Edible Oil and MicroCED™ Treatability Study for Enhanced Attenuation of cVOCs at P Area, Savannah River Site – 15364

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ABSTRACT

The purpose of the study is to evaluate the capability of **Micro**-organism **C**hlorinated **E**thene **D**estruction (MicroCED) to degrade chlorinated volatile organic compounds (cVOCs) in an existing groundwater plume at the Savannah River Site (SRS). MicroCED is similar to other commercially sold *Dehalococcoides* microbes (i.e., BAV-1 and KB-1) that are used to degrade cVOCs in support of groundwater cleanup actions. However, a primary difference is that this microbe is indigenous to the Twin Lakes area near C Area at the SRS.

The Savannah River National Laboratory (SRNL) and Clemson University have been studying the capability and toxicology of the microbe for many years. To assess the potential of MicroCED in an environmental setting, a Treatability Study [5] was developed and submitted to South Carolina Department of Health and Environmental Control (SCDHEC). Approval was received and implementation of the study was conducted during the dates of September 14, 2010 and September 30, 2011.

During September - October 2010, approximately 423,966 liters of amendments (pH buffer [AquaBupH™] and emulsified oil [EOS™]) and chase water were injected into a cVOC groundwater plume at P Area at a depth of 26 - 30.5 m below land surface. The amendments were injected to raise the groundwater pH and to convert an oxygenated groundwater system to a reducing environment better suited for MicroCED. MicroCED was injected in January and August 2011; however, only 140 liters of the 6,246 liters needed were injected. The insufficient number of growth chambers and project schedule limited the ability to grow and maintain the required amount of MicroCED needed for the study.

Groundwater monitoring of the injection wells and monitoring points were conducted monthly to evaluate and assess changes to the existing cVOC groundwater plumes. Field data collected from the injection wells demonstrated a reducing environment suitable for MicroCED growth. However, nearby monitoring points located within the injection field did not indicate a change in groundwater chemistry. Further analysis of the injected material showed that the actual radius of influence was less than assumed. This resulted in conditions favorable for MicroCED growth to be located near the injection wells versus over a larger area, as expected.

Data collected indicates biodegradation of the cVOCs via the MicroCED is encouraging but inconclusive. This could be a result of the insufficient quantity of MicroCED injected and limited timeframe of monitoring after MicroCED injections. However, considering that the data were collected within six (6) months of the first MicroCED injection it could also be plausible

that microbial growth is limited and is not measureable based on the timeframe and quantity of microbes that were injected. But, concentrations of TCE metabolic products have been observed to increase as the study progressed and were most evident after the first MicroCED injection. Due to timing of the second MicroCED injection in August 2011, there are no data available to evaluate subsequent effects of this injection on cVOC concentrations.

INTRODUCTION

Chlorinated volatile organic compound (cVOC) contamination is present in the groundwater at P Area on the Department of Energy (DOE) Savannah River Site (SRS) (Figure 1). Previous operational activities associated with the Administrative/Maintenance Building (704-P) and the Assembly Area of the P-Reactor Building (105-P) involved the use of cVOCs (i.e., trichloroethylene [TCE] and tetrachloroethylene [PCE]) for degreasing and cleaning of equipment which have resulted in soil contamination near these facilities [8]. As a result, the shallow groundwater is also contaminated with cVOCs and to some extent, degradation products (i.e., cis-1,2-dichloroethylene).

