APPENDIX B

MICROCOSM STUDY REPORT

Microcosm Study Report
Old Erie Canal Site

Abstract

The Old Erie Microcosm study was designed to determine if the complete reductive dechlorination of TCE to ethene could be stimulated at the Old Erie Canal site. A study was set up with four electron donors (lactate, ethanol, SC-80 chitin, and soybean oil) added to 120 mL bottles containing soil and groundwater obtained from the a primary source area at the site. Reduced anaerobic mineral media (RAMM) was added to selected bottles in order to determine if nutrients were required to further encourage microbial activity. A set of positive controls, containing an actively dechlorinating microbial population, was included in the study to determine if bioaugmentation would be beneficial to enhance the degradation rate. Unamended and killed controls were also included in each study. TCE was added to the killed control and donor amended bottles at a concentration of approximately 50 mg/L at the beginning of the study. Two sets of unamended control bottles were amended with 5 mg/L and 50 mg/L of TCE.

The overall conclusions that are based on an analysis of variance (ANOVA) statistical analysis are listed below:

- Enhanced bioremediation is a feasible remedial option at this site. All of the electron donors supported complete reductive dechlorination of TCE to ethene within the course of the experiment. The addition of electron donors promoted a two to three fold increase in the overall biodegradation rate over the comparable unamended control. TCE was biodegraded very rapidly in all the amended bottles. Lactate, chitin, and soybean oil promoted the fastest biodegradation of cis-dichloroethene (cis-DCE). Chitin and soybean oil promoted the fastest biodegradation of vinyl chloride (VC). In these cases, VC was biodegraded almost as fast as it was formed, so that very little was measured in the bottles. The choice of electron donor for use in a potential field application would depend on site conditions and stratigraphy.

- Complete reductive dechlorination of TCE to ethene was observed in both the unamended controls at both concentration levels. It is unusual to observe this level of activity at such a high TCE concentration and indicates robust intrinsic biological activity is currently operative at the site. This suggests that monitored natural attenuation should be an important part of the remedial strategy at this site.

- The addition of supplemental nutrients in the form of RAMM did not have a substantial positive effect on the rate or extent of TCE dechlorination. Bioaugmentation also did not have a significant effect on the results observed. These results indicate that neither supplemental nutrients nor bacteria would be required to promote the rapid biodegradation of TCE at this site.

Introduction

Accelerated anaerobic biodegradation of chlorinated solvent source areas may represent a reasonable remedial option when natural attenuation processes alone are not sufficient to mitigate risk to human health and the environment. Accelerated biodegradation involves the addition of simple and safe sources of carbon and nutrients to the subsurface in order to stimulate anaerobic bacteria capable of reductively dechlorinating chlorinated solvents like tetrachloroethene (PCE) and trichloroethene (TCE) to innocuous byproducts like ethene and ethane. Reductive dechlorination involves the step-wise replacement of individual chlorine atoms with hydrogen atoms, such that

$$\text{TCE} \rightarrow \text{cDCE} \rightarrow \text{VC} \rightarrow \text{Ethene}$$

where cDCE and VC are cis-1,2-dichloroethene and vinyl chloride, respectively.

Over a decade ago, the biological conversion of PCE and TCE to ethene or ethane by anaerobic reductive dechlorination was demonstrated both in the laboratory and in the field (Freedman & Gossett, 1989; Major, et al., 1991; de Bruin, et al., 1992). In these processes, the chlorinated compounds act as an electron acceptor, while an electron donor is required to

provide energy (McCarty, 1994). Hydrogen is generally considered to be the direct electron donor for reductive dechlorination, but is typically produced from the anaerobic oxidation of other carbon substrates, such as sugars, organic acids, or alcohols (Maymo-Gatell, et al., 1995).

Complete dechlorination of chlorinated aliphatics to ethene or ethane has been demonstrated to occur at many sites impacted by chlorinated solvents. However, this extensive dechlorination does not occur at all sites (Ellis, 1997). In rare instances, no dechlorination is observed. More commonly, partial dechlorination is observed, where the process stops at an intermediate product like cDCE, rather than proceeding all the way to ethene or ethane. This may occur for one or more of the following reasons:

- Carbon sources (electron donors), which provide energy for the dechlorinating microorganisms, are not present or are not present in sufficient quantity;
- Other important nutrients, such a nitrogen or phosphate, are lacking; and.
- The proper dechlorinating microorganisms are not present.

There are many carbon sources suitable for promoting reductive dechlorination of chlorinated aliphatics by anaerobic bacteria. Among these, sugars (e.g., molasses), organic acids (e.g.,, lactic acid or its sodium salt), and alcohols (e.g., methanol, ethanol) have been most widely used in enhanced biodegradation applications to date. Because these substrates are soluble in water and highly biodegradable, they may need to be added periodically, in continuous or batch mode. Substrate addition and maintenance of the injection system is typically the most expensive aspect of this remedial approach (Harkness, 2000).

More recently, water insoluble carbon sources have seen increasing application in enhanced biodegradation. These carbon sources biodegrade slowly over time and include substances like lactic acid polymers, soybean oil, chitin, and wood chips. Unlike water soluble substrates, these materials do not require continuous or batch additions in order to maintain their effectiveness and therefore can be cheaper to apply due to reduced substrate addition and

system maintenance costs. However, in some cases the cost of these materials can be prohibitive (Harkness, 2000).

Bacteria also require basic nutrients like nitrogen and phosphorus in order to grow. These nutrients are often present in sufficient quantities in soil and groundwater, but can be limiting in some cases. In the same way, dechlorinating bacteria are generally present in the environment, particularly those capable of dechlorinating PCE and TCE to cDCE. However, the bacteria responsible for dechlorinating cDCE through VC to ethene are more sensitive to environmental conditions, and are not present at all sites. In this case, they can be added via bioaugmentation and will grow and proliferate in the subsurface under favorable conditions (Harkness, et al., 1999, Ellis, et al., 2000).

The purpose of this study was to determine whether the complete reductive dechlorination of TCE could be stimulated in soil and groundwater from near a source area at the Old Erie Canal Site, located in the village of Clyde, NY. The study was designed to determine if an optimal carbon source (e.g., sodium lactate, ethanol, chitin, or soybean oil) exists for stimulating dechlorination at this site, and whether other nutrients (e.g., nitrogen, phosphate, or trace minerals) must also be added to promote optimal biodegradation. Finally, the study determined whether bioaugmentation (e.g., the addition of non-native dechlorinating bacteria) was necessary or beneficial to the biodegradation process. The study was performed at the General Electric Company's (GE's) Global Research Center (GRC) in Schenectady, New York, in accordance with standard practices developed in GE's partnership in the Remedial Technology Development Forum's (RTDF's) Bioremediation of Chlorinated Solvents Consortium. The microcosm work lasted for about five months.

