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Laboratory and Field Scale Bioremediation of Tetrachloroethene (PCE) Contaminated Groundwater

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Abstract

Bioremediation studies were conducted at a tetrachloroethene contaminated site located in Manhattan Kansas. A former dry cleaning facility was in operation for 30 years. Shallow and deep aquifers down gradient from contaminated site were found to be contaminated with PCE and it's degradation products all above their Maximum Contamination Limits (MCL's). PCE concentration in groundwater at the pilot study area was about 15 mg/L (ppm) in the deep zone and 1 mg/L in the shallow zone. Lab scale microcosms were prepared with different nutrients like soy oil methyl esters (SOME), yeast extract (YE), glucose, lactate, methanol and cheese whey for biostimulation experiments, and commercially available KB-1 bacterial culture was used to bioaugment PCE degradation. Biostimulation of the natural ground water and soil microflora did not completely degrade PCE as cis-DCE (c-DCE) accumulated in the sample. Bioaugmented microcosms containing YE and SOME created reducing conditions for KB-1 culture, resulting in ~ 90% dechlorination of PCE. Cheese whey microcosms with concentrations of (0.01% to 0.025%) reduced PCE, while 0.05% cheese whey and higher inhibited the KB-1 culture. This inhibition was due to a drop of pH that inhibited the bacterial culture activity. At pilot study area, tracer studies were conducted to monitor the direction and velocity of groundwater before during and after remediation experiments. Several nutrient feeding events took place during biostimulation and bioaugmentation. Results indicate that complete degradation of PCE occurred when KB-1 culture containing *Dehalococcoides* bacteria was introduced under anaerobic conditions. The total chlorinated ethenes (CEs) decreased by about 80% in the pilot study area due to bioremediation. Biodegradation of CEs continued for a long term (several months) after the addition of nutrients.

Keywords: Bioremediation; Chlorinated ethenes; PCE; Dehalococcoides.

1. Introduction

Tetrachloroethene, also known as Perchloroetheylene (PCE) is a common groundwater and soil contaminant. It's widespread application mainly at degreasing and dry cleaning sites has lead to its accumulation in natural systems (SCRD, 2007). Chlorinated solvents are considered the second most abundant contaminants after petroleum hydrocarbons (Sutfin, 1996). Acute (short term) exposure to PCE through inhalation may include irritation to upper respiratory tract and eyes, kidney dysfunction, dizziness, headache, sleepiness, and unconsciousness. While Chronic (long term) inhalation of tetrachloroethene have neurological effects, including sensory symptoms such as headaches, impairments in cognitive and motor neurobehavioral functioning and color vision decrements (U.S. EPA, 1994).

A number of abiotic processes may degrade chlorinated ethenes under both aerobic and anaerobic conditions. Abiotic pathways include hydrolysis, elimination, dehydrohalogenation and hydrolysis. Many abiotic transformations of chlorinated ethenes occur at rates that are too slow to have significance in contaminant removal (AFCEE, 2004). Bioremediation, both natural and enhanced, has proven to be a powerful approach for remediating chlorinated solvents, including PCE (Cupples et al. 2004; Lee et al. 1997; Maymo-Gatell et al. 1997). Under anaerobic conditions PCE is sequentially reduced to less-chlorinated and or non-chlorinated ethenes were chlorine atom is replaced by hydrogen in a process called reductive dechlorination that is also referred to as halorespiration (Bradley and Chapelle, 1999) (Figure 1). PCE, which contains four chlorine atoms, is sequentially degraded to TCE to DCE to VC and then to ethene. The process depends on environmental factors that include the presence of strongly anaerobic conditions, availability of fermentable substrates, generation of molecular hydrogen (H2) and the presence and viability of the appropriate microbial population to facilitate the reaction (Major et al., 2002). So far Dehalococcoides bacteria can degrade PCE to the final end products and they depend on hydrogen as the electron donor. The role of other anaerobic bacteria in

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the culture comes in providing the Dehalococcoides with the necessary hydrogen by fermenting utilizable substrates such as lactate, molasses, hydrogen releasing compound (HRC), emulsified vegetable oil, chitin, etc. (Newell et al. 2000). In this work, a biostimulation/bioaugmentation studies were carried out in lab scale microcosms and field pilot scale designed for a PCE contaminated site in Manhattan, KS (Figure 2). Biostimulation and bioaugmentation were used to enhance the rate and extent of biodegradation.