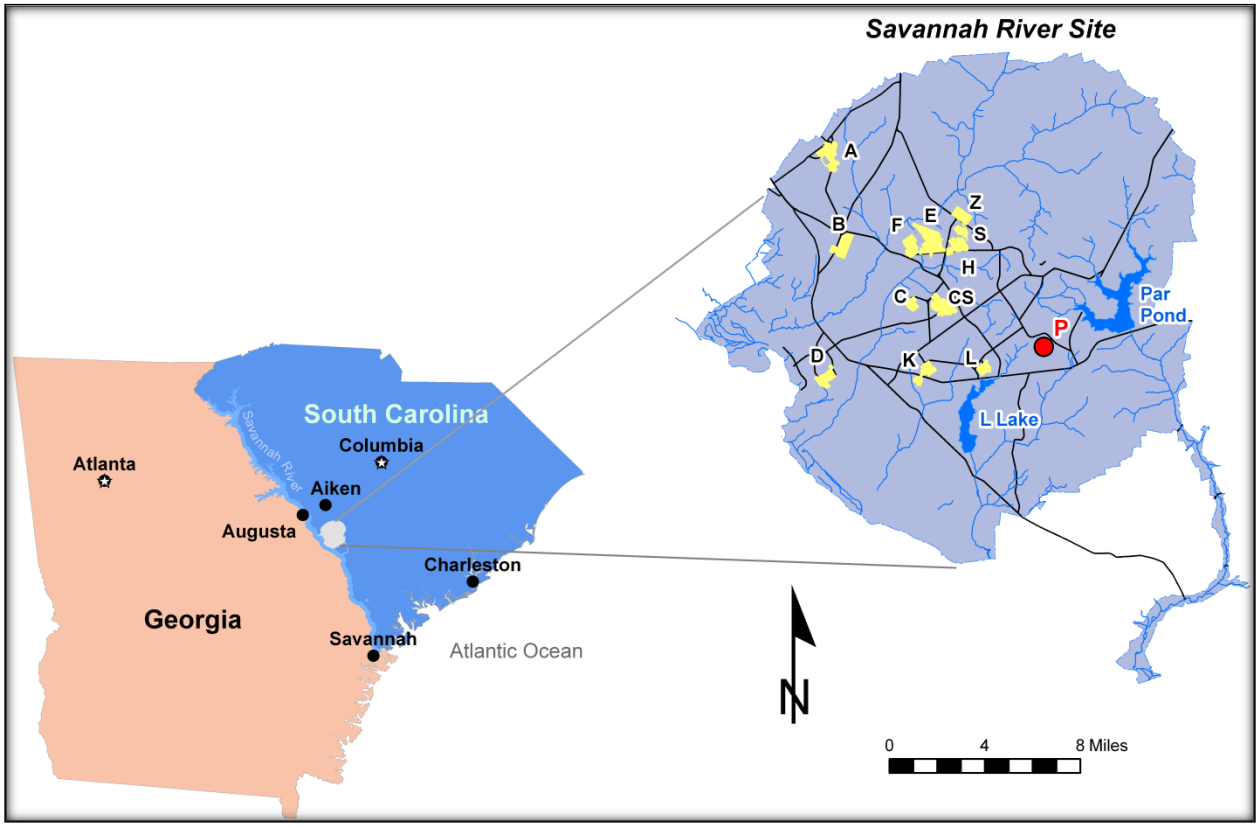

Figure 1: Location of P Area at the Savannah River Site

Early actions have been implemented to address the cVOC source areas that have been impacting the local shallow groundwater at P Area such as in-situ oxidation coupled with soil vapor extraction [6]. In an effort to address the groundwater contamination, an innovative approach is being evaluated that uses biostimulation (emulsified oil) and bioaugmentation (SRS-specific

bacteria cultures) to dechlorinate cVOCs in the groundwater and determine the viability of this technology for future applications.

Savannah River National Laboratory (SRNL) studies and existing groundwater data demonstrate very little reductive dechlorination of cVOCs is occurring in the groundwater plume underlying P Area [8, 10]. The studies indicate, however, that treatment of the cVOCs using edible oil substrates and bioaugmentation for bioremediation is appropriate and viable [1, 2, 4]. Oil and bacteria culture emplacement, along with moderate modifications to the groundwater geochemistry, should provide appropriate conditions to change the naturally aerobic aquifer conditions to anaerobic and initiate reductive dechlorination of TCE and PCE to nontoxic, safe end-products. Other degradation processes, cometabolic and abiotic, downgradient of the proposed technology treatment area are also possible [9].

Edible oils have emerged as an effective treatment at environmental waste sites as a way to enhance anaerobic bioremediation and sequestration of chlorinated solvents and related contaminants in the saturated zone [3]. Edible oil deployment serves to decrease chlorinated compound concentrations in two ways: 1) physical sequestration, which reduces effective aqueous concentration and mobility; and 2) stimulation of anaerobic, co-metabolic and abiotic degradation processes that degrade the cVOCs to less toxic, nontoxic, and/or more readily biodegradable compounds.