Materials and Methods

The soil used in this study was obtained from the saturated soil strata in the former barge turn-around area of the site, where high VOC concentrations were measured in the groundwater. The samples were obtained next to boring points GW-GP-20 and GW-GP-25. Groundwater samples obtained from GW-GP-20 in April 2002 contained 0.1 mg/L of TCE, 44 mg/L of

DCE, and 44 mg/L VC, while those from GW-GP-25 contained 71 mg/L of TCE, 170 mg/L of DCE, and 22 mg/L VC. The soil sample consisted of 2 to 3 kilograms (kg) of soil collected using a direct push rig and 2-inch diameter acetate sleeves. The acetate sleeves were capped and sealed with wax immediately upon retrieval, then packed in iced coolers and sent by overnight courier to GE GRC. Upon arrival, the soil was transferred from the sleeves to glass jars in order to limit diffusion of air through the tubes and stored under anaerobic conditions prior to use. In order to reduce variability in the experiment, all of the soil was sifted through ¼" screens to homogenize the soil and remove any larger rocks that were present. This ensured that any residual chlorinated solvents, as well as the microbial population, were evenly distributed throughout the soil.

In addition, approximately 8 Liters (L) of groundwater were obtained from well MW-6S, located in the same area as GW-GP-20 and GW-GP-25. The groundwater samples were collected in 1 L plastic or glass containers, filled to the brim with groundwater, capped, packed in iced coolers and shipped by overnight courier to GE GRC. The groundwater was stored at 4^{o}C prior to use.

The microcosm study was performed in sterile 120 milliliter (mL) serum bottles. Fifty (50) grams (g) of soil was weighed out and dispensed into each bottle in an anaerobic glove box containing an atmosphere of approximately 5 percent (%) hydrogen in nitrogen. Each serum bottle was then filled with 75 mL of non-sterile, filtered ground water that was sparged with nitrogen to remove any pre-existing solvents.

Four electron donors (sodium lactate, ethanol, chitin and soybean oil), supplemental nutrients in the form of yeast extract (YE) and reduced anaerobic mineral media (RAMM), and dechlorinating microorganisms were added to the microcosms alone and in combination to determine the optimum conditions for carrying out the reductive dechlorination of TCE. The study design is outlined in Table 1. The design consists of two sets of unamended microcosms (treatments 1 & 2) and multiple sets of microcosms where the individual electron donors are added alone (treatments 3-6) and in combination with supplemental nutrients (treatments 7-10). One of the unamended sets was spiked with 5 mg/L TCE to test the natural

attenuation capacity of the system under low TCE loadings. All other bottles were spiked with 50 mg/L TCE. This design allowed the efficacy of different electron donors to be assessed and the necessity of supplemental nutrients to be determined. In addition, one set of microcosms was amended with sodium lactate, yeast extract, supplemental nutrients and was bioaugmented with an active TCE-dechlorinating culture to act as positive controls, which ensured that there were dechlorinating bacteria available to biodegrade the solvent (treatments 11). Finally, there was also a killed control (treatment 12) to monitor non-biological losses from the bottles.

For each treatment, microcosms were set up in triplicate (Wilson, et al., 1997). The bottles were sealed with Teflon™-coated septa and aluminum crimp seals and incubated upside-down in the dark at room temperature.

Treatment	Donor	YE	RAMM	Pinellas	TCE-low	TCE - hi
1. Unamended						X
2. Unamended					X	
3. Lactate	Lactate					X
4. Ethanol	Ethanol					X
5. Chitin	SC-40					X
6. Soybean Oil	EOS					X
7. Lactate + RAMM	Lactate	X	X			X
8. Ethanol + RAMM	Ethanol	X	X			X
9. Chitin + RAMM	SC-40	X	X			X
10. SB Oil + RAMM	EOS	X	X			X
11. Positive Control	Lactate	X	X	X		X
12. Killed Control						X

Table 1 Treatments used during the microcosm study

Sodium lactate and ethanol are water-soluble substrates and were added to the microcosms each week as concentrated solutions using a gas-tight syringe. Lactate was added as 1.0 mL of 0.5 molar (M) sodium lactate solution in water, resulting in a final concentration of 5 millimolar (mM) in the microcosms. Ethanol was added as 1.0 mL of 0.75 molar (M) ethanol solution in water, resulting in a final concentration of 7.5 millimolar (mM) in the microcosms.

Chitin and soybean oil are not water-soluble substrates, and were added only at the beginning of the study. The chitin used was SC-40 (JRW bioremediation Products), a commercial bioremediation grade material consisting of 30-40 percent chitin with a calcium carbonate residual. One gram of this material was added to each bottle in a glovebox under anaerobic conditions. The soybean oil used was EOS (EOS Remediation, Inc.), a commercial emulsified soybean oil product that also contains small amounts of sodium lactate and yeast extract. In this case 0.50 g of the EOS substrate was added.

Yeast extract and RAMM was added once to designated bottles at the beginning of the study. Yeast extract was added as a concentrated solution [0.1 mL of 30 grams per liter (g/L) yeast extract], resulting in a final concentration of 30 mg/L in each microcosm. RAMM consists of a phosphate buffer, potassium and ammonium salts, and trace metals (Shelton & Tiedje, 1984) and was also added as a concentrated solution.

The positive control was bioaugmented with the Pinellas consortium, which contains bacteria able to completely dechlorinate TCE to ethane (Flanagan, et al., 1995). The Pinellas consortium was recently used to bioaugment a field test at Dover Air Force Base, resulting in the first successful use of bioaugmentation to promote the complete dechlorination of TCE to ethene in the field (Harkness, et al., 1999, Ellis, et al., 2000). Bioaugmentation took place two to three weeks after the beginning of the study, to ensure anaerobic conditions were established in the microcosms. The Pinellas consortium was added by replacing 5% of the liquid volume in the microcosms with active Pinellas bacteria growing in a low-density liquid culture containing $1x10^7$ to $1x10^8$ cells per milliliter (cells/mL). The TCE concentration in the Pinellas culture medium was monitored to ensure that all of the TCE had been degraded to ethene prior to bioaugmentation.

The killed controls was autoclaved twice and amended with mercuric chloride using a gas-tight syringe. The mercuric chloride was added as a concentrated solution (3.0 mL of 6% mercuric chloride in deionized water), resulting in a final concentration of 180 mg/L in each microcosm. The unamended controls did not receive any electron donor or nutrient amendments. Resazurin, a redox-sensitive color indicator for anaerobic conditions, was

added to all the bottles at the beginning of the experiment. No reducing agents were used in this experiment.

TCE was spiked into the microcosms at the beginning of the study using a gas-tight syringe. TCE was added from neat or saturated stock solutions to target concentrations of 50 mg/L (high dosage) for most of the bottles and 5 mg/L (low dosage) for one of the unamended controls.

VOC Analysis

VOC samples were obtained by removing 1.0 mL of liquid from the bottles using glass, gas-tight syringes. The microcosms were sampled at the start and at two-week intervals throughout the study. PCE, TCE, TCA, 11-DCE, cDCE, 11-DCA, VC, CA, and ethene/ethane were measured using a Tekmar 2016 Purge and Trap Autosampler, a Tekmar 300 Concentrator, and a Hewlett Packard 5890 Series II Gas Chromatograph (GC) equipped with a flame ionization detector (FID) and a HP-624 [30 meter (m) by 0.53 millimeter (mm) inner diameter] fused silica capillary column. Chlorinated aliphatics and ethene/ethane were quantified by comparing peak areas to standard calibration curves generated using water dilutions of a standard mixture containing TCE, cDCE, and VC obtained from Supelco, Inc., or by direct injection of known amounts of a standard gas mixture containing ethene.

