill and liners were rinsed with water and ethanol before coring as well as in between core samples to eliminate dissolved solids, sediment or biological contaminants. Dig Safe flagged all utility lines prior to obtaining the core samples.

The coring location in AOC3 was chosen near well DCF02-42 because the other wells in AOC3 were over railroad tracks in a Bald Eagle nesting conservation area. The sediment core from AOC3 was sampled near groundwater monitoring well DCF02-42 and was cored to 28.2

feet (8.6 meters) until bedrock was encountered. The saturated zone was not reached at this depth, although the deeper cores had a higher water content. Cores from AOC2 were obtained near well DCF06-40 and went to a depth of 45.3 feet (13.8 meters), which reached the saturated zone. The sediment cores were collected in one meter segments and were stored at 4°C overnight before they were used to prepare the microcosms.

The "A" horizon microcosms were filled with sediment from the 3-4 meter segment, which corresponded to the shallow permanganate injections in AOC3. The "C" microcosms were filled with the last sediment core taken (8-8.6 meters). The 2006 permanganate "deep" injections were performed at 20-26 feet (6-8 meters) in AOC3.

The bench-scale microcosm study included a positive control from AOC2, designed to replicate the previous successful degradation of PCE. In 2010, an oil emulsion was injected into AOC2 soil, near well DCF93-13, and stimulated the biodegradation of PCE. The PCE concentrations decreased, and were transformed to its degradants - 44.9 µg/L of cis-1,2,-DCE and 6.6 µg/L were measured in April 2014. This demonstrates that the native bacteria are able to bioremediate the PCE in AOC2. Therefore, the soil in AOC2 that has active bioremediation was used in the microcosm study as a positive control. The study also included a negative control, with autoclaved soil from AOC3, to act as quality control sample and to measure the effects of the microbially inactive microcosm matrix on PCE concentrations.

2.1.c. Microcosm Sampling

The microcosms contained a matrix with soil, artificial groundwater, emulsified soybean oil, and PCE, which is a chlorinated volatile organic compound (CVOC). Several steps were required to prepare the sample to be analyzed by solid phase microextraction (SPME) with gas

chromatography-mass spectrometry (GC/MS). The samples were first centrifuged to separate out the soil from the supernatant. Because PCE and its daughter products are volatile compounds, care was taken to ensure the time the sample was exposed to the air was minimized. An air tight syringe was used to transfer sample from the microcosm serum bottle to the glass centrifuge tube. After centrifuging at 4000 rpm for five minutes, a syringe was used to transfer the supernatant to five 12 mL glass vials, leaving no head space. The 12 mL samples were preserved using a pellet of mercuric chloride to kill all microbes and ensure that no further bioremediation occurred. The 12 mL samples were analyzed by GC/MS with solid phase microextraction (SPME) to determine the concentration of PCE and its degradants, and by ion chromatography (IC) and inductively coupled plasma with optical emission spectroscopy (ICP-EOS) to determine the changes in groundwater chemistry.

After the samples for the GC-MS, IC and ICP-EOS were taken from the microcosm, the remaining sample in the microcosm was used for DNA analysis. Three 13 mL samples of the soil slurry were pipetted with a disposable, sterile pipette into pre-weighed sterile centrifuge tubes. After centrifugation at 4000 rpm for 5 minutes, the supernatant was poured off, and the DNA was extracted from the remaining soil sample.

2.2 PCE and Degradation Intermediate Analysis with Gas Chromatography

The concentrations of PCE, TCE, cis-1,2-DCE and VC were measured with (GC/MS) (Agilent Technologies 7890B with 5977A MSD, Santa Clara, California) over the course of the 16 week study. Standard methods 6232B and 6040D (APHA, 2012) were used for the GC/MS analysis. Before the CVOCs in the microcosms were analyzed by GC/MS, the compounds were

extracted from each sample's water/oil matrix using a technique called solid phase microextraction (SPME), an alternative to extraction procedures such as purge and trap, headspace, and liquid-liquid extraction (Popp & Paschke, 1997). SPME was chosen as the best method to determine the concentration of CVOCs based on the equipment available in the laboratory and its low detection limits.

The SPME technique involves use of an adsorptive fiber, which is exposed to either the liquid sample or the headspace above the sample. In this study, the SPME fiber was attached to a manually operated holder with a spring loaded plunger and protective needle. When the plunger is pushed, the SPME fiber is lowered from the protective needle, and the fiber is exposed. A 75μm carboxen-polydimethylsiloxane fiber (SUPELCO, Bellefonte, Pennsylvania) was used in this study; it is a silica-based solid phase, which works best with volatile polar compounds (Popp et al., 1997). Polydimethylsiloxane-divinylbenzene and divinylbenzene-carboxen-polydimethylsiloxane fibers were compared with the carboxen-polydimethylsiloxane fiber, and more precise and accurate results were achieved with the carboxen-polydimethylsiloxane fiber when tested with a standard containing CVOCs.

The CVOCs captured on the fiber were then desorbed in the heated inlet of the GC (230°C) and analyzed using standard protocols. In this study, the fiber was exposed to the headspace, so there are two equilibria involved: (1) the partitioning between the liquid sample and the headspace, and (2) the partitioning between the headspace and the fiber. An increase in temperature produces an increase in the amount of a given CVOC in the headspace, but a decrease in the efficiency of adsorption from the headspace to the fiber. Thus, there is an optimum temperature, which varies for each compound. It also varies with the amount of EOS in the liquid sample, and that amount decreased with time as the study proceeded. The boiling

points of the CVOCs are 121.1°C for PCE, 86.7°C for TCE, 55°C for DCE, and -13.4°C for VC (Giaya, Thompson, & Denkewicz Jr, 2000). The boiling points were considered when determining the optimal temperature for extraction with the SPME fiber. 55°C was used as the extraction temperature because it was around the midpoint of the boiling points, and the emulsified oil in the sample burned and turned brown above 60°C.

To perform the extraction, each 12 mL sample obtained during microcosm sampling was poured into a 15 mL vial containing 1 gram of salt. The salt was used to increase the ionic strength and promote volatilization of the CVOCs to the headspace in the vial. The vial was capped with a silicon/polytetrafluoroethylene septum, and partially submerged in a water bath (on a Fisher Scientific Isotemp heat and stir plate) to maintain temperature. The SPME fiber was inserted into the vial and kept there during the 10 minute extraction. During the extraction of each sample, a temperature of 55°C ± 1°C was maintained. A calibration curve was made with standards containing EOS, so that the concentrations of CVOCs in the samples could be determined from the peak areas of the standards. An internal standard, 1,2-dichloroethane, was also added to each sample (at 20 ppb), so that the calculated CVOC concentration could be adjusted based on the relative response of the compound of interest to the internal standard. A method of standard additions was tested to compare the results using the internal standard. A known aliquot of standard (containing PCE, TCE, cis and trans-DCE and VC) was added to selected samples, and measured to determine the response of the standard. This method was repeated at different concentrations of standard to get a plot of the amount added versus the peak area measured by GC/MS. A linear regression was performed on the plot and the concentration of a given contaminant in the sample was determined by extrapolating the line of fit to the

concentration axis at zero signal. Because the data did not fit the linear regression well, this method (standard additions) was not successful.

The method detection limits (MDLs) for aqueous samples were determined using standard method 1030C with seven replicate artificial groundwater samples spiked with the compounds of interest at concentrations near the reported MDL (APHA, 2012). The detection limits for PCE, TCE, trans-DCE, cis-DCE, and VC in aqueous samples are reported in Table 4. The low MDL levels are indicative of a very sensitive instrumental response and a very sensitive analysis for aqueous samples relative to the concentrations of interest (initial concentrations of 50 and 150 ppb in the microcosms). These MDLs are applicable to the groundwater samples obtained from the monitoring wells at Fort Riley.

Table 4: Method detection limits for chlorinated compounds in aqueous samples

Compound	MDL (ppb)
VC	0.15
trans-DCE	0.15
cis-DCE	0.10
TCE	0.13
PCE	0.17

MDLs were also determined for PCE, TCE, cis-DCE, trans-DCE, and VC with the soybean oil emulsion present, and are shown in Table 5. The MDLs for the two-phase system were higher than for the aqueous system, but SPME with GC/MS was quite sensitive to the CVOCs at the concentrations present at the Ft. Riley site and used in the microcosm study (initial concentrations of 50 and 150 ppb). However, there were difficulties determining CVOC concentrations due to inconsistent partitioning of the CVOCs into the oil emulsion as it degraded over time. For this reason, biodegradation was documented with the appearance of the

degradants of PCE, and changes in DNA concentration and terminal electron acceptors, instead of focusing on the measured concentration of PCE over time.

Table 5: Method detection limits for chlorinated compounds from EOS-containing microcosms

Compound	MDL (ppb)
VC	1.36
trans-DCE	0.87
cis-DCE	0.78
TCE	1.28
PCE	2.23

2.3 Quantification of DNA and 16s rRNA Genes

Quantification of DNA and the 16s rRNA was performed during the microcosm study to measure the total bacterial concentration in the soil over time. Sediment cores from Ft. Riley were sampled for DNA to represent the DNA at Week 0 in the microcosms. Five 1 gram samples were taken from each soil horizon using a flame sterilized utensil and transferred to a 2 mL microcentrifuge tube and stored at -80°C. To sample sediment from the microcosms, soil slurry was pipetted with a sterile pipette into a centrifuge tube, and then the supernatant was removed from the sample, and the soil sample was used for DNA analysis. Using sterile utensils, 1 gram of sediment from each microcosm was weighed, placed into a 2 mL microcentrifuge tube, and stored at -80°C until DNA extraction was performed.

A PowerSoil DNA Kit from MoBio was used to isolate the DNA from the sediment samples using the recommended protocol with final DNA elution in 60 µL of DNase-free water. A PowerClean DNA Kit from MoBio was used to rid the DNA of PCR inhibitors from the soybean oil emulsion. The DNA concentrations were measured using a Qubit Fluorometer (Life

Technologies, Carlsbad, California) with the double-stranded DNA assay. The DNA extracts were stored in a -80°C freezer until quantitative polymerase chain reaction (qPCR) was performed. The DNA was measured in nanograms of DNA per gram of soil.

qPCR was performed on the DNA extract to amplify the gene of interest and quantify the gene copies of total bacteria. Primers, or short strands of nucleic acid, are used in qPCR as a starting point to synthesize DNA. The 16s rDNA gene was quantified using primers and a fluorescent probe (shown in Table 6) (Harms et al., 2003) using the procedure published by Dr. Belinda Sturm and her colleagues (W. Zhang, Sturm, Knapp, & Graham, 2009).

Table 6: Primers and probe used for 16S rRNA assay

Target gene	Primer name	Sequence	Source
16S rRNA	16S_Forward (1055f)	ATGGCTGTCGTCAGCT	Harms et al.
	16S_Reverse (1392r)	ACGGGCGGTGTGTAC	Zhang et al.
	16S_probe (16STaq1115)	CAACGAGCGCAACCC	

The 16S rDNA analysis used 1 x LD Taq Mastermix (Applied Biosystems) 600 nM primers (as shown in Table 6), 250 nM TaqMan probe, and 2 ng of template. qPCR for 16S rDNA was performed on six dilutions of each sample to determine and control the presence of inhibitory substances. The lowest dilution that amplified the rDNA without inhibition was used for the rest of the samples. A BioRad iCycler with an iCycler iQ fluorescence detector and software version 2.3 performed the qPCR reactions. A calibration curve using a tenfold serial dilution of plasmid DNA standard (3×10^1 to 3×10^5 gene copies/reaction) was made to calculate the gene concentrations. For each qPCR plate a standard curve consisting of five dilutions run in duplicate was generated. A plasmid standard was used for the 16S rDNA assay (Harms et al., 2003). The quantitative detection limit (qLOD) for the 16S rDNA assay was 60 gc/µL DNA. The final concentration of gene copies per gram of soil was calculated from the gene copies

detected in each microliter of DNA, and was multiplied by the dilution factor of DNA for a given sample and the volume of DNA extracted from one gram of original soil sample.