Biodegradation of an organic substrate such as soybean oil depletes the aquifer of oxygen and other terminal electron acceptors and creates conditions conducive to anaerobic degradation processes. The organic substrate is fermented to produce hydrogen, which is then used as an electron donor for anaerobic dechlorination. TCE and PCE degrade fairly readily once anaerobic conditions are created. Additionally, specific dechlorinating organisms (*Dehalococcoides*) also need to be present in order for these solvents to degrade completely to ethene. There are commercially available *Dehalococcoides* organisms available, such as KB-1 and BAV-1, which are used frequently around the United States. However, SRS has identified, evaluated, and cultivated an SRS-specific culture that originated from the Twin Lakes near C Area at the SRS [1]. This SRS-specific dechlorinating enrichment culture, referred to as **Micro**-organism **C**hlorinated **E**thene **D**estruction (MicroCED), is capable of completely degrading chlorinated ethenes to ethene and carbon dioxide (CO_2) and chloride ion with only transient accumulation of intermediate byproducts [7].

Objectives

The overall objective of the study was to assess the performance of the deployment strategy for long-term attenuation of the cVOCs in the groundwater [5]. Specific objectives for the treatability study include the following:

- Evaluate emulsified oil distribution and buffering capacity;
- Assess the extent and rate of change from aerobic to anaerobic;
- Quantify cVOC degradation and the associated degradation rates;
- Assess degradation daughter products and their subsequent degradation;
- Assess degradation pathways (reductive dechlorination, cometabolism, abiotic);

- Assess the distribution and effectiveness of MicroCED and sufficient amount of biomass;
- Determine if additional means are needed to stimulate and/or maintain attenuation (i.e., geochemistry modifications, oil addition, nutrient addition, etc.);
- Assess the ability of the oil and MicroCED deployment to stabilize and shrink the groundwater plume in the treatment zone and to provide a sustainable treatment to meet the 5 ppb groundwater standard for TCE/PCE; and
- Determine long-term operation, maintenance and monitoring requirements for full scale application.

Technical Approach

Using an array of previously installed five (5) injection wells, the dissolved cVOC contamination in the transmissive zone of the shallow water table (approximately 26-30.5 m below surface) was injected with Emulsified Oil Substrate (EOS™) and AquaBupH™ from EOS Remediation, LLC to promote reducing conditions (i.e., anaerobic). Nutrients (i.e., vitamin B-12) that are beneficial for cVOC degradation were also injected. Once reducing conditions were reached, the study area was inoculated with a SRS-specific dechlorinating enrichment culture (MicroCED). Monitoring was conducted through an array of sampling and analytical techniques at the injection wells and eight (8) monitoring points to measure appropriate oil placement, maintenance of appropriate geochemistry and microbial activity, and attenuation of TCE and PCE groundwater contamination.

Design Approach

The design for an edible oil deployment for remediation of cVOCs in the P Area groundwater at the SRS toward a natural attenuation condition derives from two mechanisms, partitioning and degradation, combined with standard hydrology and engineering calculations. Based on the current state of P Area, deployment of edible oil and MicroCED represents a promising approach to reducing concentrations and facilitating the transition from a passive treatment of an aerobic transmissive zone to a naturally sustainable anaerobic environment thus allowing for continuous treatment of residual contamination that moves into the transmissive zone.

Groundwater plume configuration of P Area influenced the assumptions used in developing the design. The area targeted exhibits the highest cVOC groundwater concentrations within the shallow water table [8]. The shallow water table groundwater plume is approximately 18 m thick and ranges from a depth of 15 to 33.5 m below land surface.

The groundwater plume is underlain by a semi-confining clay layer commonly referred to as the “tan clay”. Notably, the design utilizes wells and monitoring points for access to the central part of the plume located in the transmissive zone (approximately 26-33.5 m below ground surface) (Figure 2) [8]. Each of the injection wells and monitoring points are constructed to the same screen zone depth of 26-30.5 m below land surface. The injection wells are spaced approximately 9 m apart in an effort to maximize the introduction of amendments into the study site and to provide conditions favorable for bioaugmentation [5].