2. Materials and Methods

Ground water sampling

Ground water samples were collected from monitoring wells using a three stage pump. Three water samples were collected from each monitoring well with 5 ft (1.5 m) spacing between them, across the lower portion of the screened zone. Shallow wells were sampled at 18 (5.5 m), 23 (7 m) and 28 (8.5 m) ft below ground surface (bgs), while water from the deep wells was collected at 42 ft (13 m), 47 ft (14.3 m) and 52 ft (16 m) bgs. This sampling allowed detection of a concentration gradient in the monitoring wells and /or it could be considered as triplicate sampling from the same well. Order of sampling was always from the top to the bottom of the well. Samples were collected in 16.5 mL glass vials, filled to the top, and then immediately capped with mininert caps. Vials were then transported to the lab, and 1 mg/L resazurin added as a redox indicator. A 5 mL sample was taken out of the vial with a syringe leaving 5 mL of headspace. While liquid removed, the cap was loosened to allow air replacement. Vials were manually shaken and let stand at least an hour before head space analysis. The water removed was preserved in glass vials, closed with screw caps and stored in a cold room (4ºC) for ion analyses the same day.

3. Microcosms Preparations

Reagents and Supply

Chemicals used in this research included, D-Glucose (Fisher Scientific, Fair Lawn, NJ), Yeast Extract (Sigma-Aldrich, Inc. St. Louis, MO), Soy Oil Methyl Ester (AG Environmental Products, L.L.C.), Methanol (Certified A.C.S. Fisher scientific Co. Fair lawn NJ), Lactic acid (Sigma-Aldrich, Inc. St. Louis, MO), Cheese Whey (AlmaCreamery, KS), Resazurin (Baltimore Biological Laboratory Inc. Baltimore, MD). Chlorinated ethenes were obtained from: PCE (Certified A.C.S., Fisher Scientific Co. Fairlawn NJ), TCE (Aldrich Chemical Co. Inc., Milwaukee WI), cis-1,2-DCE (Chem. Service. West Chester PA. Purity 99.4%), VC (Chem. Service. West Chester PA), methane (Matheson Gas Products, A division of Will Ross Inc., E. Rutherford, NJ). The microbial culture KB-1, which was used in microcosm experiments was kindly provided by SiREM laboratories in Ontario, Canada. A 20 L batch of of KB-1 was purchased from SiREM for the pilot study (Figure 2.3). Hamilton gas tight syringes (Hamilton Company, Reno, Nevada) were used to inject gas phase samples into the gas chromatograph with 26 gauge needles, (SUPELCO, Bellefonte, PA). The clear glass 16.5 mL vials fitted with mininert Teflon caps were obtained from SUPELCO (Bellefonte, PA).

4. Water Microcosms

Water microcosms were prepared from different monitoring wells, at different times, to study the appropriate combinations of nutrients for biostimulation and bioaugmentation studies. Water was collected from wells into 1 L glass bottles, filled to the top, immediately capped, and brought to the lab. Microcosms were prepared by adding treatment nutrients to 16.5 mL glass vials, and then transferring water collected from the site so that final volume was 11 mL. Resazurin was added (1 mg/L) as a redox indicator. Vials were flushed with either argon or nitrogen to maintain anaerobic head space and sealed with mininert Teflon caps. Then 0.5 mL of PCE saturated gas was introduced to the treatment vials for water collected from monitoring wells other than MW-5 (which already contained high PCE). The vials were shaken and allowed to equilibrate for a few hours to overnight before analysis. Vials were maintained under ambient conditions for the duration of the experiment. Headspace analysis was done with a gas chromatograph. The KB-1 bacterial consortium from SiREM was used for bioaugmentation. The culture is sensitive to oxygen, so microcosms were first stimulated with nutrients before adding 10 µL of KB-1, usually after 2-3 days, when resazurin was reduced from blue to colorless. Redox potential at the colorless stage of resazurin is -100 mV or lower, which is optimum for KB-1 microorganisms.

5. Cheese Whey Microcosms

Several microcosm experiments were prepared using liquid cheese whey. Cheese whey contains 5% lactose and therefore different concentrations were used in microcosms ranging from 0.01 to 0.5 percent of lactose content. Whey was used as sole nutrient and carbon source, or amended with soy oil methyl esters. One set used cheese whey that was filtered through 0.22 µm filter (CAMEO 25 NS Nylon filter) to eliminate microbial competition with KB-1. Microcosms with and without KB-1 were compared. Water from monitoring wells 5D, 8D and 9D was used for different sets of vials and the same method was used, as described above in water microcosms section. All microcosms were prepared in 16.5 mL clear glass vials, topped with mininert caps. Total volume of liquid phase was 12 mL. Resazurin (1 mg/L) was added as a redox indicator, and vials were flushed with nitrogen gas (30 s) after preparation and sealed immediately.