2.4 Groundwater Sampling from Wells at Ft. Riley

Groundwater samples from the monitoring wells listed in Table 7 from 2014 were collected in April, 2015 to characterize the groundwater chemistry associated with the wells and to determine the levels of contaminants present. The concentrations of terminal electron acceptors in AOC3 were of main interest to determine if there is a reduction potential for CVOC bioremediation, which requires the VOC to serve as an electron acceptor. A few wells in AOC1 and AOC2 were tested for comparison of geochemical parameters, since there is active bioremediation in this area. The groundwater monitoring wells that were tested are listed in Table 7 along with their location (AOC) and the concentrations of CVOCs (PCE, TCE, DCE, and VC). A pump controller and compressor rented from Field Environmental were used to sample the groundwater from the wells. Water was pumped from the wells at less than 500 mL per minute and was purged until the pH and temperature of the water coming out of the well were constant (± 0.2 pH units and ± 0.5°C) for five minutes. The pH, temperature, conductivity and dissolved oxygen were measured in the water coming out of the well using a YSI 556 Multi Probe System. Samples from the wells were filtered using a 144 mm Polycarbonate In-Line Filter Holder (Geotech Environmental Equipment, Inc.) containing a 1.2 µm glass fiber prefilter and a 0.2 µm membrane filter. The groundwater samples were collected in triplicate and stored at 4°C until analyzed.

Table 7: CVOC concentrations for selected groundwater wells measured in April 2014 (US Army Corps of Engineers, 2014)

AOC	Well ID	PCE (ppb)	TCE (ppb)	cis-1,2-DCE (ppb)	VC (ppb)
AOC1	DCF92-01				
AOC2	DCF06-40	0.71 J	0.50 J	12.6	0.33 U
AOC2	DCF93-13	0.26 U	0.41 J	44.9	6.6
AOC2	DCF02-41	0.26 U	0.30 U	74.4	0.57 J
AOC3	DCF02-42				
AOC3	DCF06-25	37.6	3.9	5.2	0.33 U
AOC3	DCF02-44A	22.3	3.9	6	0.33 U
AOC3	DCF02-44C	22	3.7	5.8	0.33 U

U: Analyte was not detected at or above the reported result
J: Analyte was positively identified; the reported result is an estimate

2.5 Measurements of Geochemical Parameters

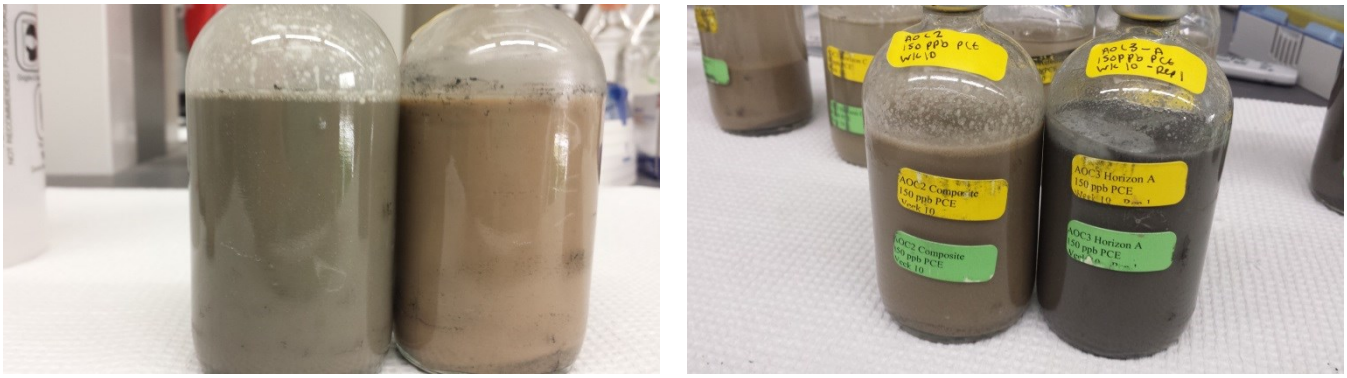
The dissolved ferrous and ferric ion concentrations were measured in field using filtered groundwater samples. The colorimetric ferrozine method was used with Hach method 8147 reagents. Dissolved orthophosphate levels were analyzed on a spectrophotometer at 880 nm using the PhosVer3 (Ascorbic Acid) Hach method 8048, which is a USEPA approved method. Samples not analyzed in the field were taken to a lab at the University of Kansas for geochemical analysis. Alkalinity measurements were taken within two days using a Fisher Titrimeter®II Automatic Titration System and 0.02 N hydrochloric acid. Total organic carbon (TOC) was measured with a Torch Carbon Analyzer (Teledyne Tekmar, Mason, Ohio) on unfiltered samples. Cation analysis was performed on a Perkin Elmer Optima 5300DV ICP-EOS to determine the concentrations of Na^{+}, Ca^{+2}, Mg^{+2}, K^{+}, and Fe. The samples were filtered then acidified using 2% HNO_3 prior to analysis. Anion analysis was performed on filtered samples using a Dionex ICS-3000 IC to determine concentrations of Cl^{-}, SO_4^{-2}, NO_3^{-}, and PO_4^{3-}.

Chapter 3: Results

3.1 Bench Scale Microcosm GC-MS Analysis

The microcosm study was performed over the course of 16 weeks with sampling occurring at 0, 1, 2, 3, 4, 6, 8, 12, and 16 weeks, for a total of 9 sampling points. The appearance of the microcosms was noted during the course of the study. The AOC2 and dead control microcosms, and the AOC3 horizon C microcosms, remained a milky brown color over the course of the study, while the AOC3 horizon A microcosms got darker in color starting at Week 3. The milky color, which came from the soybean oil emulsion, was lost over time in AOC3-A. A picture of the AOC3-A microcosm and dead control is included below in Figure 5. A sulfide odor was also noticed in Week 3 in the AOC3-A and AOC2 microcosms.

Figure 5: (Left) Week 3 AOC3A microcosm. The microcosm on the right is the dead control and the one on the left is AOC3A, which shows a darker color and loss of the milky appearance from the soybean oil emulsion. (Right) By week 10, the milky color of AOC3A on the right was completely gone, and the microcosm appeared black.



At the beginning of the study (Week 0), the microcosms were spiked with a solution PCE, made from the neat chemical. Therefore, evidence of biodegradation in the microcosms can be shown by the appearance of the degradants TCE, cis-DCE, trans-DCE, and VC. The anaerobic pathway for biodegradation is shown in Figure 6 (Parsons, 2004). The kinetics for

each step are shown in Table 8 from a reference textbook (Haston & McCarty, 1999). The slowest steps in the reductive dehalogenation process are the conversion of cis-DCE or trans-DCE to VC and VC to ethylene, so there is potential for DCE and VC to accumulate during bioremediation.

Figure 6: Degradation of PCE by reductive dehalogenation (Parsons, 2004)

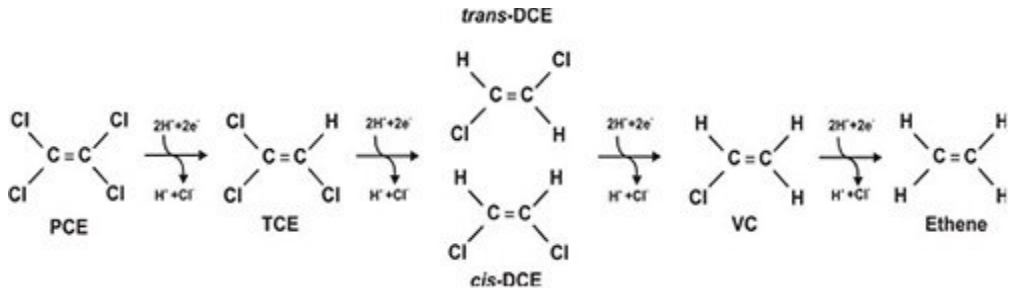


Table 8: Kinetic constants for 38 mg/L PCE culture at 25°C

	kX **± 95% CI (μM/day)**	**K_s values ± 95% CI (μM)**
PCE	77 ± 5	0.11 ± 0.04
TCE	59 ± 11	1.4 ± 0.9
cis-DCE	14 ± 3	3.3 ± 2.2
VC	13 ± 3	2.6 ± 1.9

In Figure 7 through Figure 11, the concentrations of PCE, TCE, cis-DCE, trans-DCE and vinyl chloride are shown for the AOC3-A, AOC3-C and live and dead control (positive and negative control) microcosms. In each figure, the top graph shows results for the AOC3-A microcosm at a 150 ppb initial PCE concentration, the middle graph for AOC3-C at 150 ppb PCE, and the lower graph for both AOC2 (positive control) and the dead control made from autoclaved AOC3 soil and containing an initial PCE concentration of 150 ppb. The 50 ppb microcosms did not show any degradation during the study, as evidenced by the absence of

biodegradation intermediates. Non-detects are reported as one-half of the detection limit of the compound.

The partitioning of PCE into the emulsified soybean oil phase and the change in partitioning behavior over time impacted the concentrations of PCE presented in Figure 7. The concentration of PCE for AOC3-C and AOC2 increased over time, and occasionally increased beyond the initial spike concentration of 150 ppb PCE. The PCE concentration in AOC3-A and the AOC3 dead control decreased towards the end of the study. As later explained in Chapter 4, decreases in PCE concentration were not considered to provide a reliable indication that bioremediation occurred, since the PCE results were evidently being compromised by variability in the partitioning of PCE into the oil phase. Instead, the presence of PCE degradation products (TCE, DCE, and VC) was used as evidence for biodegradation of PCE.

The microcosms with sediment from AOC3 Horizon A with an initial PCE concentration of 150 ppb showed the highest concentrations of biodegradation products. In both replicates of the AOC3 Horizon A 150 ppb microcosms, biodegradation intermediates were observed. During the first two weeks 1-4 ppb of TCE, 1-4 ppb trans-DCE, and 2 ppb cis-DCE were measured in both replicates. Increasing concentrations of vinyl chloride (2-9 ppb) were consistently observed starting at Week 6 in the AOC3 Horizon A microcosms. Biodegradation intermediates were also observed in the AOC3 Horizon C microcosms for Weeks 1 and 2, as well as Week 12, when 3.2 ppb TCE, and 2.1 ppb trans-DCE were measured by GC/MS. The AOC2 microcosm showed low levels of degradants during the first two weeks, but no degradation intermediates were detected for the remainder of the study.

The presence of biodegradation intermediates – TCE, trans-DCE, cis-DCE, and vinyl chloride – is evidence that biological remediation of the PCE occurred in the microcosms containing sediment from AOC3-A and AOC3-C. Blanks run with the SPME fiber were consistently clean, with no PCE or degradation products measured, which assures the detection of biodegradation intermediates in the AOC3-A and AOC C microcosms is true. According to the kinetic constants, the reaction from DCE to VC is the slowest step in reductive dehalogenation of PCE, so the higher concentrations of vinyl chloride observed toward the end of this study are as expected when bioremediation is in progress.