The P Area geology and groundwater chemistry required creative application of treatment reagents to exploit the site characteristics. The site-specific application was essential to generating a deployment zone that has the appropriate geometry necessary to intercept contaminants and effectively treat the mobile groundwater plume in the test area. The design also relied heavily on the results of SRNL laboratory bench-scale column experiments, scientific literature, and design tools provided to the environmental service industry in guidance documents, short courses, and manufacturer spreadsheets.

The groundwater at P Area is characterized as well-oxygenated with little or no organic matter and exhibits a groundwater pH of 5. Under these conditions, anaerobic degradation is not likely to occur. However, the actions undertaken to support a reducing environment were: 1) injection of emulsified vegetable oil and pH buffers in the core of the groundwater cVOC plume; and 2) inoculation of the treatment zone with MicroCED. The emulsified vegetable oil serves to stimulate the formation of the appropriate conditions needed to support the MicroCED (i.e., providing carbon and nutrients, reducing oxygen, and adjusting pH). Buffering of the groundwater provides for optimal pH conditions to support microbial activity. Inoculation with MicroCED will create an active bioremediation "reactor" within the transmissive zone which will degrade existing groundwater cVOC contamination.

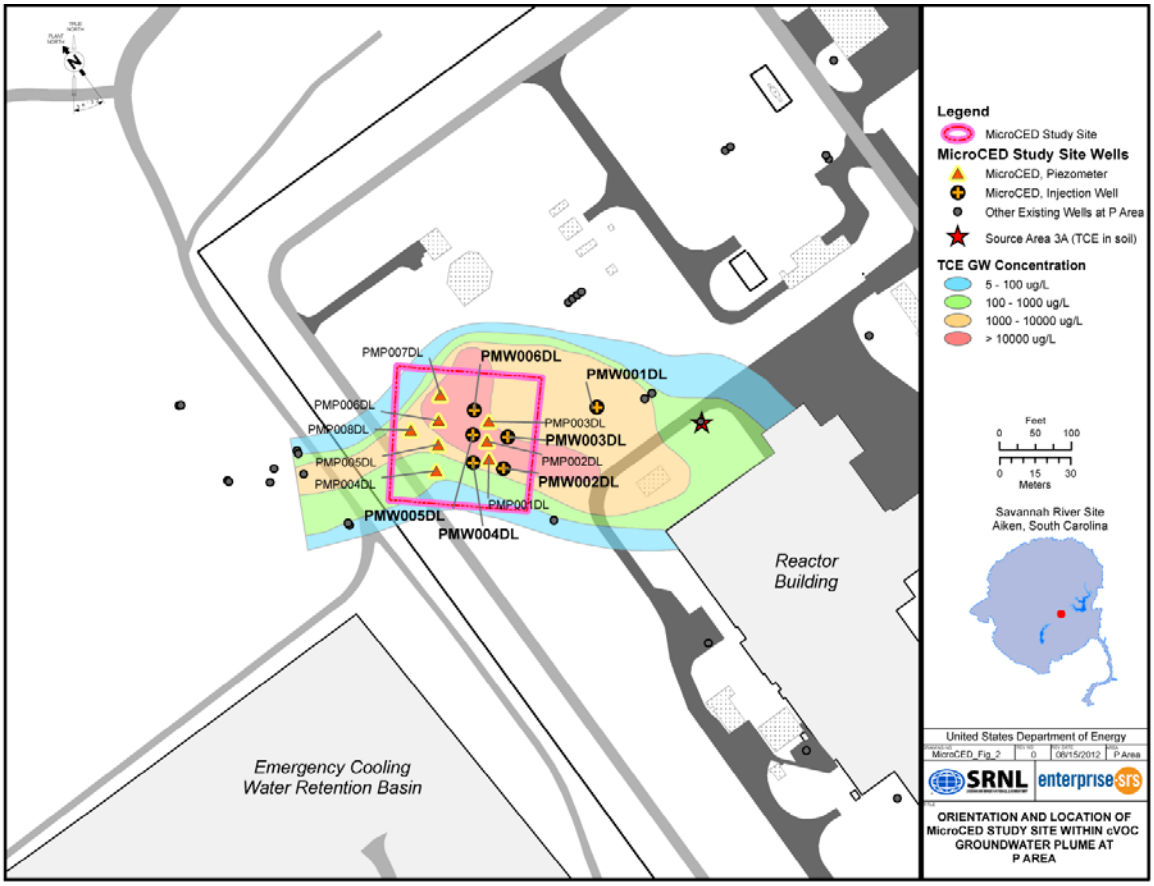

Figure 2: Orientation and Location of Study Site within cVOC Groundwater Plume at P Area