Other Biological Indicators

In addition to VOC analysis, other indicators of biological activity were monitored during the study. Resazurin is a redox-sensitive color indicator for anaerobic conditions. Resazurin is blue under oxidizing conditions, pink under mildly reduced conditions, and clear under more strongly reduced conditions (e.g. –100 mV). Therefore, the color of the water in the bottles was monitored, especially early in the experiment.

Biologically mediated sulfate reduction produces hydrogen sulfide, which reacts readily with soluble (ferrous) iron in solution to form a distinctive black precipitate. Given that sulfate

and iron are present in the groundwater, this precipitate is a clear indication that sulfate-reducing conditions are present in the bottles. Sulfate reduction is an indicator that strongly reducing conditions are present in the microcosms. Excessive sulfide-reduction can be inhibitory to dechlorinating activity, but this has typically only been observed where sulfate levels are very high in the groundwater.

Under the most highly reduced conditions, carbon dioxide is reduced to methane gas by methanogenic bacteria. This activity is significant because reductive dechlorination of cDCE and VC to ethene and ethane typically occurs under these most highly reduced conditions. Therefore, any gas produced in the microcosm bottles was collected and measured on a weekly basis during the study.

Results and Discussion

Biological activity was observed in the bottles throughout the duration of the experiment. The indicator in all of the bottles went from pink to clear within the first two days of the experiment. Significant gas production was observed in the majority of the bottles. The black precipitate indicative of sulfate reduction was observed in all of the lactate, chitin, and soybean oil-amended bottles during the course of the study.

Time Course Analysis

The graphs of the VOC results over time are provided in Figures 1-5. TCE was completely dechlorinated to cis-DCE prior to the first sampling point (three days) in all bottles, except the killed control (Figure 1c). The killed control bottles were the only set that did not achieve complete reductive dechlorination of TCE to ethene during the course of the experiment. The majority of the bottles went to completion within the first 67 days of the study. The exception to this was the unamended control containing 50 mg/L TCE, which required 124 days to reach complete dechlorination to ethene.

Most of the cis-DCE was dechlorinated to VC as of day 30 in the lactate amended bottles (Figure 2a). Complete dechlorination of VC to ethene was observed by day 47 in two of the

bottles. The remaining bottle went to only ethene as of day 67. Cis-DCE and VC were completely dechlorinated to ethene in 47 days in both the lactate and RAMM amended bottles (Figure 2b) and bioaugmented controls (Figure 2c).

Complete dechlorination of cis-DCE and VC to ethene was observed as of the 67-day sampling period in both the ethanol amended bottles (Figure 3a) and bottles containing ethanol and RAMM (Figure 3b).

The time to complete dechlorination to ethene observed for the three bottles amended with chitin ranged from 30 to 67 days (Figure 4a). Very little VC production was observed in these bottles during the experiment. Complete dechlorination of cis-DCE and VC to ethene was observed as of the 47-day sampling period in the chitin amended bottles with RAMM present (Figure 4b). In this case a small amount of VC was observed.

Complete dechlorination of cis-DCE and VC to ethene was observed in the soybean oil amended bottles as of day 47 of the study (Figure 5a). Similar results were observed when nutrient were present (Figure 5b), with one bottle reaching completion as early as day 30.

In the unamended control containing 5 mg/L TCE, cis-DCE was completely dechlorinated to VC in all bottles by day 47 of the study (Figure 1a). The VC in one bottle in this set was completely reduced to ethene by day 47 of the study, while the other two bottles went to completion by day 67. Cis-DCE went completely to VC in the unamended control with 50 mg/L TCE (Figure 1b) by day 95 of the study. The VC is these bottles was completely reduced to ethene as of day 124. . It is unusual to observe this level of activity in unamended controls, particularly at such a high TCE concentrations. These results indicate that substantial intrinsic biodegradation of TCE and its daughter products is currently operative at the site and suggest that monitored natural attenuation should play an important role in the remedial strategy at this site.

Finally, a reduction in TCE with a subsequent increase in cis-DCE and VC was observed in the killed control bottles during the first 30 days of the study, suggesting even autoclaving

and the addition of mercuric chloride was not sufficient to completely kill off this robust dechlorinating population. However, bioactivity ceased after this point and the TCE and cis-DCE levels remained approximately constant throughout the duration of the study.

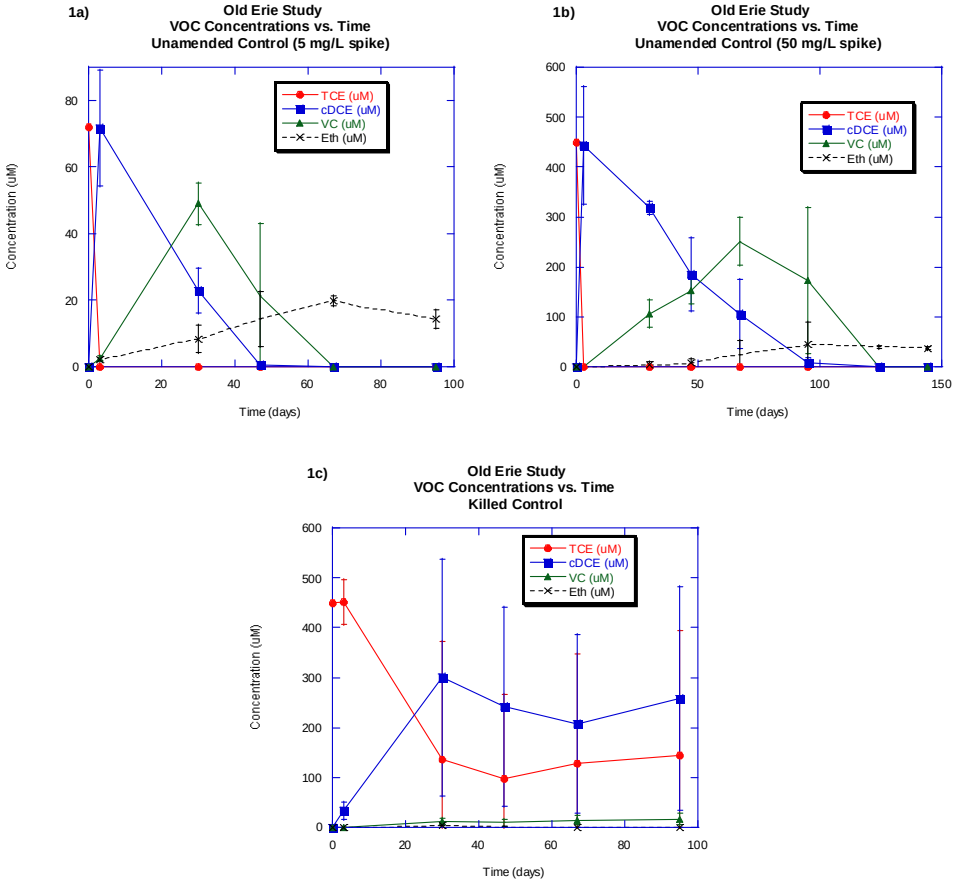
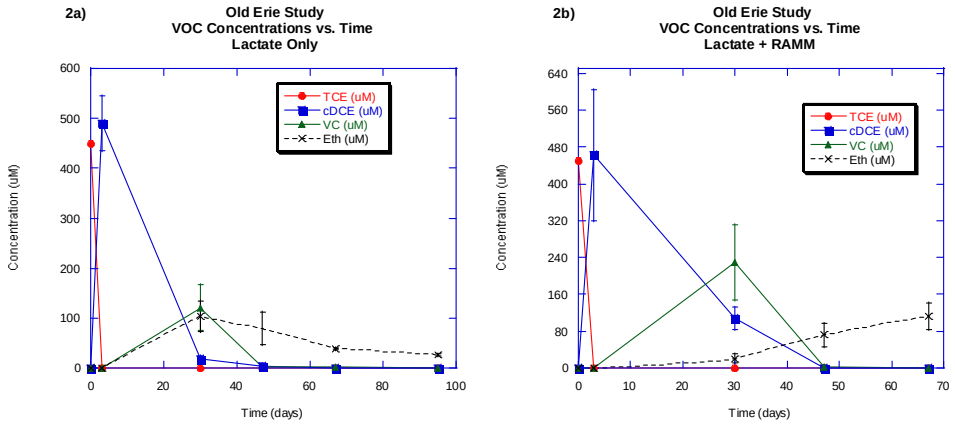