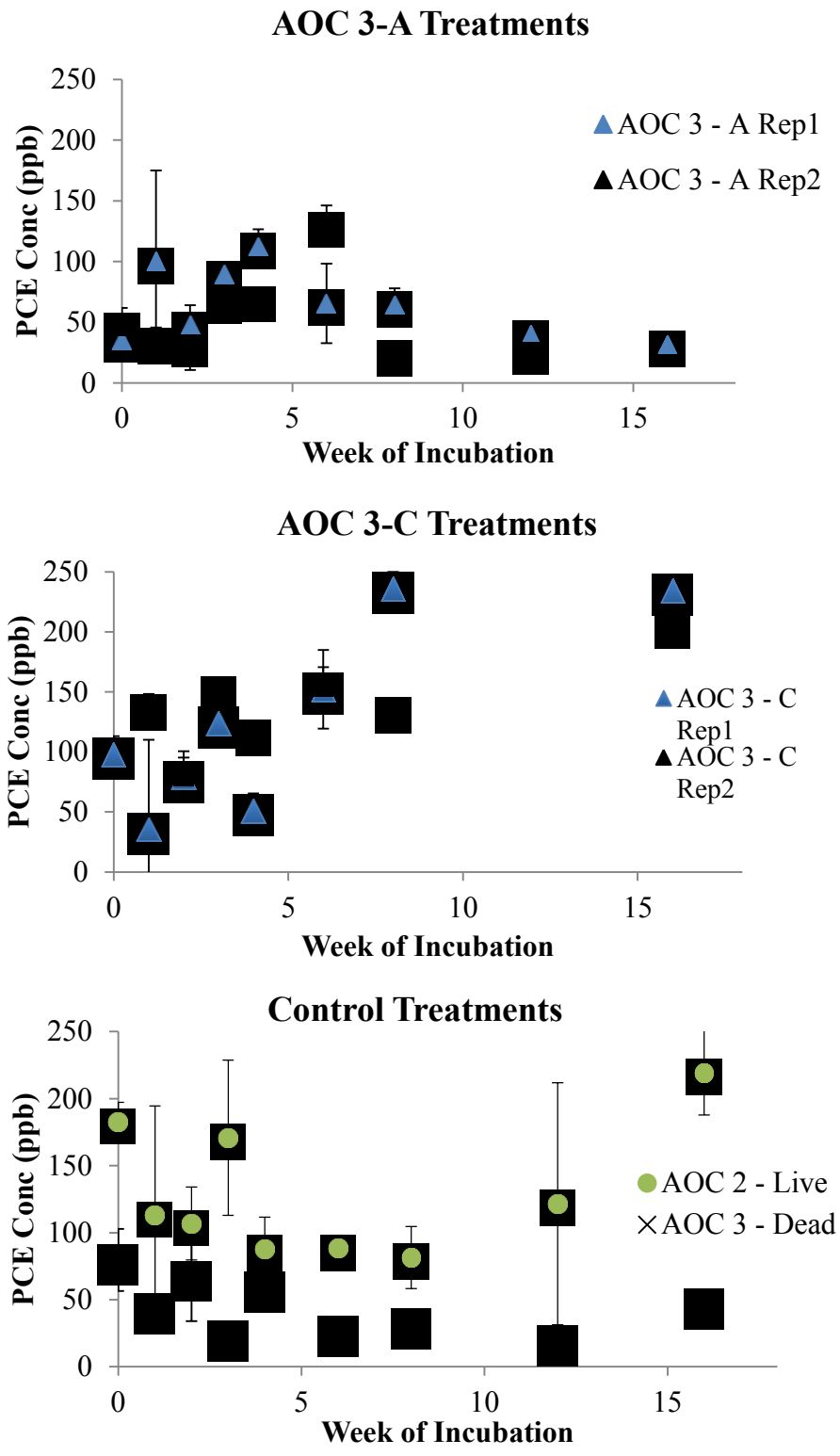


Figure 7: PCE concentrations measured by GC-MS during the 16 week study. The plot at the top shows the AOC3-A 150 ppb microcosms. The middle plot is the AOC3-C 150 ppb microcosms. The bottom plot shows the controls – AOC2 (live) and AOC3 (dead). Non-detects were measured as one half the limit of detection.

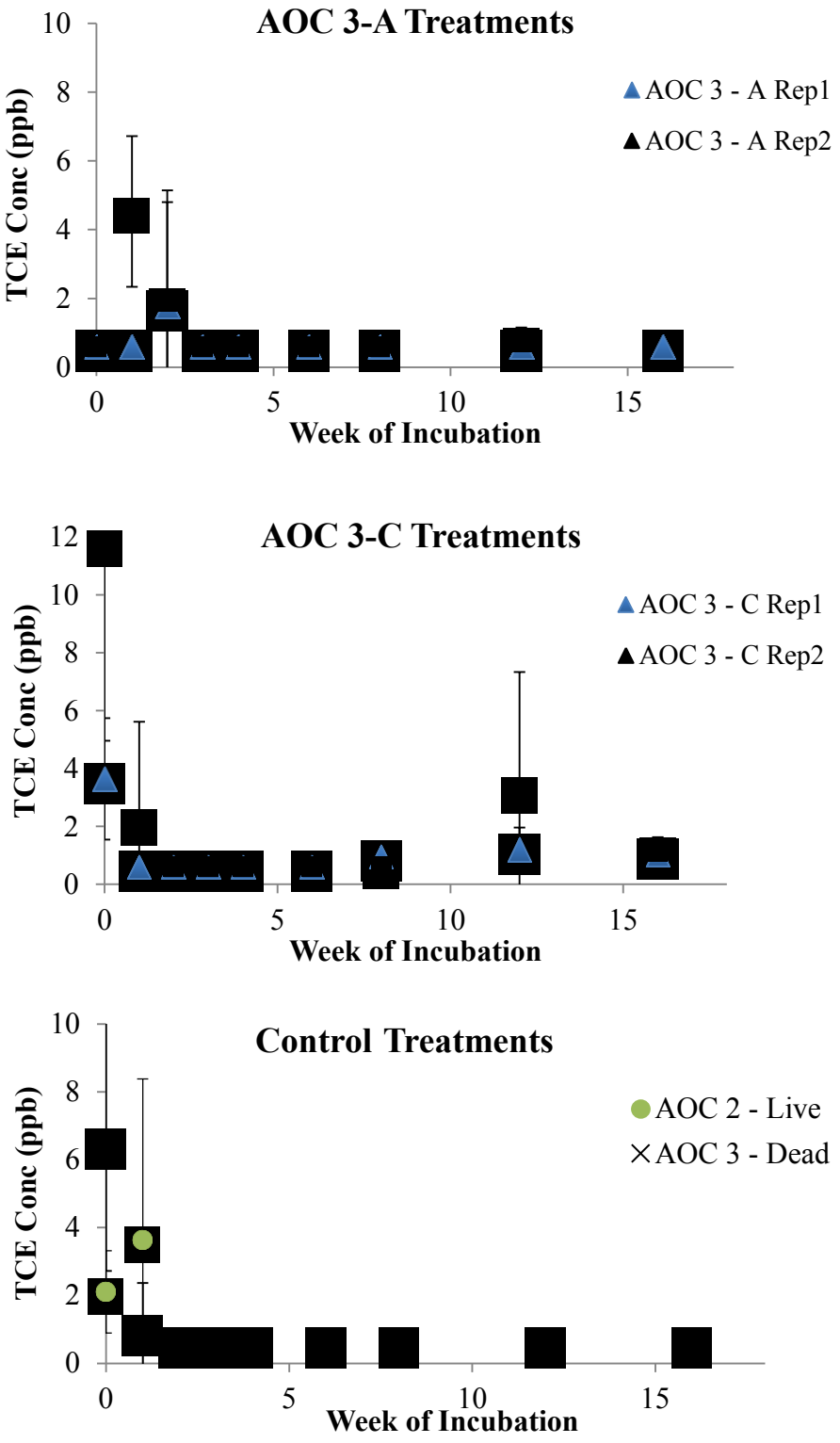


Figure 8: TCE concentrations measured by GC-MS during the 16 week study. The plot at the top shows the AOC3-A 150 ppb microcosms. The middle plot is the AOC3-C 150 ppb microcosms. The bottom plot shows the controls – AOC2 (live) and AOC3 (dead). Non-detects were measured as one half the limit of detection.

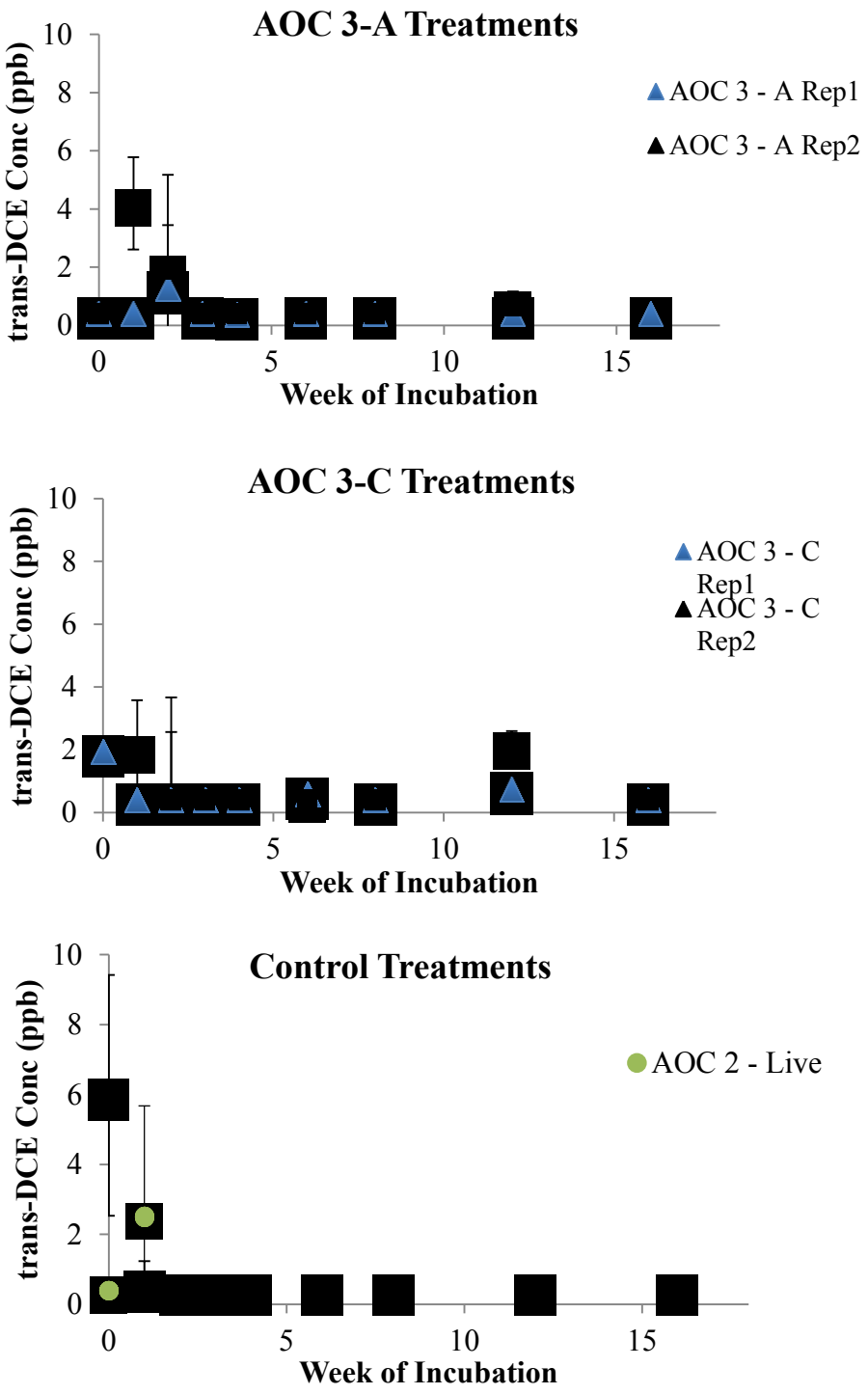


Figure 9: trans-DCE concentrations measured by GC-MS during the 16 week study. The plot at the top shows the AOC3-A 150 ppb microcosms. The middle plot is the AOC3-C 150 ppb microcosms. The bottom plot shows the controls – AOC2 (live) and AOC3 (dead). Non-detects were measured as one half the limit of detection.