DESCRIPTION

Based on site conditions, laboratory bench-scale studies, scientific literature, and design tools (i.e., Substrate Estimating Tool for Enhanced Anaerobic Bioremediation of Chlorinated Solvents, v1.2), approximately 13,249 liters of EOS™ and 8,706 liters of AquaBupH™ were determined to be needed for the study site. Additionally, to adequately distribute the material within the aquifer, additional water would be needed for injection. Once reducing conditions were reached, the study area would be inoculated with an SRS-specific dechlorinating enrichment culture (MicroCED).

Overall, deployment of the material into the subsurface at the test site was conducted using water extracted from a nearby well, a transfer pump, a metering system (i.e., Dosatron), and flexible hoses. Visual monitoring of the system during injections included recording flow rates and pressure readings at the metering system and at the injection wellhead. Electronic flow readings and pressures were also recorded via a data logger for a more detailed analysis. To ensure adequate placement of the material, injections were conducted under constant pressure. Figure 3 depicts the layout of the equipment used at the injection site.

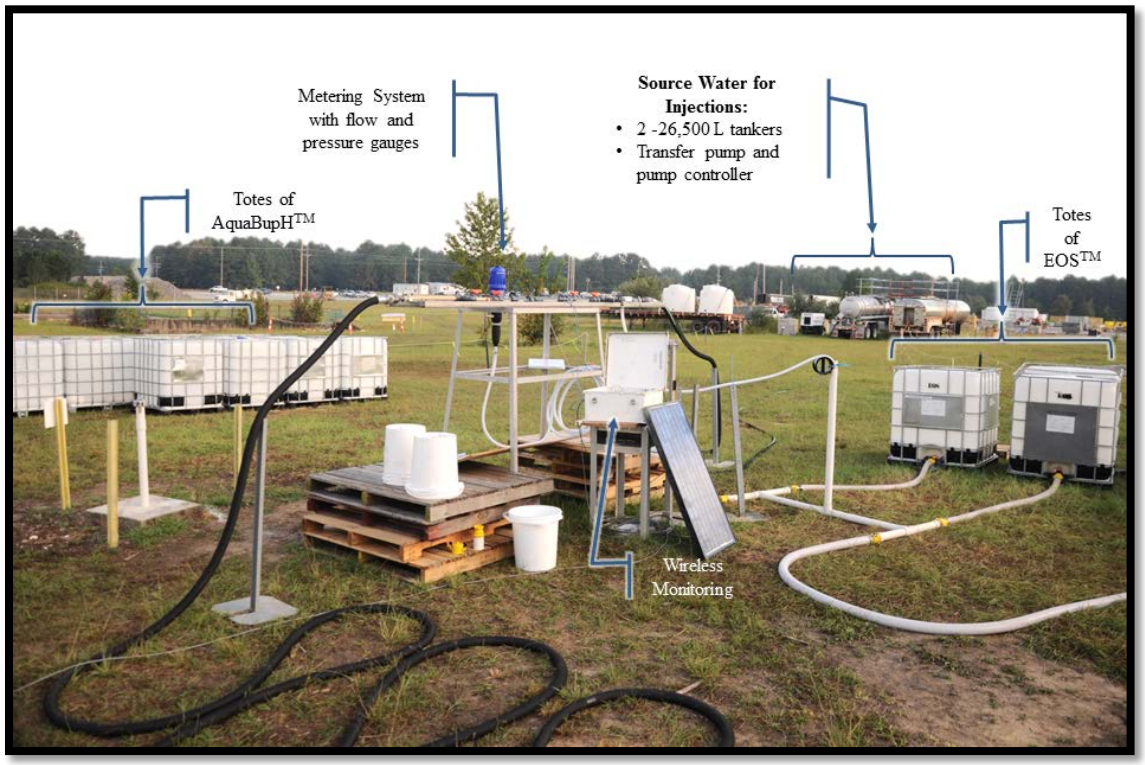

Figure 3: Layout Overview of Injection Site

Amendment and Microbial Injections

The rate of injection of the amendments varied amongst the five (5) injection wells even though the wells were closely spaced (within 9 m) from one another and screened in the same horizon.