Figure 1 VOC results for **a)** Unamended control (5 mg/L) **b)** Unamended Control (50 mg/L) **c)** Killed Control



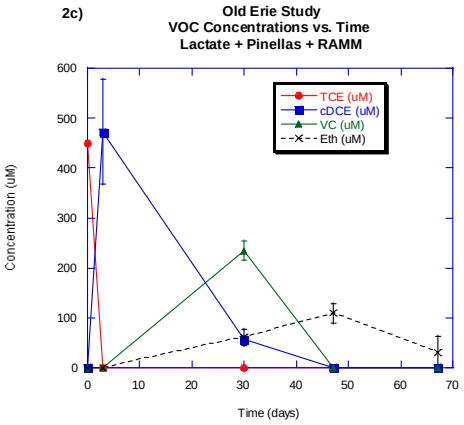


Figure 2 VOC results for **a)** Lactate **b)** Lactate + RAMM **c)** Lactate + Pinellas + RAMM

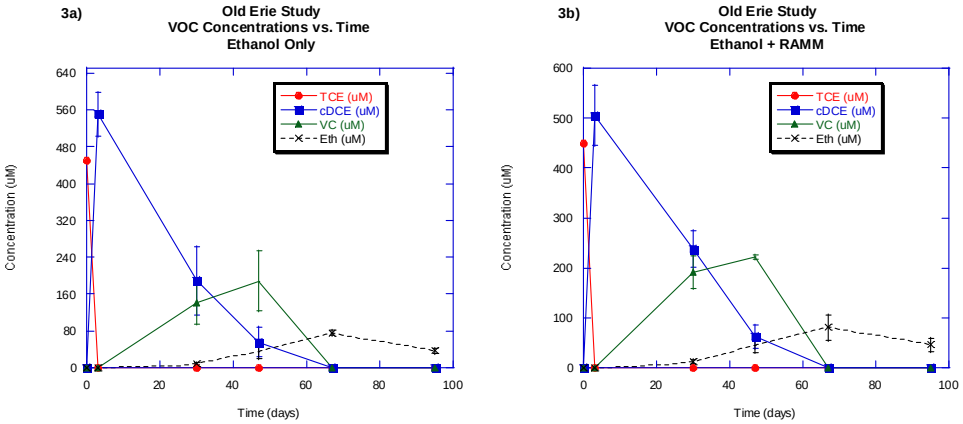


Figure 3 VOC results for **a)** Ethanol **b)** Ethanol + RAMM

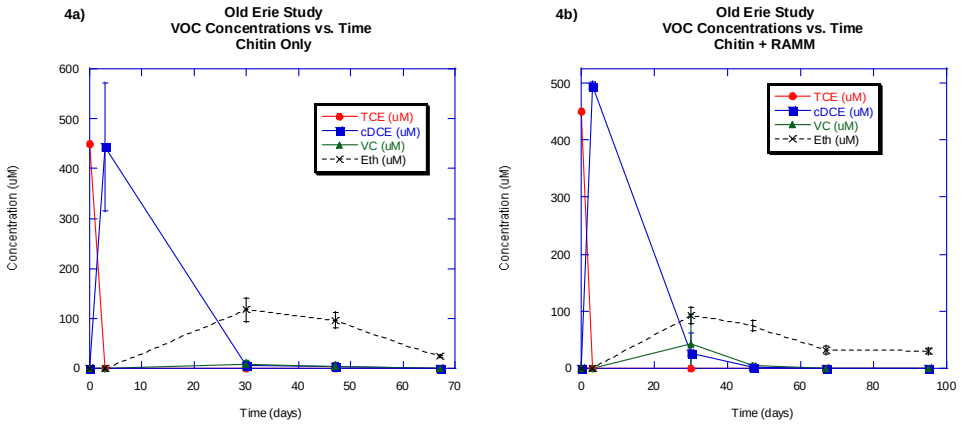


Figure 4 VOC results for **a)** Chitin **b)** Chitin + RAMM

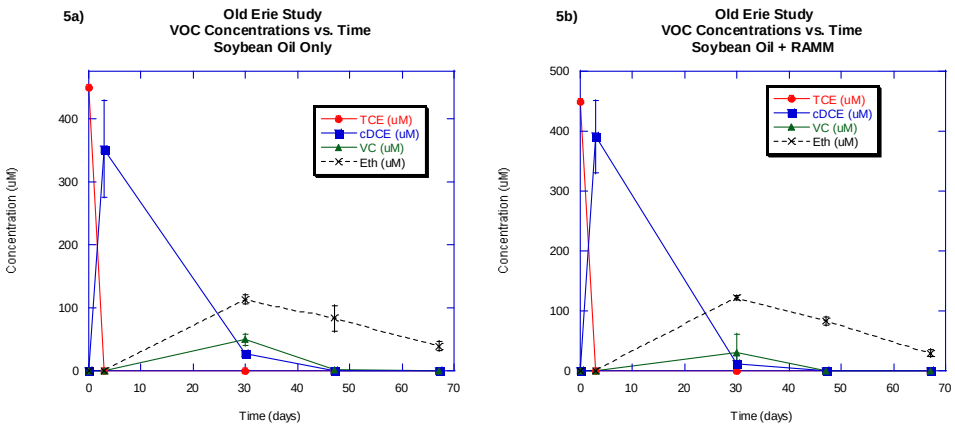
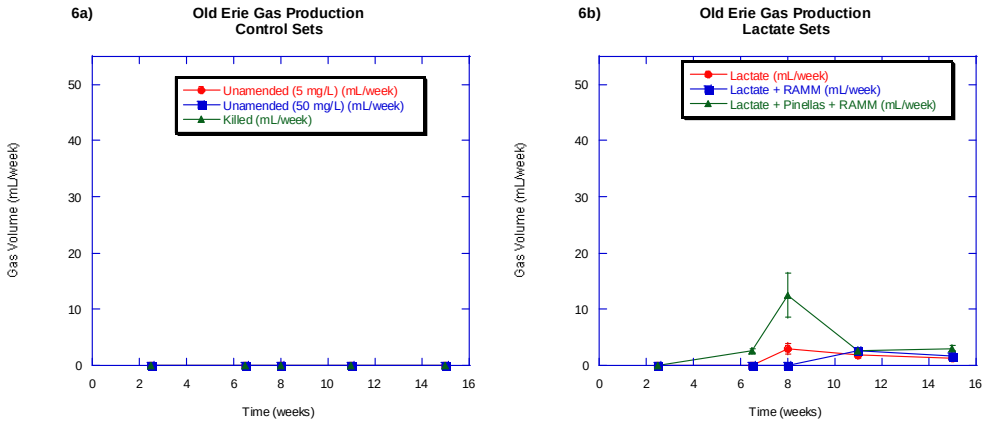