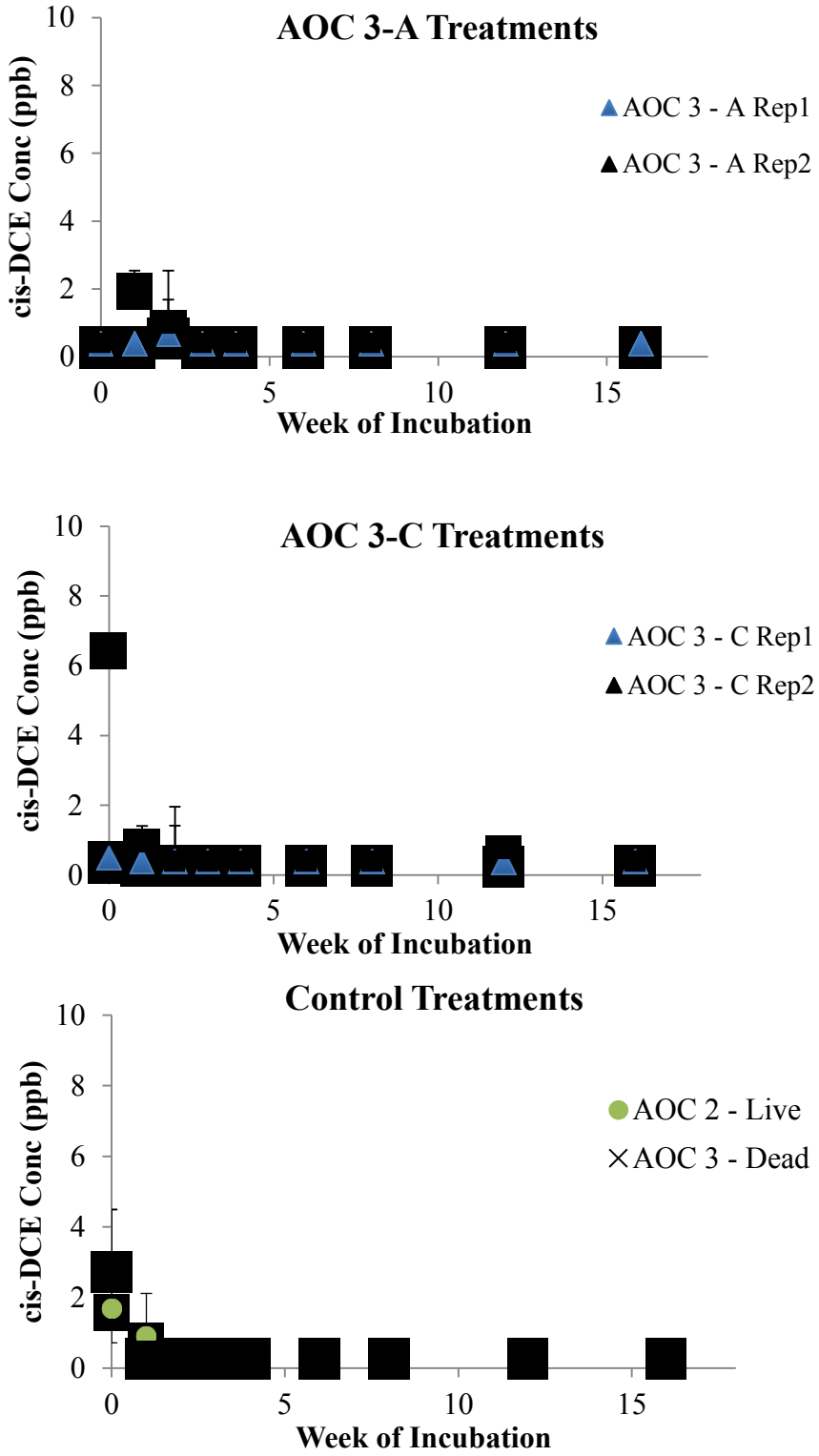


Figure 10: cis-DCE concentrations measured by GC-MS during the 16 week study. The plot at the top shows the AOC3-A 150 ppb microcosms. The middle plot is the AOC3-C 150 ppb microcosms. The bottom plot shows the controls – AOC2 (live) and AOC3 (dead). Non-detects were measured as one half the limit of detection.

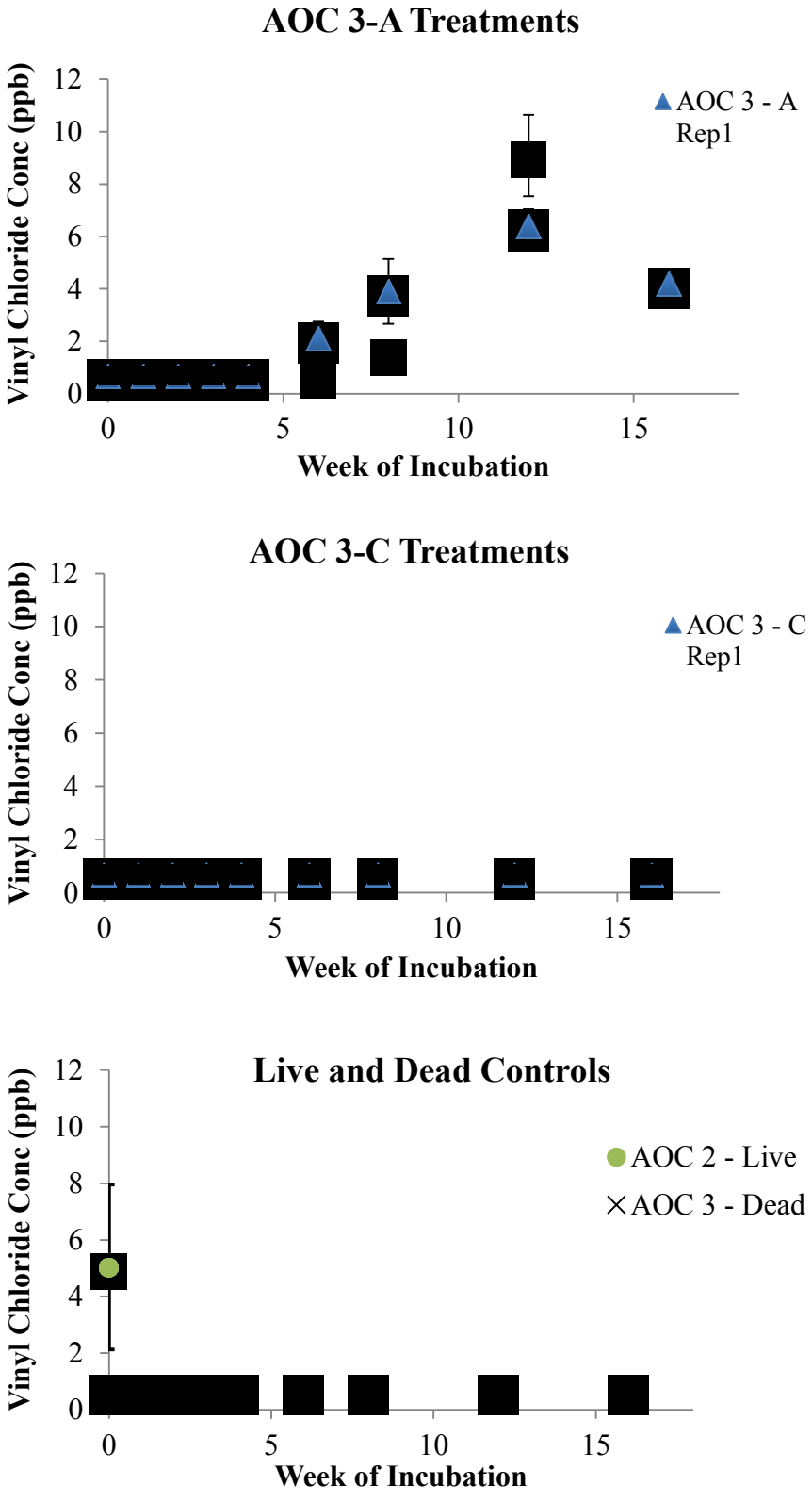


Figure 11: Vinyl chloride concentrations measured by GC-MS during the 16 week study. The plot at the top shows the AOC3 A 150 ppb microcosms. The middle plot is the AOC3 C 150 ppb microcosms. The bottom plot shows the controls – AOC2 (live) and AOC3 (dead). Non-detects were measured as one half the limit of detection.

3.2 Microbial Growth Evidenced by DNA and 16s rRNA Quantification

DNA was extracted from the microcosms during Weeks 0, 3, 6, 12, and 16. Quantitative PCR was performed in order to quantify the total number of copies of 16S rRNA, a universal gene for bacteria. The bacterial gene concentrations in the microcosms were quantified as gene copies per gram of soil (gc/ g soil), and are shown in Figure 12. The DNA data show that autoclaved (dead) controls did not support bacterial growth. AOC3-A microcosms contained the highest concentrations of DNA during the majority of the study (Weeks 0 through 12) and dropped off at the ending measurement taken at 16 weeks. The drop in bacterial concentration in AOC3-A from Week 12 to 16 also corresponds to a decrease in VC concentration. In Week 12, the concentrations of VC in AOC3 Horizon A ranged from 6.4 to 9.1 ppb, and dropped to 4.2 ppb in Week 16. It is possible that the VC that had been produced starting in Week 6 was toxic to the bacteria, and inhibited growth from Week 6 to the end of the study. As shown in a study by Wackett et al, the metabolism of TCE inhibited the growth of *P. putida* F1 due to the cellular toxicity of the intermediates formed (Wackett & Householder, 1989). Other bacteria commonly found in the soil that bioremediate PCE are likely also inhibited by the degradants formed in the dechlorination of PCE. The DNA results agree with this hypothesis as the bacteria concentration started decreasing in Week 6.

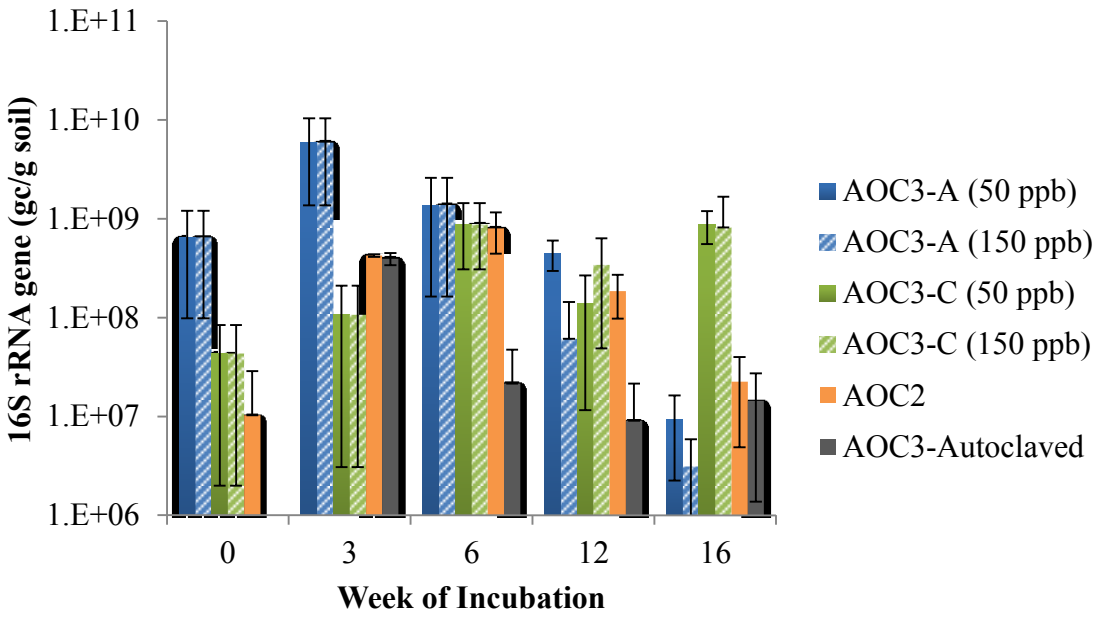


Figure 12: Total DNA concentrations in soil harvested from the microcosms over time

3.3 Biogeochemistry Analysis by ICP-EOS and Ion Chromatography

The terminal electron acceptors nitrate (NO_3^-), ferrous iron, and sulfate (SO_4^{2-}), and bicarbonate (HCO_3^-), and phosphate (PO_4^{3-}) were analyzed for each sampling point in the 16 week study. Ferrous iron was measured as total dissolved Fe, because the only viable source of dissolved iron at neutral pH is microbially mediated reduction of sediment-bound iron oxyhydroxides (Konhauser, 2007). After oxygen is depleted, subsurface organoheterotrophic microbes use sulfate as the electron acceptor. A redox tower is shown in Figure 13, where the terminal electron acceptors with the highest redox potential are preferred. Nutrient depletion during biodegradation in the microcosms was assessed by examining the dissolved phosphate concentrations. Bicarbonate was used to determine the amount of organic carbon that was converted into inorganic carbon.

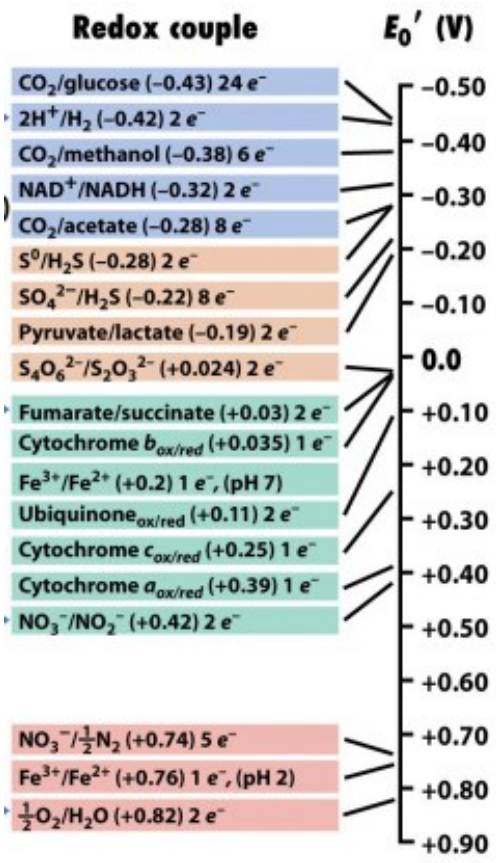


Figure 13: Redox tower (Madigan, Martinko, Dunlap, & Clark, 2009)

The bicarbonate concentrations (shown in Figure 14) increased in all microcosms except those for the AOC3 Horizon C 50 ppb treatment and the dead control. This indicates that organic carbon was converted to inorganic carbon in these microcosms through microbial oxidation of the emulsified soybean oil or PCE and its biodegradation intermediates. The AOC3-A and AOC3-C 150 ppb microcosms showed the greatest increases in HCO_3^- over the study, supporting other evidence of bioremediation seen in these microcosms, i.e., the presence of degradation intermediates.