The amount of time needed to inject the amendments in each well ranged from three (3) to ten (10) days with all the wells exhibiting a reduction in injection rates as time progressed. This is partly due to the viscosity and particle sizes of the material and the lithologic sediments present at the test site. Table I summarizes the quantity of material injected, observed injection rates, and durations.

TABLE I: Injection summary

INJECTION WELL	TOTAL AMOUNT INJECTED* (liters)	RATE OF INJECTION (lpm)		DURATION (days)
		Max	Min	
PMW002DL	95,642	14	6.1	10
PMW003DL	79,766	5.3	3.8	7.2
PMW004DL	81,091	10.2	4.9	5.6
PMW005DL(1)	89,991	22.7	14	3
PMW006DL	76,503	19.3	5.7	4

*Includes amendments and chase water
(1) Well redeveloped prior to injection of amendments

Bioaugmentation at the study site was conducted with the injection of MicroCED once reducing conditions were reached in the aquifer. The MicroCED was grown and maintained at Clemson University under the direction of Dr. David Freedman. Knowing that the current growth process at Clemson University could not sufficiently grow the needed quantity of microbes (6,246 liters) within the timeframe needed for the study, two approaches for microbe injections were performed. The first approach involved the direct injection of MicroCED from canisters that were used to grow and maintain the culture from Clemson. Each canister held approximately 20 liters of MicroCED with a cell density of 10^{11} cells/liter (Figure 4). The second approach involved the growth of the MicroCED in 5-1,249 liter double-lined collapsible containers. The later approach was an experimental approach to determine if the culture could be grown under field conditions.

Two separate injections (January and August 2011) of the MicroCED culture were conducted with a total injected amount of 140 liters. The duration between injections allowed for the necessary time needed (approximately 6 months) to grow a sufficient amount (10^{11} cells/liter) of MicroCED between injections.

Because the direct injections would not provide the quantity of MicroCED needed during the project schedule, a novel approach was developed prior to implementation of the study in which 5-1,249 liter double-lined bags and collapsible containers (herein referred to as totes) were setup at the site. The double-lined bags were placed in collapsible containers for support and filled via an opening on top of the bags. Each tote received various amounts of cVOC laden groundwater from the study site, pH adjusted with AquaBupH™ and amended with EOS™ (Figure 5).

Once the contents of the totes indicated a reducing environment, approximately 3.78 liters of MicroCED was introduced into each tote. The totes were monitored and maintained to allow

microbial growth to occur. Periodic pH adjustments were made based on field data collected. Dissolved oxygen, pH, and temperature measurements were routinely collected.

Figure 4: Photograph of Canisters Containing the MicroCED

Figure 5: Photograph of a Tote Before (left) and After (right) Filling with Groundwater and Amendments

Approximately six (6) weeks after inoculating the totes with MicroCED, microbial samples were collected from each tote and submitted to an offsite laboratory for analysis. Results from the samples were inconclusive. The results may not have been indicative of microbial activity because of the following:

- Insufficient time between inoculation and sampling to allow for adequate microbe growth;
- Inadequate mixing of totes after introduction of MicroCED and during maintenance;
- Microbial samples were collected near the bottom of the totes and as a result the sampling may have been biased because of inadequate mixing of the totes; and
- Stratification could be occurring within the totes where conditions were more conducive for microbial activity.

However, during tote maintenance, headspace and liquid samples were collected from the totes to evaluate changes that may be occurring. The data indicated that reductive dechlorination activity may be occurring as shown by an increase in methane concentrations in the tote headspace and decrease in TCE concentrations with buildup of degradation products.