6. Pilot Study

Bioremediation Implementation

Nutrient injection

Table 1 lists the amount of each supplement and the tracer added to the nutrient solution during the injection on August 18, 2005. Glucose (40 g) and yeast extract (10 g)

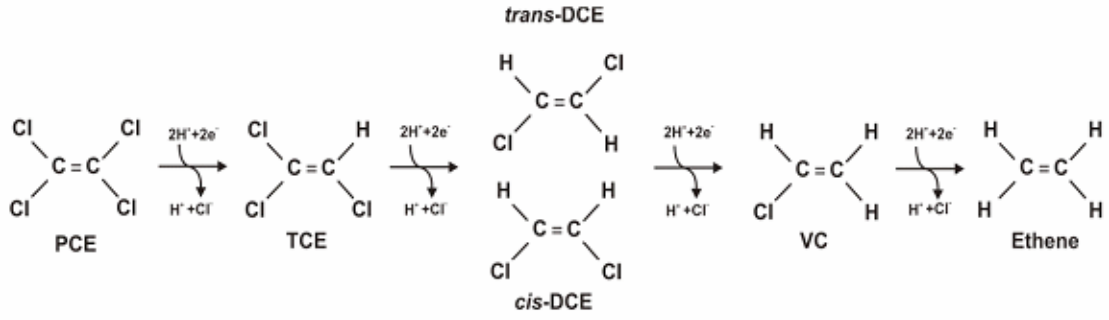

Figure 1. Sequential reduction of PCE to ethene by anaerobic reductive dechlorination adapted from (AFCEE, 2004).