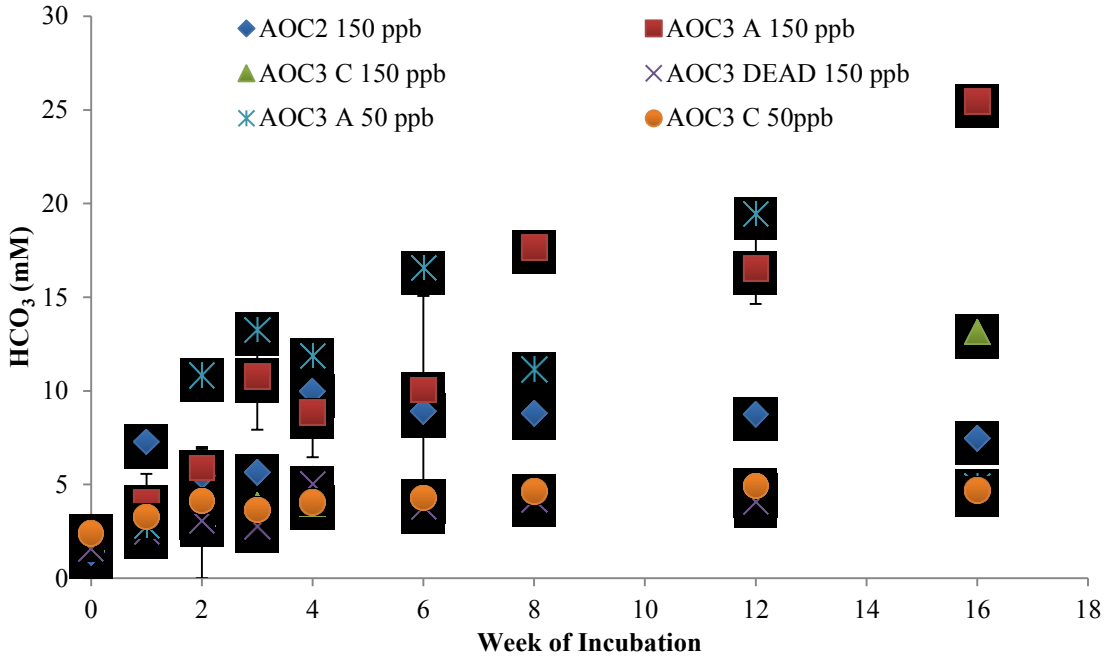


Figure 14: Bicarbonate concentrations in the microcosms over time

The dissolved phosphate concentrations were low in all of the microcosms as shown in Figure 15. In the AOC3-A 150 ppb microcosms, the concentration of dissolved phosphate increased from Week 0 to 6, although there was high variability in the data. At Week 16, there was no measurable amount of dissolved phosphate available in the AOC3-A and C 150 ppb microcosms. Phosphate was likely the limiting nutrient in the microcosms.

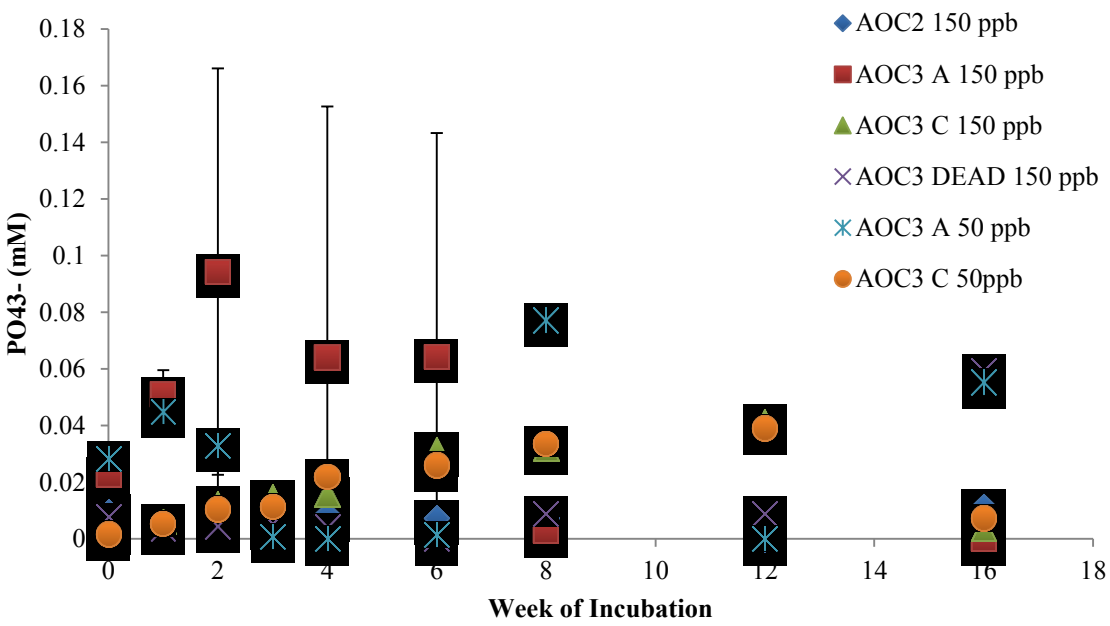


Figure 15: Phosphate concentrations in microcosms over time

The concentrations for the terminal electron acceptors (TEAs) nitrate, iron (II), and sulfate are shown in Figure 16 through Figure 18. The MDLs for these parameters are listed in Table 11. The data for these TEAs were consistent with the bicarbonate data and CVOC data because the TEA concentrations indicate a decrease in redox potential over the course of the study. As the organic carbon was oxidized to bicarbonate, the terminal electron acceptors were reduced. Nitrate concentrations were low for all of the microcosms and were around the concentration of the method detection limit, except for the dead control which showed slightly a slightly higher concentration of nitrate for most of the study. The nitrate added to the microcosms with the artificial groundwater was 0.05 mM (shown in Table 3), whereas the concentrations of nitrate beginning in Week 1 are less than 0.05 mM. The rapid drop in nitrate concentration between Week 0 and Week 1 indicates it was used as a terminal electron acceptor for the conversion of organic carbon to inorganic carbon. There was some variation of nitrate in

Weeks 6, 12 and 16 due to analytical error and the low levels of nitrate initially present in the microcosms.

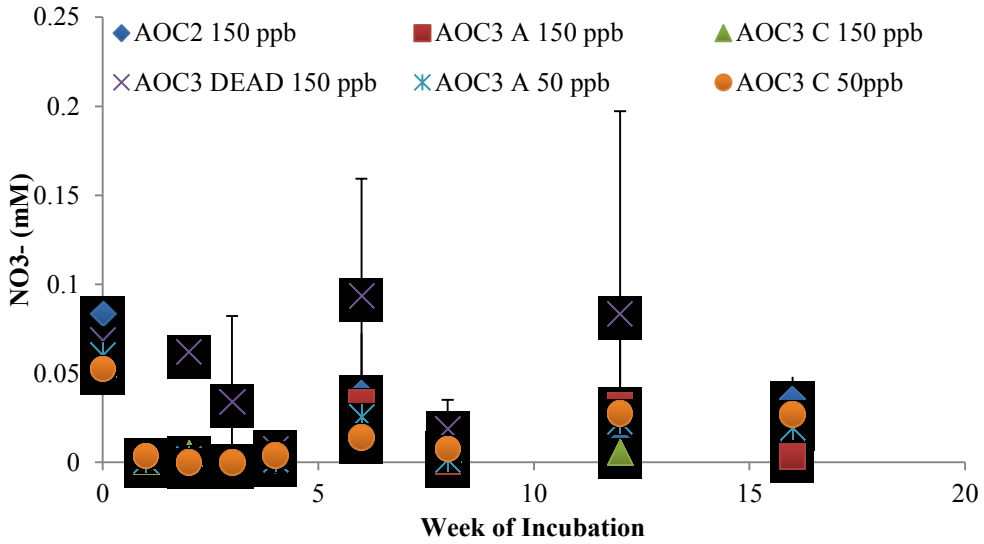


Figure 16: Nitrate concentrations in the microcosms over time

Iron can change oxidation states from ferric iron to ferrous iron when it is microbially reduced. The AOC3-A 150 ppb microcosms showed an increase in ferrous iron around Week 8, indicating there was microbial iron reduction occurring. The ferrous iron concentration decreased to below the detection limit in Week 12, which is likely due to precipitation of iron sulfide minerals after sulfate reduction. The microcosms AOC3-A 50 ppb and AOC3-C 150 ppb showed the presence of ferrous iron in Weeks 12 and 16, however, there was high variability in the AOC3-C 150 ppb data, so it is unclear if there was microbial activity towards the end of the experiment, or if the results are anomalous.

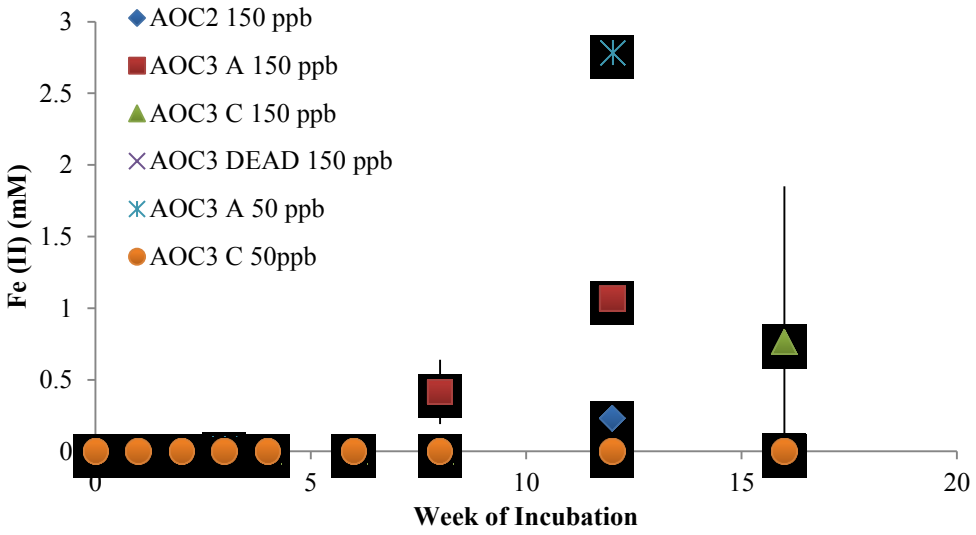


Figure 17: Iron (Fe(II)) concentrations in microcosms over time

Figure 18 shows the concentrations of sulfate in the microcosms over time. The concentration of sulfate in AOC3-A started rapidly decreasing in Week 6, when CVOC degradant concentrations increased. The other microcosms did not exhibit this decrease in sulfate concentration except for an anomalous data point for AOC3-A 50 ppb in Week 6.