Contents of the totes were injected in August 2011. A total of 2,831 liters were injected.

DISCUSSION

Groundwater sampling was conducted at the study site before, during, and after injections of amendments and microbes. The sampling strategy was designed to optimize data collected to meet the study objectives, while minimizing analytical costs. Measurements were made in wells within the treatment zone and outside the treatment zone (for background information) and in representative downgradient wells (to evaluate the distal treatment zone). Samples were collected prior to injections and monthly after injections [7]. Table II lists the analytes monitored at the test site.

TABLE II: List of analytes and sample frequency

Category	Analyses	Frequency
Light Hydrocarbons	Acetylene, butane, carbon dioxide, ethane, ethylene, ferric and ferrous iron, isobutane, methane, nitrogen, oxygen, propane, and propylene	Monthly
Stable C Isotopes	Compound Specific Isotope Analysis (CSIA) (specifically for c12DCE, PCE, TCE, and VC)	Semi-annual
cVOCs	1,1,1-trichloroethane, benzene, carbon tetrachloride, chloroform, VC, c12DCE, fluorotrichloromethane, methylene chloride, PCE, toluene, trans-1,2-dichloroethylene, and TCE	Monthly
Field	pH, conductivity, DO, ORP, temperature, and turbidity	Monthly
Geochemistry	Ammonia, soluble iron, sulfate, nitrate, alkalinity, TOC	Monthly

*background well
ǂ furthest downgradient well
DO: Dissolved Oxygen
ORP: Oxygen-Reduction Potential
TOC: Total Organic Carbon
TCE: trichloroethylene
PCE: tetrachloroethylene
VC: vinyl chloride
c12DCE: cis-1,2-dichloroethylene
CSIA: compound-specific isotope analysis

Preliminary Results
Injection Wells

As expected, groundwater geochemistry at the injection wells was changed as a result of amendment injections. Groundwater pH was raised to over 8, dissolved oxygen (DO) values decreased to near or below 1 mg/L while oxygen-reduction potential (ORP) values went from being strongly positive to strongly negative. Total Organic Carbon (TOC) concentrations also increased along with ferric and ferrous iron concentrations. All these changes can be attributed to the injection of the amendments as no MicroCED had been injected at this time.

Concentrations of cVOCs were also affected by injection of the amendments. At the injection wells, TCE and cis-1,2-dichloroethylene (c12DCE) concentrations decreased (based on the average result) by 97% and 40%, respectively. The decrease in concentrations can possibly be attributed to displacement of the groundwater from the injections and also partial partitioning of the cVOCs into the emulsified oil. An increase in degradation products such as vinyl chloride, ethane, and ethylene were also observed (Table III).

Data collected three months post first MicroCED injection indicated an increase in vinyl chloride, ethane, ethylene, and chloride production. A marked increase in methane was also observed. These observed changes indicate limited microbial activity but also continued reductive dechlorination associated with the emulsified oil. Physical conditions (i.e., pH, ORP, and DO) needed for maintaining a reducing environment was relatively stable.

TABLE III: Data results

	INJECTION WELLS			INTERIOR MONITORING POINTS			DOWNGRADIENT MONITORING POINTS		
	Before Injections	After Injections	After 1st MicroCED	Before Injections	After Injections	After 1st MicroCED	Before Injections	After Injections	After 1st MicroCED
Constituent	Average of Result	Average of Result	Average of Result	Average of Result	Average of Result	Average of Result	Average of Result	Average of Result	Average of Result
CHLORIDE (AS CL) (mg/L)	6.15	20.96	103.93	2.79	5.56	6.01	2.91	4.19	4.56
CIS-1,2-DICHLOROETHYLENE (ug/L)	231.46	138.38	98.35	145.67	143.19	168.52	251.73	314.8	339.78
DISSOLVED OXYGEN (mg/L)	2.68	0.68	1.43	2.21	3.46	3.14	2.82	2.83	3.42
ETHANE (ug/L)	0.53	3.99	15.1	0.08	0.09	0.16	0.06	0.05	0.04
ETHYLENE (ug/L)	0.878	3.57	284.59	0.42	0.2	0.24	0.35	0.184	0.15
FERRIC IRON (mg/L)	0.33	0.46	1.56	- -	0.21	0.22	0.1	0.19	0.37
FERROUS IRON (mg/L)	2.03	3.32	8.42	- -	- -	1.38	- -	- -	0.52
METHANE (ug/L)	10.82	15,904	22,114	4.17	4	3.51	6.66	10.23	5.79
OXYGEN-REDUCTION POTENTIAL (mV)	197.72	-144.28	-134.98	214.35	252.87	211.4	211.23	275.55	238.26
pH	5.31	7.61	7.69	5.2	5.02	4.96	4.98	4.98	4.82
TOTAL ORGANIC CARBON (mg/L)	0.87	1,335.00	2,165.14	1.02	4.17	5.65	1.13	4.14	5.24
TRICHLOROETHYLENE (TCE) (ug/L)	10,311	233.59	276.42	7,050	14,288	16,131	5,091	10,222	11,019
VINYL CHLORIDE (ug/L)	2.27	6.94	249.85	- -	6.28	4.18	1.56	3.35	3.64