Figure 5 VOC results for **a)** Soybean Oil **b)** Soybean Oil + RAMM

Gas Production

In addition to measuring the levels of VOCs present in the bottles, the production of gas over time was measured periodically by venting the headspace of each of the bottles. The results are shown in the graphs in Figure 6. The greatest amount of gas production was observed in the soybean oil-amended sets (Figure 6e), with the maximum production observed around week 11 of the experiment. Gas production in the other amended sets peaked between weeks 8 and 11 and continued throughout the experiment (Figures 6b, 6c, 6d). No measurable gas was produced in the controls (Figure 6a) during the course of the experiment. The gas volumes observed throughout the study further support the observation that the site is extremely biologically active.



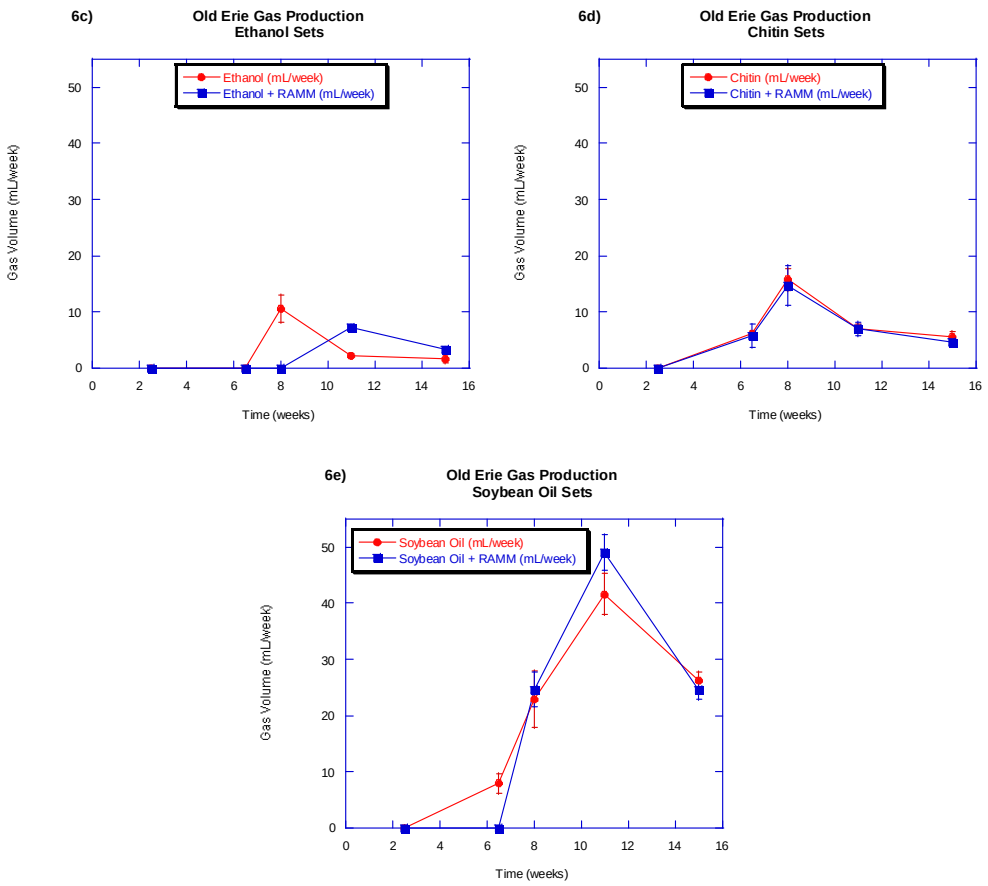


Figure 6 Gas Production for **a)** Controls **b)** Lactate sets **c)** Ethanol sets **d)** Chitin sets **e)** Soybean oil sets

CVOC Ratio Analysis

The following molar ratio was used to measure the performance of the microcosms:

$$\frac{\text{Total moles CVOC at day } i}{\text{Total moles CVOC at day 0}}$$

where *i* represents the day of sampling for which the ratio was being determined and Total CVOC represents the total concentration of CVOCs at the given point in time. All CVOC concentrations were in uM. Using this ratio, the most successful bottles were those where the ratio approached zero by the end of the experiment, indicating that all the VOCs were degraded and only ethene remained.

Graphs of the CVOC ratio results over time are provided in Figures 7-11. The graphs clearly support the observation that all of the treatments were effective, with the exception of the killed control, since they all went to zero during the course of the experiment. Each graph shows the CVOC ratio in each bottle, as well as the average of each set, so that bottle-to-bottle variability can be seen. In general, the bottle-to-bottle consistency in this study was quite good.

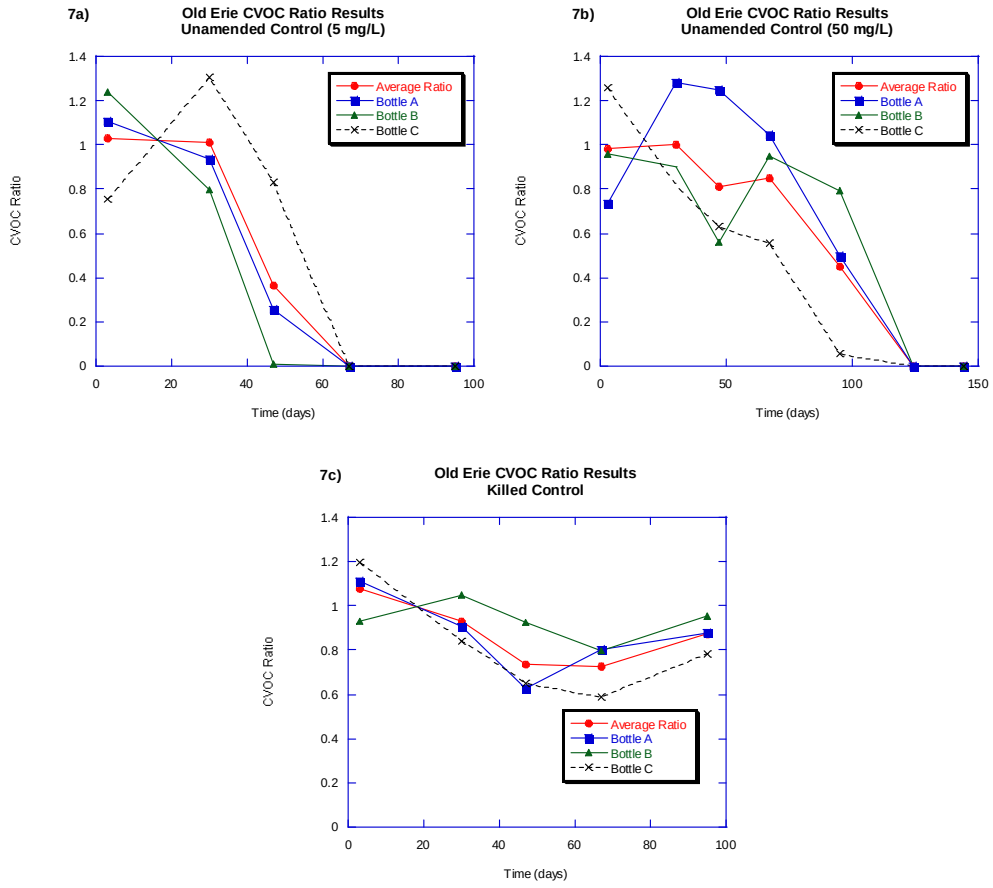


Figure 7 CVOC ratio results for **a)** Unamended Control (5 mg/L) **b)** Unamended Control (50 mg/L) **c)** Killed Control