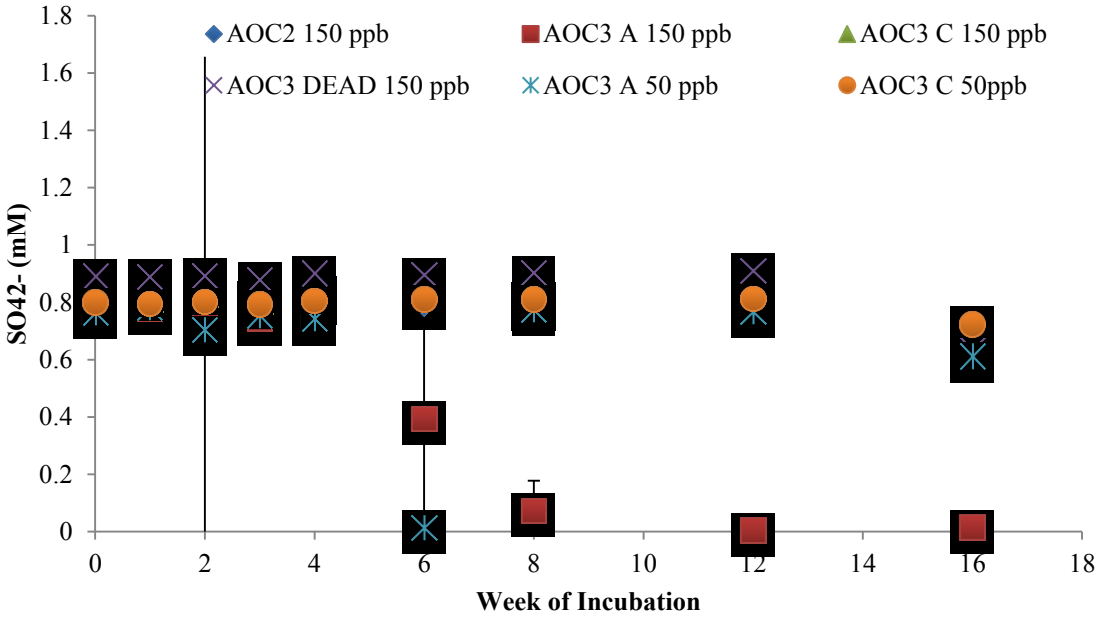


Figure 18: Sulfate concentrations in microcosms over time

In the AOC3-A 150 ppb microcosms the decrease in nitrate concentration, presence of ferrous iron, and use of sulfate is consistent with the redox reactions that occur during microbial oxidation of organic carbon. The geo-chemistry in the microcosms compares favorably with the DNA and GC-MS data, also indicating that microbial degradation was occurring during Weeks 6 – 12. Although the air was purged with nitrogen before the microcosms were sealed, it appears that it took until approximately Week 6 for the microcosms to become fully anaerobic. During Week 3, there was an increase in DNA concentration in AOC3-A 150 ppb, followed by degradants concentrations that appeared in Week 6. The concentration of phosphorus needed for microbial metabolism likely limited the growth of bacteria capable of biodegradation. Reductive dechlorination of PCE occurs under conditions that support nitrate and iron reduction, but degradation of TCE and Vinyl Chloride requires strongly reducing conditions that happens through sulfate reduction or methanogenesis. (Vogel, Criddle, & McCarty, 1987).

3.4 Groundwater Monitoring at Ft. Riley

Prior to the setup of the microcosms, groundwater sampling was performed on the monitoring wells. For each monitoring well the following tests were performed in the field: water level, pH, temperature, dissolved oxygen, conductivity, ORP, and dissolved ferrous and ferric iron. Samples brought back to the lab were tested for alkalinity, TOC, cations, anions, and terminal electron acceptors. The results are shown in Table 9 through Table 11, and agree with the results of the groundwater monitoring performed in 2014. Well DCF02-42 was a dry well, so no data on aqueous geochemistry could be obtained. The MDLs shown in Table 11 were determined for an aqueous system.

Table 9: Field parameters for groundwater wells sampled on March 27, 2015

Well	AOC	Water Level (ft)	Volume Purged (L)	pH (S.U.)	Temp (°C)	DO (mg/L)	Conductivity (µS/cm)	Alkalinity (mg $CaCO_3$ /L)	ORP
DCF92-01	1	42.1	14	6.42	14.54	3.36	1.322	463.9	119
DCF06-40	2	40.3	10	6.28	14.57	0.93	1.833	852.9	14.5
DCF02-41	2	19.3	10	6.51	13.48	1.18	1.547	657.9	-59.3
DCF93-13	2	35.8	10	6.45	14.1	2.57	1.618	480.6	-42.2
DCF02-44A	3	22.2	8	6.46	13.17	4.53	1.555	562.9	160.5
DCF02-44C	3	22.2	8	6.55	13.41	1.90	1.287	489.4	143.7
DCF06-25	3	21.8	8	6.49	13.48	2.28	1.459	530.4	158.5
DCF02-42	3	Dry Well	-	-	-	-	-	-	-

The CVOC concentrations in the monitoring wells were similar to those measured previously in 2014. In AOC3 there continue to be high concentrations of PCE (from greater than 15 ppb to 42 ppb), and much lower concentrations of bioremediation intermediates (TCE, cis-DCE, trans-DCE, and VC). In AOC2, where there is active bioremediation occurring, the PCE concentrations were much lower, ranging from 2-5 ppb, and there were degradation intermediates present. The concentrations of cis-DCE included the highest observed, ranging from 12-50 ppb. The lower concentrations of PCE and higher concentrations of intermediates confirms that active bioremediation is taking place in AOC2.

Table 10: PCE and degradation concentrations during field sampling

Well	AOC Location	PCE (ppb)	TCE (ppb)	cis-DCE (ppb)	trans-DCE (ppb)	VC (ppb)	Alkalinity (mg $CaCO_3$ /L)	TOC (mg/L)
DCF92-01	1	3.8	2.35	0.12	0.16	<0.15	464	2.16
DCF06-40	2	5.49	4.03	12.2	0.23	0.33	853	3.36
DCF02-41	2	2.29	2.35	49.8	1.14	0.81	658	1.95
DCF93-13	2	3.69	2.89	41.6	2.25	<0.15	481	2.22
DCF02-44A	3	15.6	2.31	1.79	<0.15	<0.15	563	2.50
DCF02-44C	3	34.7	3.59	2.6	0.7	<0.15	489	1.52
DCF06-25	3	42.1	6.92	4.97	0.92	<0.15	530	1.88

The geochemical data collected in AOC2 and AOC3 show redox conditions in the groundwater consistent with being driven by microbes. As shown in Table 11, nitrate has been depleted in AOC2 where active bioremediation is occurring, which suggests there are nitrate reducing conditions. In AOC1 and AOC3, however, there are still appreciable levels of nitrate present in the groundwater. The levels of ferrous iron in AOC2 also support the evidence of reducing conditions in the groundwater. The geochemistry suggests that iron reduction is ongoing. The sulfate concentrations in the wells are quite high, so sulfate reduction is not significant at current conditions. The levels of phosphate are low in AOC2, which could suggest microbial activity or that phosphate is tied up in the solid phase (ex. ferrous phosphate or ferric phosphate). Most natural groundwaters have very low phosphorus concentrations, which limits microbial growth; therefore, microbial communities may tightly cycle phosphate resulting in low phosphate concentrations (Vogel et al., 1987). The geo-chemical parameters all indicate there are moderate reducing conditions in AOC2 that support reductive dehalogenation of PCE. Stronger reducing conditions such as sulfate reducing conditions may be necessary for the conversion of VC to ethene (Rogers & Bennett, 2004). Sulfate is present in sufficient quantities in the groundwater, so sulfate reducing conditions may happen in time as iron is depleted from the sediments. Sulfate reducing conditions could potentially be stimulated by adding additional organic carbon to the aquifer, provided that sufficient nutrient concentrations are also available, which might require nutrient addition.

Table 11: Cation, anion and terminal electron acceptor concentrations

Well	AOC	Si	SiO_2	Ca	Mg	Na	K	Sr	Ba	Fe (II)
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
DCF 92-01	1	3.67	7.85	218	40.4	96.2	11.9	1.09	0.063	ND
DCF 06-40	2	4.56	9.75	286	57.9	175	11.6	1.56	ND	16.2
DCF 02-41	2	5.11	10.9	250	66.1	113	6.03	6.38	0.107	9.58
DCF 93-13	2	3.06	6.56	237	41.2	160	11.6	1.22	0.046	3.13
DCF 02-44A	3	3.36	7.18	245	56.9	123	7.34	3.51	0.149	ND
DCF 02-44C	3	3.06	6.54	199	50.4	92.1	7.86	3.51	0.118	ND
DCF 06-25	3	3.36	7.20	214	50.4	134	11.9	3.17	0.107	ND
MDL		10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-2}

Well	AOC	Alkalinity	Cl	SO_4	NO_3-N	F	Br	PO_4-P
		(mg $CaCO_3$ /L)	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
DCF 92-01	1	464	299	121	4.09	0.23	0.19	0.065
DCF 06-40	2	853	348	191	1.08	0.31	0.32	ND
DCF 02-41	2	658	291	233	0.01	0.24	0.22	ND
DCF 93-13	2	481	429	119	0.01	0.24	0.19	ND
DCF 02-44A	3	563	384	137	3.57	0.34	0.21	0.075
DCF 02-44C	3	489	282	130	3.10	0.32	0.19	0.068
DCF 06-25	3	530	349	122	5.07	0.30	0.22	0.036
MDL		10^{-2}	10^{-3}	10^{-3}	$3x10^{-3}$	10^{-3}	10^{-3}	$5x10^{-3}$

Chapter 4: Discussion

4.1 Evidence of PCE Degradation through GC-MS Analysis and Data Quality

As shown in Figure 7, the PCE data did not show any trends, which was attributed to inconsistent partitioning of the PCE into the EOS soybean oil. The presence of degradation intermediates was taken as evidence of PCE degradation in the AOC3-A and C 150 ppb microcosms. There were greater concentrations of vinyl chloride in the AOC3-A 150 ppb microcosms, so it appears biodegradation occurred to the greatest extent in the AOC3-A 150 ppb treatment. The emulsified soybean oil in the microcosms interfered with the PCE data because of partitioning of the PCE into the oil phase. The difficulties of measuring the PCE and degradant concentrations are explained in the following paragraphs.

Analysis of PCE and its degradants was more challenging for samples from the microcosms because the EOS product created a multi-phase system with variable partitioning of the CVOCs. Specifically, the soybean oil contained in the EOS (approximately 60% by weight) is largely insoluble and forms a separate organic phase, and the CVOCs of interest have a higher affinity for the organic oil phase than for the artificial groundwater. Also, as the samples are being analyzed using SPME, the oil phase competes for CVOCs with the gas phase in the headspace of the extraction vials. When soybean oil is used in the field, CVOCs sorb, or partition, into the oil phase, retarding mobilization of the contaminants through the aquifer as groundwater is being remediated (EOS Remediation, 2015); however, this also reduces CVOC concentrations in the water phase, complicating groundwater monitoring efforts. The partitioning of the CVOCs makes it difficult to produce consistent analytical results.

At the beginning of the experiment, the emulsified soybean oil in the microcosms started as a white homogenized milky substance. It was observed during sampling of the microcosms that the appearance of oil changed during the course of the experiment and became clearer and formed visible oil droplets. This is likely due to the substrate and emulsifiers in the EOS® being depleted (consumed) by the microbes. The oil droplets likely formed because the emulsion was thermodynamically unstable, so the oil droplets gradually coalesced. Some of the PCE results from the samples measured greater than the 150 ppb of PCE that was added to the microcosm, according to the GC/MS results. This is probably because the calibration curve was made with the white, milky EOS at the beginning of the study, and as the oil emulsion changed over time, there was less partitioning of the PCE to the oil-containing liquid sample, producing greater concentrations in the headspace, greater amounts adsorbed by the fiber, and greater instrumental responses, hence greater calculated PCE concentrations.

Sampling the microcosms to obtain reproducible GCMS results was challenging. The microcosms contained a mixture of artificial groundwater, soil, EOS, and CVOCs. In order to analyze the samples by SPME the samples were centrifuged to remove the soil and preserved by adding mercuric chloride to ensure that no further biodegradation occurred before the samples were analyzed. Three 12-mL samples were taken from each microcosm. The soybean oil floated to the top when the microcosm sample was centrifuged, so it was often difficult to get three samples that contained the same amount of oil. Since the CVOCs partition into the oil, the results from the SPME analysis had low repeatability; however, this was the only method that was able to detect levels of contaminants at the ppb level.

In addition to sampling and analytical challenges, there were likely abiotic losses in the microcosm, as demonstrated by the rapid decrease of PCE in the dead control, which was shown

in Figure 12 to have no growth in bacteria over time. Pfeiffer et al. showed that the injection of soybean oil into the subsurface will result in rapid decreases in chlorinated ethene concentrations due to abiotic losses and partitioning of the compounds into the soybean oil (Pfeiffer, Bielefeldt, Illangasekare, & Henry, 2005).

As explained in the experimental methods section, the method used to determine CVOCs was optimized as much as possible by experimenting with different extraction temperatures and times, inlet GC-MS temperatures and times, SPME fiber types, and an internal standard was used to help correct for the loss of the analyte during sample preparation.

4.2 Comparison of Biodegradation with Soybean Oil to Literature

A bioremediation study by Long and Borden (2006) used soybean oil as the organic substrate in biodegradation of chlorinated solvents. Short chain fatty acids are often used as the carbon source in bioremediation studies, but they are quickly depleted. The immobility of soybean oil in an aquifer is advantageous because it provides a longer lasting substrate for reductive dechlorination. Long and Borden performed laboratory column studies to determine the effects of emulsified soybean oil addition on PCE degradation in a stimulated permeable reactive barrier. Samples from the reactor were collected in a syringe from a port in the reactor. The PCE, TCE and DCE and VC were analyzed with a GC equipped with an FID detector after concentrating the sample in a purge and trap concentrator. The sensitivity of the FID as well the ability to eliminate any contact between the sample and external air prior to injection into the sampling port produced more reliable results for their study (Long et al., 2006).