Interior Monitoring Points

Results from sampling conducted prior to injections demonstrate elevated concentrations of TCE and c12DCE with low concentrations of ethane, vinyl chloride, and TOC as also exhibited by the injection wells. DO and ORP values indicate the groundwater is well-oxygenated. After

injection of the amendments, overall cVOC concentrations remained unchanged. Additionally, groundwater pH was unchanged.

TCE concentrations did increase from before to after injections which may be attributed to displacement of contaminated groundwater associated with the amendment injections while c12DCE concentrations remained unchanged. An increase in concentrations for chloride, TOC and VC were observed as a result of the amendment injections.

Data collected post first MicroCED injection did not indicate microbial activity as groundwater geochemistry remained unchanged.

Overall, little or no change was observed at the interior monitoring points even though these monitoring points are located within the injection field.

Downgradient Monitoring Points

Results from the sampling demonstrated elevated concentrations of TCE and c12DCE with low concentrations of ethylene, ethane, VC, and TOC at the study site prior to injections. Additionally, DO and ORP values indicate the groundwater is well-oxygenated.

Monitoring results after amendment and the first MicroCED injection indicated that the concentrations of cVOCs remained unchanged. TCE concentrations were observed to have increased which may be attributed to displacement of contaminated groundwater associated with the amendment injections. Groundwater pH was also unaffected and remained constant during the monitoring period. DO and ORP also remained unchanged other than fluctuations in data that might be associated with instruments and sampling variability.

CONCLUSIONS

Subsurface lithology, lack of well development, and material injected all influenced the performance of the injections wells. Overall, all the amendments were injected for a total quantity injected of 166,055 liters (amendments plus water), 1,052 liters of B-12 (B-12 plus water), and 235,483 liters of chase water for a total injected quantity of 425,223 liters.

Two separate injection campaigns of MicroCED were conducted in 2011. The amount of MicroCED from these two injections did not meet the total needed to support the study. A limitation in capacity to support microbial growth and project schedule were key factors in supplying the needed quantity of MicroCED for the test. To support the additional need, an approach was implemented to evaluate the potential to grow MicroCED in the field. Success was not measurable and limited sampling schedules did not allow enough data collection prior to injection. However, a total of 140 liters of MicroCED were injected with an additional 2,972 liters of inoculated water from the totes.

Data analysis of the field data demonstrated that changes were occurring at the site. However, even though changes in DO, ORP, TOC, and other constituents were observed that suggest a reducing environment, not all the injection wells or monitoring points monitored reacted the

same to the injection of the amendments. Naturally, the injection wells exhibited the greatest changes. However, the monitoring points did not indicate significant changes by the end of the year. Some minor increases in TOC and decreases in DO and ORP were observed but the overall condition of the groundwater remains oxygenated with the exception near the injection wells.

Changes in cVOC concentrations were observed during the sampling events. Decreases in TCE and c12DCE concentrations could be attributed to natural processes (i.e., dilution dispersion) and partitioning into the emulsified oil. Microbial degradation may be occurring but data collected is inconclusive. This can be attributed to the limited quantity of microbes injected and monitoring timeframe needed to determine long-term effects.

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