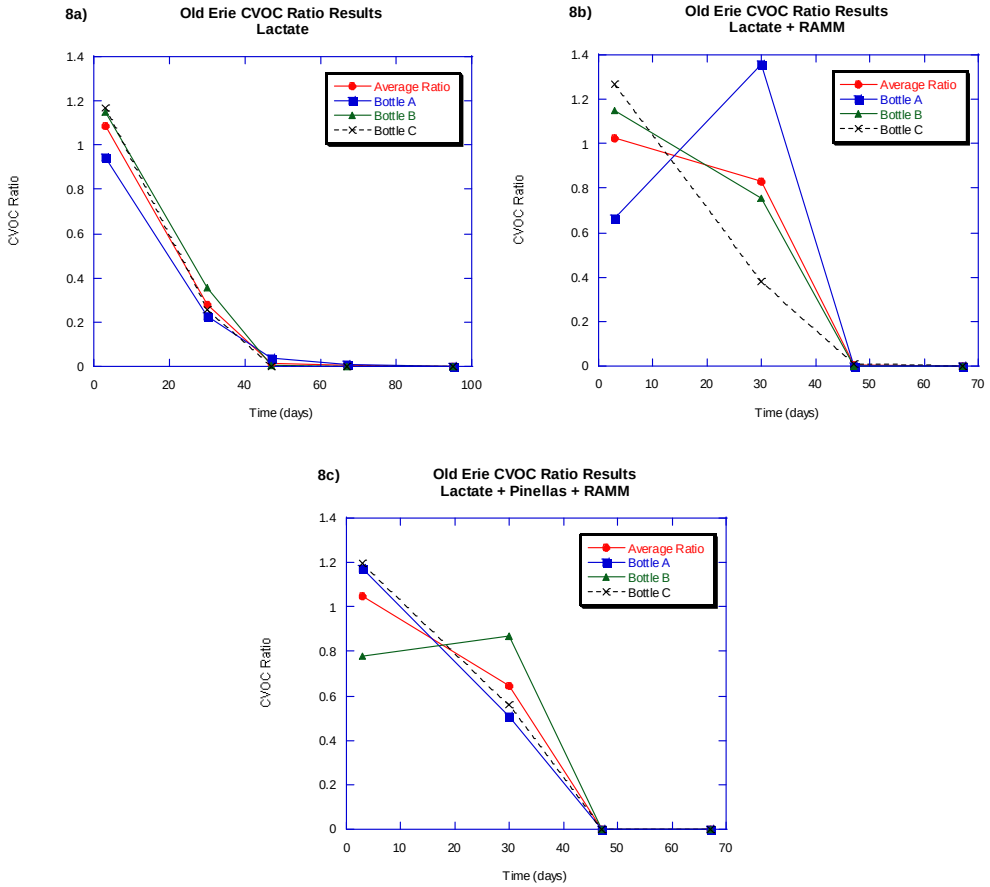


Figure 8 CVOC ratio results for **a)** Lactate **b)** Lactate + RAMM **c)** Lactate + Pinellas + RAMM