The study of Long & Borden was successful with complete biodegradation of PCE to ethene, although the columns had to be augmented with a culture of bacteria partway through the experiment to stimulate the reductive dechlorination from cis-DCE to ethene. The degradation of

PCE to cis-DCE was quite rapid in the columns but bioaugmentation with *dehalococcoides ethenegenes* strain 195 was needed to achieve complete biodegradation. Long and Borden found that the single injection of emulsified soybean oil was effective in generating strongly reducing conditions for over 14 months.

A field study performed by Borden (2007a) injecting EOS® into wells to form a biologically active permeable reactive barrier for concurrent biodegradation of perchlorate and chlorinated solvents. The study, which lasted for 2.5 years also indicated that the oil injection generated strongly reducing conditions. As with the column studies, Borden found that the degradation of PCE to cis-DCE was relatively quick, but little VC or ethene was generated, indicating that the biodegradation had stopped with cis-DCE (Borden, 2007a). Both of these studies indicated that bioaugmentation may be necessary in order for complete biodegradation of PCE to ethene to occur.

4.3 Degradation Potential of In-Situ Microbial Community

The DNA data from the microcosms showed that there was growth in the AOC3-A 150 ppb microcosms during the beginning of the experiment. The DNA concentrations then decreased as vinyl chloride was produced in the microcosm. An active microbial community is necessary for bioremediation, and increasing levels of bacteria in AOC3-A at the beginning of the experiment indicated that the bacteria were capable of reproducing. The decline in bacterial concentration after the presence of vinyl chloride was observed shows that the site may need to be augmented with a culture of VC degrading bacteria to sustain biodegradation. Bacterial concentrations increased over time in AOC3-C (with a small drop in Week 12), which showed an active community, although not many degradation intermediates were observed. The microbial

community in AOC3-C could have consisted of a larger portion of bacteria that were unable to degrade PCE. The microbial community in AOC2 grew from Week 0 to Week 6, and then declined. There were no degradation intermediates measured in AOC2, so the decline in bacteria may have been caused by a limiting nutrient concentration, such as phosphorus. It is also possible that the permanganate treatments performed in AOC3-Area in 2006 decreased the natural bacterial concentration in the soil. If so, perhaps the site could benefit from increased levels of bacteria to further promote biodegradation.

The strand *Dehalococcoides etheneogenes* 195 is known to degrade PCE to vinyl chloride and eventually ethane gas (Ritalahti et al., 2006). The last step in the transformation is quite slow, which would explain the accumulation of vinyl chloride in the microcosms. Other strains of bacteria such as *Dehalococcoides etheneogenes* BAV1 is known to more rapidly degrade vinyl chloride to ethene. Field studies that used bioaugmentation of *dehalococcoides* strands showed improved bioremediation as evidenced by increased rates of ethene gas production (Ritalahti et al., 2006). Companies such as EOS Remediation offer bioaugmentation cultures capable of degrading chlorinated solvents, such as the BAC-9 culture, which contains 10^{10} cells/L of *Dehalococcoides mccartyi* and enzymes in water based media (EOS Remediation, 2015).

In future studies, it would be beneficial to perform qPCR studies with a primer that targets the genes on bacteria that can perform reductive dehalogenation on PCE. Ritalahti et al. used the reductive dehalogenase (RDase) genes to target three specific strands of *Dehalococcoides* to characterize the bacterial community in their samples. The qPCR targeted three specific strains of *Dehalococcoides ethenogenes* RDase genes: the TCE to VC RDase strain gene (tceA), the VC to ethene strain (bvcA), and TCE to ethene dechlorination gene

(vcrA). The qPCR with RDase as described by Ritalahti et al. allows for the quantification of as few as 50 to 100 tceA, bvcA or vcrA gene targets per PCR volume. The specificity of primers and probes targeting regions of the RDase genes met the criteria of Primer Express software with an amplicon length of 50 to 150 base pair and probe melting temperature of 58 to 60°C (Ritalahti et al., 2006). The decision of whether to bioaugment the AOC3 site with *Dehalococcides* inoculum could be made after performing further qPCR testing to quantify the amount of bacteria capable of reductive dechlorination naturally present at the site.

4.4 Geochemical Parameters and Electron Donor Source

The geo-chemical parameters that were measured in the microcosms showed that increased sulfide concentrations might be beneficial for the degradation of vinyl chloride, since it needs highly reducing conditions. The phosphate concentrations declined towards the end of the study, so increased levels of phosphate are recommended to optimize microbial growth. One of the more important components for cell growth is the electron donor, which for this study was the soybean oil. Other studies have successfully used lactate, acetate, or propionate as the carbon source for the electron donor for PCE reduction (Lendvay et al., 2003). The increase in bicarbonate concentration in all microcosms suggested that the microbes were oxidizing the organic carbon contained in the soybean oil to inorganic carbon.

Conditions that promote the growth of methanogens are necessary in order to complete reductive dechlorination all the way to the end product ethene. Degradation intermediates such as vinyl chloride are toxic to animals and humans, causing cancer, liver failure, and negative central nervous system and reproductive effects (EPA, 2000). Ethene is the only degradation product acceptable to leave remaining in the environment. Common bacteria that are used to convert

PCE degradation intermediates to ethene are *Methanobacterium thermosautotrophicum*, *Methanococcus deltae*, and *Methanococcus thermolithotrophicus* (Freedman & Gossett, 1989). Freedman and Gossett found that methanogens greatly improved the degradation of PCE, especially the last step of vinyl chloride to ethene (Freedman et al., 1989). *Dehalococcoides* prefer hydrogen gas as their electron donor, so providing high concentrations of H_2 creates optimal conditions for the growth of *Dehalococcoides*. Other strains of dechlorinating bacteria, such as *dehalospirillum* and *desulfitobacterium* can use a broader spectrum of electron donors such as acetate, methanol, and formate (Aulenta, Majone, & Tandoi, 2006). To determine the best electron donor to sustain bioremediation, it would be beneficial to perform qPCR to characterize the bacterial community native to the site.

Studies show that the last step of degradation from vinyl chloride to ethene is the rate limiting step, and an electron donor is necessary for complete degradation of PCE to occur. Freedman et al found that methanol was a more effective electron donor for methanogens, with more ethene gas formed than when formate, acetate or glucose was used (Freedman et al., 1989). Soybean oil is composed of around 46-53% linoleic acid, which has the chemical formula $C_{18}H_{32}O_2$ (Howell & Collins, 1957). The advantage with a longer chain carbon source is slower degradation over time which sustains the carbon source necessary to degrade PCE completely to ethene (Coulibaly & Borden, 2004). Soybean oil is also less soluble and adsorb on sediments, so it remains in the aquifer longer. Once vinyl chloride accumulates in the groundwater, it would be beneficial to add methanol as the primary electron donor instead of soybean oil to promote the growth of methanogens that can more readily degrade the vinyl chloride to ethene.

The nutrient and terminal electron acceptor concentrations at Fort Riley are sufficient to initiate PCE degradation. Because the degradation of vinyl chloride to ethene is the rate limiting

step in reductive dechlorination, it may accumulate. Highly reducing conditions are necessary in order for biodegradation of vinyl chloride to ethene to occur. AOC2 has moderate reducing conditions, which is likely occurring by nitrate reduction, evidenced by the low levels of nitrate in the groundwater. As vinyl chloride accumulates, the addition of a carbon source, such as methanol, in AOC2 and AOC3 may promote more strongly reducing conditions.

Chapter 5: Conclusions and Recommendations

The microcosm studies showed that the microbial community from AOC3-A soils was able to degrade PCE with the use of soybean oil as an electron donor. Both AOC3-A and C soils showed active microbial communities with DNA analysis, however only AOC3-A was able to degrade PCE to VC under the conditions in the microcosms. The AOC3-A 150 ppb microcosms performed PCE degradation under nitrate, iron, and sulfate reducing conditions. The nutrient and terminal electron acceptor concentrations at Fort Riley in AOC3 appear to be adequate to initially support PCE degradation, however phosphate concentrations could limit microbial growth over time.

Soybean oil has the potential to stimulate bioremediation at the site, especially since it is degraded slowly, and adsorbs to the sediments, thereby providing a carbon source for months while bioremediation is occurring. It is recommended that the soybean oil be injected into AOC3-A and C where active microbial communities capable of degrading PCE live. The EOS Pro soybean oil from EOS Remediation is the recommended emulsified soybean oil, as its small droplets penetrate through sediment pores (Borden, 2007b). Bioaugmentation with a strain of

dehalococcoides could help stimulate biodegradation if the production of vinyl chloride inhibited bacterial growth, as possibly suggested by the results of this study.

The injection of the soybean oil should not be performed near well DCF02-42, as it is a dry well, and it is recommended that the injection be performed either in the saturated zone or in the overlying vadose zone in the areas where contaminated sediments are to be bioremediated. Access to some wells in the AOC3 may be difficult as it is near a railroad and in a bald eagle nesting conservation area. A ramp will have to be built to get over the railroad into AOC3 for the heavy vehicles needed for soybean oil injection.

Phosphate concentrations should be monitored while bioremediation is occurring in case supplemental phosphate needs to be added to sustain microbial communities. The bioremediation of vinyl chloride to ethene, which is the rate limiting step of degradation of PCE needs highly reducing conditions. The addition of methanol as an electron donor may stimulate the growth of methanogens, which are more capable of degrading vinyl chloride to ethene. Monitoring of terminal electron acceptors is recommended to track the progress of bioremediation in AOC3.

Bibliography:

APHA, AWWA, WEF,. (2012). Standard methods for the examination of water and wastewater. *American Public Health Association Publisher. Washington, DC, 1496p*.

Aulenta, Federico, Majone, Mauro, & Tandoi, Valter. (2006). Enhanced anaerobic bioremediation of chlorinated solvents: environmental factors influencing microbial activity and their relevance under field conditions. *Journal of Chemical Technology and Biotechnology, 81*(9), 1463-1474.

Borden, Robert C. (2007a). Concurrent bioremediation of perchlorate and 1, 1, 1-trichloroethane in an emulsified oil barrier. *Journal of Contaminant Hydrology, 94*(1), 13-33.

Borden, Robert C. (2007b). Effective distribution of emulsified edible oil for enhanced anaerobic bioremediation. *Journal of Contaminant Hydrology, 94*(1), 1-12.

Cleasby, JL, Baumann, ER, & Black, CD. (1964). Effectiveness of potassium permanganate for disinfection. *Journal (American Water Works Association), 56*(4), 466-474.

Coulibaly, Kapo M, & Borden, Robert C. (2004). Impact of edible oil injection on the permeability of aquifer sands. *Journal of Contaminant Hydrology, 71*(1), 219-237.

EOS Remediation, LLC. (2015). www.eosremediation.com.

EPA. (2000). Vinyl Chloride Hazard Summary. (https://www3.epa.gov/airtoxics/hlthef/vinylchl.html).

Ferris, FG, Phoenix, V, Fujita, Y, & Smith, RW. (2004). Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20 C in artificial groundwater. *Geochimica et Cosmochimica Acta, 68*(8), 1701-1710.

Freedman, David L, & Gossett, James M. (1989). Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. *applied and Environmental Microbiology, 55*(9), 2144-2151.

Gates-Anderson, Dianne D, Siegrist, Robert L, & Cline, Steven R. (2001). Comparison of potassium permanganate and hydrogen peroxide as chemical oxidants for organically contaminated soils. *Journal of Environmental Engineering, 127*(4), 337-347.

Giaya, Arjan, Thompson, Robert W, & Denkewicz Jr, Raymond. (2000). Liquid and vapor phase adsorption of chlorinated volatile organic compounds on hydrophobic molecular sieves. *Microporous and mesoporous materials, 40*(1), 205-218.

Google Maps. (2016). Fort Riley.

Harms, Gerda, Layton, Alice C, Dionisi, Hebe M, Gregory, Igrid R, Garrett, Victoria M, Hawkins, Shawn A, Robinson, Kevin G, & Sayler, Gary S. (2003). Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. *Environmental science & technology, 37*(2), 343-351.

Haston, Zachary C, & McCarty, Perry L. (1999). Chlorinated ethene half-velocity coefficients (Ks) for reductive dehalogenation. *Environmental Science & Technology, 33*(2), 223-226.