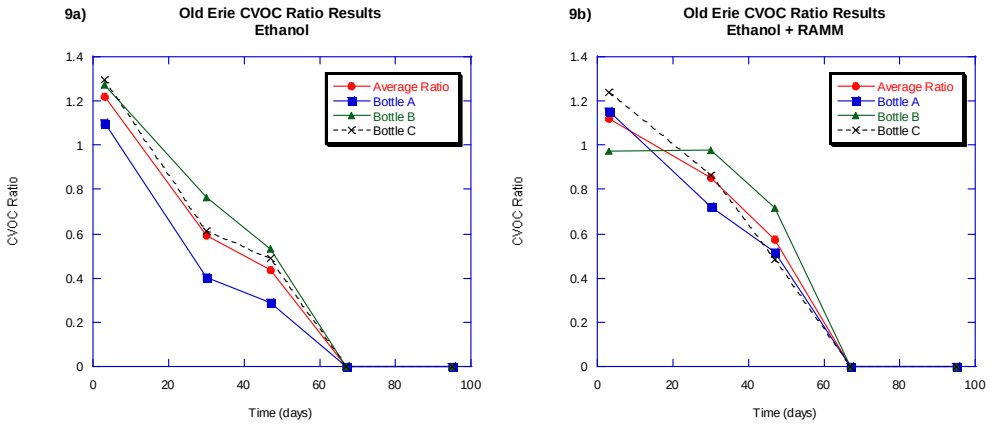


Figure 9 CVOC ratio results for **a)** Ethanol **b)** Ethanol + RAMM

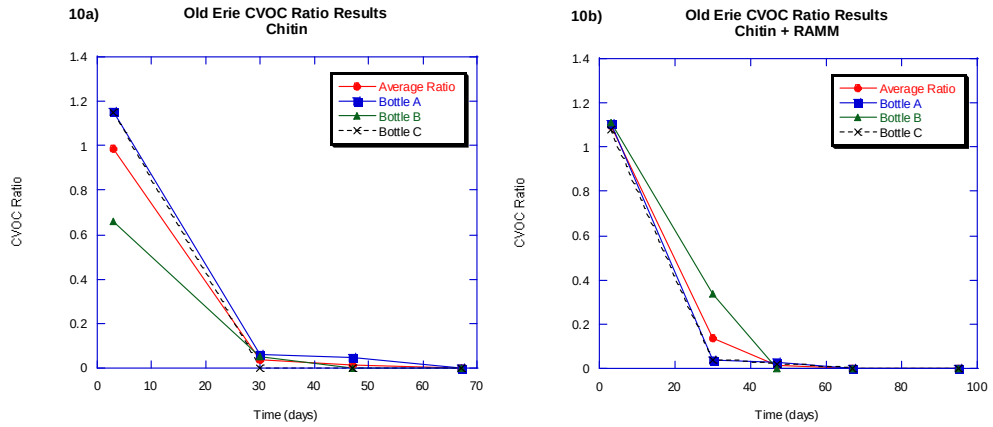


Figure 10 CVOC ratio results for **a)** Chitin **b)** Chitin + RAMM

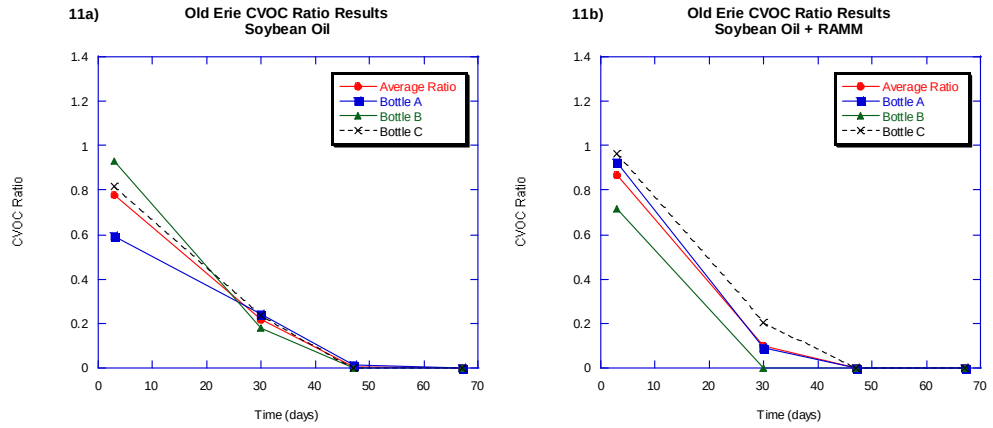


Figure 11 CVOC ratio results for **a)** Soybean Oil **b)** Soybean Oil + RAMM

Statistical analysis

Two metrics were used to subject the data to statistical analysis. The CVOC ratio calculated at the end of the study is a measure of the extent or completeness of degradation in each treatment. The time required to reach the zero endpoint in the CVOC ratio is a relative measure of degradation rate. After the CVOC ratio and time to zero endpoint (>97% CVOC removal) were determined for each set, a statistical analysis of each study was performed in Minitab in order to determine the statistical significance of the donor and RAMM terms and the first-order interaction between donor and RAMM. A confidence level of 95% was used to determine the statistical significance of a given factor. The design-of-experiment (DOE) set-up is shown in Table 2.

The results of the statistical analysis of the DOE are shown in the Appendix. The DOE was analyzed according to the two response variables, the CVOC ratio and the time to zero endpoint. None of the terms (donor, RAMM, donor-RAMM interaction) were found to be statistically significant when using the CVOC ratio for the analysis (Table A-1). This is reasonable, since all the bottle sets went to completion during the course of the study, and indicates all conditions were effective in promoting complete dechlorination of TCE to ethene.

However, when the results were analyzed according to the time to endpoint (Table A-2), the donor term was found to be statistically significant (p-value = 0.001). This is primarily due to the fact that all of the ethanol bottles went to completion at day 67, which was longer than the average time to endpoint observed for the other donor treatments. This is evident from both the endpoint values in Table 2 and from the graphs of the interaction effects provided for each analysis in the Appendix (Figures A-1 and A-2). Both show that the time to complete dechlorination was slightly slower using ethanol as the electron donor. However, since this rate difference was relatively small and all of the electron donors were effective in encouraging the complete reductive dechlorination of TCE to ethene, the choice of electron donor for use in a potential field application would depend primarily on site conditions and stratigraphy.

The RAMM term and the donor-RAMM interaction term were not statistically significant in either analysis, indicating that supplemental nutrients did not have a significant effect on the rate or extent of dechlorination. Therefore additional nutrients should not be required at this site.

Donor	RAMM	CVOC ratio at endpoint	Time to endpoint
Lactate	No	0.0082	67
Lactate	No	0.0051	47
Lactate	No	0	47
Lactate	Yes	0	47
Lactate	Yes	0	47
Lactate	Yes	0.012	47
Ethanol	No	0	67
Ethanol	No	0	67
Ethanol	No	0	67

Ethanol	Yes	0	67
Ethanol	Yes	0	67
Ethanol	Yes	0	67
Chitin	No	0	67
Chitin	No	0	47
Chitin	No	0	30
Chitin	Yes	0.026	47
Chitin	Yes	0	47
Chitin	Yes	0.02	47
Soybean Oil	No	0.012	47
Soybean Oil	No	0	47
Soybean Oil	No	0	47
Soybean Oil	Yes	0	47
Soybean Oil	Yes	0	47
Soybean Oil	Yes	0	47

Table 2 DOE set-up for Old Erie study

Effect of bioaugmentation

In order to determine if the addition of Pinellas bacteria to the positive controls was a statistically significant factor in the experiments, t-tests were run between the lactate + RAMM and lactate + Pinellas + RAMM bottle sets. The tests were run using both the CVOC ratio and the time to zero endpoint as response variables. The results of these analyses are shown in the Appendix. Neither analysis showed a statistically significant difference between the two sets in terms of the rate or extent of dechlorination observed. This indicates that bioaugmentation would not be required at this site.

Rate constant analysis

In order to compare the performance of the unamended and amended sets, rate constants for cis-DCE degradation were calculated using Scientist, which is a computer program capable of solving differential equations. The computation was performed assuming the contaminants biodegrade according to first order kinetics. The following equations were used to determine the first order rate constants:

Equation 1: $d[TCE]/dT = -K_{TCE}*[TCE]$

Equation 2: $d[cis\text{-}DCE]/dT = K_{TCE}*[TCE] - K_{cis\text{-}DCE}*[cis\text{-}DCE]$

Equation 3: $d[VC]/dT = K_{cis\text{-}DCE}*[cis\text{-}DCE] - K_{VC}*[VC]$

Where [TCE], [cis-DCE] and [VC] are the concentrations of the contaminants at a given point in time, T represents time and K_{TCE}, $K_{cis\text{-}DCE}$ and K_{VC} are the first order rate constants for the three contaminants. The data from the microcosm experiments and initial estimates for the rate constants were required for Scientist to perform the first order rate constant calculations.

The results are in Tables 3 and 4, which show the rate constants in units of day^{-1}. The rate constants for TCE are not reported. The results for these rate constants were not meaningful because the biodegradation of TCE was too rapid to measure accurately.. The comparison between the amended and unamended rate constants and the half-life, in days, for cis-DCE and VC in each set are included. The rate constant for the unamended control with 50 mg/L was used for the comparison to the amended sets. The rate constant for the killed control set was not evaluated because very little dechlorination was observed during the course of the experiment.

The rate constant for cis-DCE biodegradation in the unamended control with 50 mg/L was 0.02 day^{-1}, which corresponded to a half-life of about 35 days. The cis-DCE biodegradation rates observed in the amended bottles were about two to three times greater, with half-lives ranging from 10 to 20 days. In general, the lactate, chitin, and soybean oil promoted the fastest cis-DCE biodegradation. The biodegradation promoted by ethanol was somewhat slower. These rate data are very consistent with the time to overall degradation shown in Table 2.

The rate constant for cis-DCE biodegradation in the unamended control with 50 mg/L was also 0.02 day^{-1}, corresponding to a half-life of about 33 days. The rate constants for VC biodegradation ranged from two to several hundred fold faster in the amended microcosms than in the comparable unamended control. Biodegradation of VC was particularly rapid in the chitin and soybean oil microcosms. In these bottles it was biodegraded almost as fast as it

was formed. Biodegradation of VC in the lactate and ethanol amended microcosms proceeded about twice as fast as the unamended control.

Set	$K_{cis\text{-}DCE}$ (day^{-1})	$K_{cis\text{-}DCE}$ Amended/ $K_{cis\text{-}DCE}$ Unamended	Half Life for cis-DCE (days)
Unamended – 5	0.045	--	15.40
Unamended – 50	0.02	--	34.66
Lactate	0.068	3.40	10.19
Ethanol	0.04	2.00	17.33
Chitin (SC-40)	0.07	3.50	9.90
Soybean Oil	0.064	3.20	10.83
Lactate + RAMM	0.051	2.55	13.59
Ethanol + RAMM	0.034	1.70	20.39
Chitin + RAMM	0.066	3.30	10.50
Soybean Oil + RAMM	0.069	3.45	10.05
Lactate + Pinellas + RAMM	0.06	3.00	11.55
Killed Control	--	--	--

Table 3 Rate constant analysis for cis-DCE

Set	K_{VC}	K_{VC} Amended/ K_{VC} Unamended	Half Life for VC (days)
Unamended - 5	0.037	--	18.73
Unamended - 50	0.021	--	33.01
Lactate	0.096	4.57	7.22
Ethanol	0.055	2.62	12.60
Chitin (SC-40)	16.1	766.67	0.04
Soybean Oil	2.2	104.76	0.32
Lactate + RAMM	0.061	2.90	11.36
Ethanol + RAMM	0.043	2.05	16.12
Chitin + RAMM	3.64	173.33	0.19
Soybean Oil + RAMM	4.78	227.62	0.15
Lactate + Pinellas + RAMM	0.06	2.86	11.55
Killed Control	--	--	--

Table 4 Rate constant analysis for VC

Conclusion

The primary objective of the Old Erie study was to determine the feasibility of enhanced bioremediation in reductively dechlorinating TCE to ethene. The experiments studied the

effects of the addition of four different electron donors (lactate, ethanol, chitin, and soybean oil), supplemental nutrients (RAMM), and bioaugmentation with bacteria.

Enhanced bioremediation is a feasible remedial option at this site. All of the electron donors tested in the study supported the complete biodegradation of TCE to ethene during the course of the experiment. The addition of electron donors promoted a two to three fold increase in the overall biodegradation rate over the comparable unamended control. Lactate, chitin, and soybean oil promoted the fastest biodegradation of cis-DCE. Chitin and soybean oil promoted the fastest biodegradation of vinyl chloride (VC). In these cases, VC was biodegraded almost as fast as it was formed, so that very little was measured in the bottles. The electron donor selected for use in a field application would depend on the site conditions and stratigraphy.

The rate and extent of TCE dechlorination was not positively affected by the addition of either RAMM or Pinellas bacteria. Both unamended control sets went to completion during the course of the study, indicating robust intrinsic biodegradation activity is currently operative at the site. Therefore, monitored natural attenuation should be integral part of the remedial strategy at this site.

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Appendix

General Linear Model

```
Factor     Type Levels Values
Donor     fixed      4 1 2 3 4
RAMM      fixed      2 1 2

Analysis of Variance for CVOC rat, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS       F      P
Donor         3  0.0001978  0.0001978  0.0000659    1.74  0.200
RAMM          1  0.0000447  0.0000447  0.0000447    1.18  0.294
Donor*RAMM    3  0.0003411  0.0003411  0.0001137    3.00  0.062
Error        16  0.0006070  0.0006070  0.0000379
Total        23  0.0011907
```

Table A-1 DOE analysis according to CVOC ratio

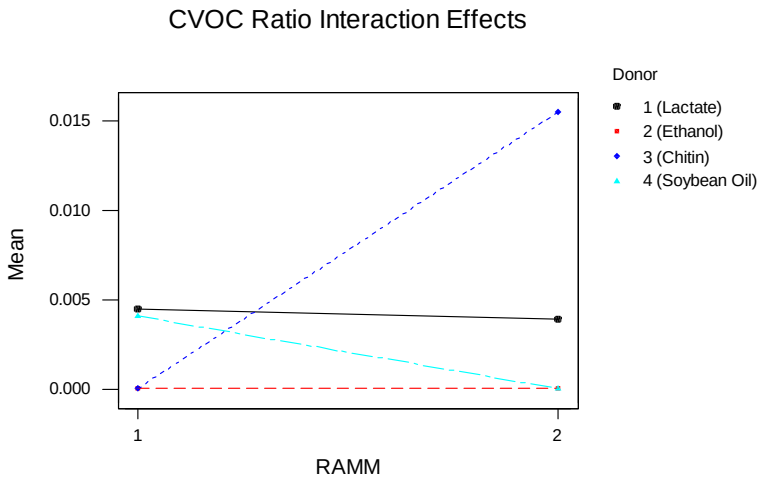

Figure A-1 Interaction effects according to CVOC ratio

General Linear Model

```
Factor     Type Levels Values
Donor     fixed      4 1 2 3 4
RAMM      fixed      2 1 2

Analysis of Variance for Time to, using Adjusted SS for Tests

Source       DF     Seq SS     Adj SS     Adj MS       F      P
Donor         3    1854.83    1854.83     618.28    8.64  0.001
RAMM          1      66.67      66.67      66.67    0.93  0.349
Donor*RAMM    3      49.67      49.67      16.56    0.23  0.873
Error        16    1145.33    1145.33      71.58
Total        23    3116.50
```

Table A-2 DOE analysis according to the time to endpoint

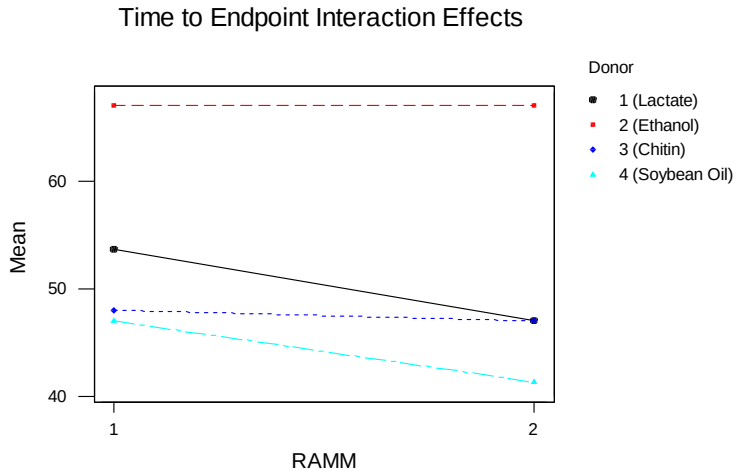

Figure A-2 Interaction effects according to time to endpoint

Two Sample T-Test and Confidence Interval

```
Two sample T for lr cvoc vs lpr cvoc

          N      Mean     StDev   SE Mean
lr cvoc   3   0.00391   0.00678    0.0039
lpr cvoc  33.3333E-095.7735E-093.3333E-09

95% CI for mu lr cvoc - mu lpr cvoc: ( -0.0129,  0.0207555675)
T-Test mu lr cvoc = mu lpr cvoc (vs not =): T = 1.00  P = 0.42  DF = 2
```

Table A-3 T-test between lactate + RAMM and lactate + Pinellas + RAMM according to CVOC ratio

Two Sample T-Test and Confidence Interval

```
Two sample T for lr end vs lpr end

         N      Mean     StDev   SE Mean
lr end   3     47.00      1.00      0.58
lpr end  3     47.00      1.00      0.58

95% CI for mu lr end - mu lpr end: ( -2.27,  2.27)
T-Test mu lr end = mu lpr end (vs not =): T = 0.00  P = 1.0  DF = 4
```

Table A-4 T-test between lactate + RAMM and lactate + Pinellas + RAMM according to time to endpoint