Howell, Robert W, & Collins, FI. (1957). Factors affecting linolenic and linoleic acid content of soybean oil. *Agronomy Journal, 49*(11), 593-597.

Konhauser, K. O. (2007). Introduction to Geomicrobiology. *Wiley Blackwell*, 440.

Lendvay, JM, Löffler, Frank E, Dollhopf, M, Aiello, MR, Daniels, G, Fathepure, BZ, Gebhard, M, Heine, R, Helton, R, & Shi, J. (2003). Bioreactive barriers: a comparison of bioaugmentation and biostimulation for chlorinated solvent remediation. *Environmental Science & Technology, 37*(7), 1422-1431.

Long, Cameron M, & Borden, Robert C. (2006). Enhanced reductive dechlorination in columns treated with edible oil emulsion. *Journal of contaminant hydrology, 87*(1), 54-72.
Madigan, Michael T., Martinko, John M., Dunlap, Paul V., & Clark, David P. (2009). Brock Biology of Microorganisms. *Pearson*.
Parsons. (2004). Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. *AFCEE, NFEC, ESTCP*, 457.
Pfeiffer, Patricia, Bielefeldt, Angela R, Illangasekare, Tissa, & Henry, Bruce. (2005). Partitioning of dissolved chlorinated ethenes into vegetable oil. *Water research, 39*(18), 4521-4527.
Popp, P, & Paschke, A. (1997). Solid phase microextraction of volatile organic compounds using carboxen-polydimethylsiloxane fibers. *Chromatographia, 46*(7-8), 419-424.
Ritalahti, Kirsti M, Amos, Benjamin K, Sung, Youlboong, Wu, Qingzhong, Koenigsberg, Stephen S, & Löffler, Frank E. (2006). Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple Dehalococcoides strains. *Applied and Environmental Microbiology, 72*(4), 2765-2774.
Rogers, Jennifer Roberts, & Bennett, Philip C. (2004). Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. *Chemical Geology, 203*(1), 91-108.
United States EPA Region 7 Kansas City. (2010). Fort Riley Kansas (ID#KS6214020756), 1-7.
US Army Corps of Engineers. (2014). 2014 Annual Groundwater Monitoring Report.
Vogel, Timothy M, Criddle, Craig S, & McCarty, Perry L. (1987). ES&T critical reviews: transformations of halogenated aliphatic compounds. *Environmental Science & Technology, 21*(8), 722-736.
Wackett, Lawrence P, & Householder, Steven R. (1989). Toxicity of trichloroethylene to Pseudomonas putida F1 is mediated by toluene dioxygenase. *Applied and environmental microbiology, 55*(10), 2723-2725.
Zhang, Hubao, & Schwartz, Franklin W. (2000). Simulating the in situ oxidative treatment of chlorinated ethylenes by potassium permanganate. *Water Resources Research, 36*(10), 3031-3042.
Zhang, Wen, Sturm, Belinda SM, Knapp, Charles W, & Graham, David W. (2009). Accumulation of tetracycline resistance genes in aquatic biofilms due to periodic waste loadings from swine lagoons. *Environmental science & technology, 43*(20), 7643-7650.

Appendix A: Permanganate Injections

Tab -1
Vadose Zone Sodium Permanganate Injection
Pilot Study Report
DCF Study Area
Fort Riley, Kansas

Injection Location	Date	Injection Depth (feet bgs)	Injection Interval	3% NaMnO4 Volume Injected (gallons)	3% NaMnO4 Volume Injected by Interval (gallons)	NaMnO4 Mass (pounds)	Comments
VI-1	1/31/2006	5	shallow	40	150		
		8	shallow	50			
		11	shallow	60			
		14	intermediate	30	70		
		17	intermediate	40			
		20	deep	80	245		Lifted tip 4" twice due to high pressure
		23	deep	80			
		26	deep	85			
		28	saturated	98	98		
				Total	563	363	
VI-2	2/1/2006	5	shallow	80	240		
		8	shallow	80			
		11	shallow	80			
		14	intermediate	35	70		
		17	intermediate	35			
		20	deep	50	150		
		23	deep	50			
		26	deep	50			
				Total	460	296	
VI-3	2/1/2006	6	shallow	31	245		Daylighted
		8	shallow	134			
		11	shallow	80			
		14	intermediate	45	90		
		17	intermediate	45			
		20	deep	70	210		
		23	deep	70			
		26	deep	70			
				Total	545	351	
VI-4	2/2/2006	5	shallow	0	0		Daylighted
		7	shallow	0			Daylighted
VI-4 (offset)	2/2/2006	5	shallow	0	0		Daylighted
		7	shallow	0			Daylighted
				Total	0	0	

Table 3-1a.xls

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Tab -1
Vadose Zone Sodium Permanganate Injection
Pilot Study Report
DCF Study Area
Fort Riley, Kansas

Injection Location	Date	Injection Depth (feet bgs)	Injection Interval	3% NaMnO4 Volume Injected (gallons)	3% NaMnO4 Volume Injected by Interval (gallons)	NaMnO4 Mass (pounds)	Comments
VI-1	1/31/2006	5	shallow	40	150		
		8	shallow	50			
		11	shallow	60			
		14	intermediate	30	70		
		17	intermediate	40			
		20	deep	80	245		Lifted tip 4" twice due to high pressure
		23	deep	80			
		26	deep	85			
		28	saturated	98	98		
				Total	563	363	
VI-2	2/1/2006	5	shallow	80	240		
		8	shallow	80			
		11	shallow	80			
		14	intermediate	35	70		
		17	intermediate	35			
		20	deep	50	150		
		23	deep	50			
		26	deep	50			
				Total	460	296	
VI-3	2/1/2006	6	shallow	31	245		Daylighted
		8	shallow	134			
		11	shallow	80			
		14	intermediate	45	90		
		17	intermediate	45			
		20	deep	70	210		
		23	deep	70			
		26	deep	70			
				Total	545	351	
VI-4	2/2/2006	5	shallow	0	0		Daylighted
		7	shallow	0			Daylighted
VI-4 (offset)	2/2/2006	5	shallow	0	0		Daylighted
		7	shallow	0			Daylighted
				Total	0	0	

Tab. -1
Vadose Zone Sodium Permanganate Injection
Pilot Study Report
DCF Study Area
Fort Riley, Kansas

Injection Location	Date	Injection Depth (feet bgs)	Injection Interval	3% NaMnO4 Volume Injected (gallons)	3% NaMnO4 Volume Injected by Interval (gallons)	NaMnO4 Mass (pounds)	Comments
VI-8	2/3/2006	6	shallow	9	16.6		Daylighted
		7	shallow	1.5			Daylighted
		9	shallow	2.5			Daylighted
		11	shallow	2.3			Daylighted
		12	shallow	1.3			Daylighted
		15	intermediate	64	66		Daylighted
		17	intermediate	2			Daylighted
		20	deep	148	449		
		22	deep	169			
		24	deep	132			
				Total	532	342	
VI-9	2/3/2006	6	shallow	90	376		
		8	shallow	80			
		10	shallow	126			
		12	shallow	80			
		14	intermediate	60	174		
		16	intermediate	60			
		18	intermediate	54			
				Total	550	354	
VI-10	2/27/2006	5	shallow	0	0		Daylighted
		8	shallow	0			Daylighted
		10	shallow	0			Daylighted
		12	shallow	0			Daylighted
		15	intermediate	0	0		Daylighted
		18	intermediate	0			Daylighted
		21	deep	0	0		Daylighted
		30	saturated	550	550		
				Total	550	354	
VI-11	2/27/2006	5.5	shallow	200	550		Daylighted
		7.5	shallow	0			
		11	shallow	350			
				Total	550	354	

Table 3-1
Vadose Zone Sodium Permanganate Injection
Pilot Study Report
DCF Study Area
Fort Riley, Kansas

Injection Location	Date	Injection Depth (feet bgs)	Injection Interval	3% NaMnO4 Volume Injected (gallons)	3% NaMnO4 Volume Injected by Interval (gallons)	NaMnO4 Mass (pounds)	Comments
VI-12	2/28/2006	5.5	shallow	0	350		Daylighted
		8	shallow	0			Daylighted
		11	shallow	350			
		15	intermediate	0	200		Daylighted
		18	intermediate	200			
				Total	550	354	
VI-13	2/28/2006	5.5	shallow	0	0		Daylighted
		8	shallow	0			Daylighted
		11	shallow	0			Daylighted
		15	intermediate	0	0		Daylighted
		18	intermediate	0			Daylighted
		21	deep	182	182		
		24	deep	0			Daylighted
		27	saturated	0	0		Daylighted
		30	saturated	0			Daylighted
				Total	182	117	
VI-14	2/28/2006	5	shallow	120	361		Balance remaining from VI-13
		12	shallow	241			
				Total	361	233	
VI-14	3/1/2006	12	shallow	550	550		
				Total	550	354	
VI-15	3/1/2006	15	intermediate	550	550		
				Total	550	354	
VI-16	3/1/2006	7	shallow	450	450		
		15	intermediate	100	100		
				Total	550	354	
VI-17	3/1/2006	7	shallow	0	0		Daylighted
		9	shallow	0			Daylighted
		12	shallow	0			Daylighted
		15	intermediate	0	0		Daylighted
		18	intermediate	0			Daylighted
		21	deep	0	550		
		24	deep	550			
				Total	550	354	

Table 3-1
Vadose Zone Sodium Permanganate Injection
Pilot Study Report
DCF Study Area
Fort Riley, Kansas

Injection Location	Date	Injection Depth (feet bgs)	Injection Interval	3% NaMnO4 Volume Injected (gallons)	3% NaMnO4 Volume Injected by Interval (gallons)	NaMnO4 Mass (pounds)	Comments
				Grand Total	11,471	7,388	

$NaMnO_4$ - Sodium Permanganate
Daylighted - NaMnO4 came to the surface due to low permeability at the injection interval
bgs - below ground surface
" - Inch
% - Percent

Appendix B: EOS Product Information

EOS Remediation, LLC

Experience you can rely on, Products you can trust™

EOS PRO

PRODUCT INFORMATION SHEET
Emulsified Oils Family

Description

EOS PRO is a nutrient-enriched, DoD-validated, emulsified vegetable oil (EVO). **EOS PRO** is engineered to quickly stimulate microbial activity while providing long-term nourishment to enhance anaerobic bioremediation of chlorinated solvents, nitrates, perchlorate, energetics, acid mine drainage, and other recalcitrant chemicals in contaminated groundwater. **EOS PRO** can also be used to reduce redox sensitive metals and radionuclides. The negative surface charges on the droplets combined with small droplet size promote effective transport in the subsurface.

EOS PRO benefits include:

- Biostimulating vitamins and nutrients
- Rapidly-biodegradable substrates to "jump start" bacterial growth
- Slow release biodegradable substrates to promote long-term biological activity
- Engineered for effective transport in the subsurface
  - Small oil droplet size
  - Negative surface charge
- Neutral pH
- Extensive third-party validation

EOS PRO incorporates the patented **EOS®** technologies that clients have trusted for more than a decade. Domestic supply *made in the USA* with US farmed soybeans.

Chemical & Physical Properties

Oil Emulsion Concentrate: EOS PRO	**Typical**
Refined and Bleached US Soybean Oil (% by wt.)	59.8
Rapidly Biodegradable Soluble Substrate (% by wt.)	4
Other Organics (emulsifiers, food additives, etc.) (% by wt.)	10
Specific Gravity	0.96 - 0.98
pH (Standard Units)	6 - 7
Median Oil Droplet Size (microns)	1.0
Organic Carbon (% by wt.)	74
Mass of Hydrogen Produced (lbs. H_2 per lbs. **EOS PRO**)	0.25

Packaging

Shipped in 55-gallon drums, 275-gallon IBC totes or bulk tankers (40,000 lbs.)

Handling & Storage

EOS PRO is shipped as a ready-to-use concentrated emulsion that can be diluted with water in the field to prepare a high quality suspension for easy injection. **EOS PRO** has a low viscosity and can be distributed with commonly available pumps or by continuous metering with a diluter (e.g., Dosatron™). Dilution ratios for **EOS PRO** typically range from 4:1 to 20:1 (water: **EOS PRO**) depending on site conditions. **EOS PRO** injections should be followed with additional chase water to maximize distribution of **EOS PRO** into the formation.

For best performance, use **EOS PRO** within 60 days of delivery and store at a temperature between 40°F (4°C) to 100°F (38°C).

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www.eosremediation.com

Rev. 06.12.2014