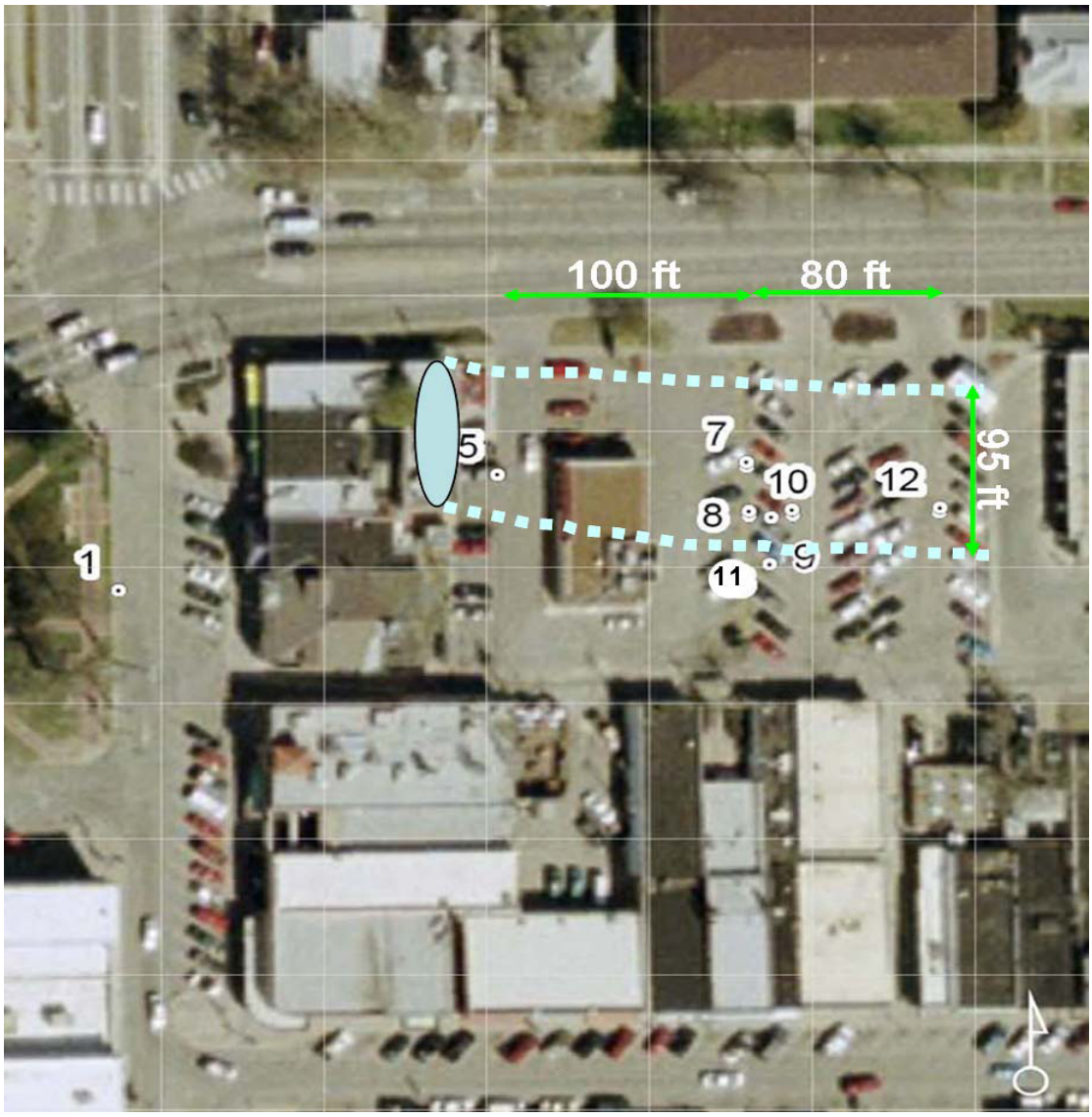

Figure 2. GIS aerial map showing location of PCE source (Oval shape), direction and width of contaminating plume (Dashed lines) in the pilot study area.

Table 1. Amount of SOME, lactate, yeast extract and KBr added in the nutrient solution for injection on August 18, 2005.

	Deep Zone		Shallow Zone	
Water (L)	188		197	
Nutrient/Tracer	**Mass (kg)**	**Concentration (%)**	**Mass (kg)**	**Concentration (%)**
SOME	8	4	2	1
Lactate	0.8	0.4	0.8	0.4
YE	2	1	0.2	0.1
KBr	0.2	0.1	0.2	0.1

were added to approximately 200 L of groundwater on August 15, 2005 and incubated to obtain the anaerobic chase water. Soy oil methyl ester (SOME) acts as a slow electron donor while lactate and glucose (in chase water) act as fast electron donors to reduce the redox potential of groundwater. Yeast extract (YE) was added as a source of vitamins and minerals. Groundwater from MW-10S and MW-10D was pumped into four 55 gal barrels (approximately 200 L each) for preparing nutrient solution and anaerobic chase water. Injection of nutrient solution and chase water into the injection wells was carried out on Thursday, August 18, 2005 (considered as day 0). The injection process is explained in detail in Santharam (2008).

7. KB-1 injection

The KB-1 culture was purchased from SiREM, Ontario, Canada. After determining that the area around the injection wells had become reduced [based on the measured oxidation reduction potential (ORP) and dissolved oxygen (DO) values], and after migration of the tracer to down-gradient wells, a culture of KB-1 was injected through the injection wells, on October 13, 2005. The injection of KB-1 was preceded, as well as followed by, injection of low concentration of nutrient water. Each injection well first received 50 L of nutrient solution. The nutrient solution was prepared using groundwater from MW-9S and MW-9D that had been incubated, from October 10, 2005, with 1 L of SOME, 40 g of glucose, 0.5 L of lactate and 200 g of yeast extract in a 55 gal (approximately 200 L) barrel. Each injection well received 5 L of KB-1 culture; Flow of KB-1 was achieved by pressurizing the KB-1 vessel using a nitrogen cylinder. Injection of KB-1 was followed by injection of 50 L of the anaerobic chase water. The anaerobic chase water was prepared similar to the nutrient solution, described above, but without SOME and lactate.

8. Fall 2006

After demonstrating that lactic acid and SOME were successful in reducing PCE levels in samples collected from the pilot study area, we planned to use cheese whey as nutrient source in the field. Groundwater from MW-10 was used to dilute cheese whey for injection. Cheese whey inhibition at higher concentrations presents a problem in the field because higher concentrations are often introduced to account for dispersion that occurs within the aquifer. In the microcosm studies, the optimal concentration of cheese whey for PCE degradation (with and without KB-1) was 0.025%, with inhibition occurring at concentrations greater than 0.1%.

If we consider a 10-50 fold dilution, injection of about 0.5% cheese whey would disperse to the effective concentration range of 0.05% to 0.01%. In microcosms, 0.1% cheese whey resulted in a long lag time before reduction of PCE. While 0.05% whey supplies very little carbon, it is sufficient to fully reduce the levels of PCE typically observed in the field. Cheese whey contains about 50 g/L of sugars (mostly lactose). Dilution of cheese whey to 0.5% results in a concentration of about 250 mg/L or approximately 1.4 mM glucose equivalent. Depending on detailed reaction paths, this may be enough to reduce more than 1.4 mM of PCE because each glucose molecule provides a dozen active hydrogen molecules (H_2) as NADH, and each PCE needs four H_2. Theoretically, therefore, 0.05% cheese whey contains sufficient reducing power for 130 μM PCE.

On July 31, 2006, one day before the fourth nutrient injection, one liter of cheese whey, 40 g of glucose and 10 g of yeast extract were added to approximately 200 L of

groundwater pumped into a 55 gal barrel. The resulting concentration of cheese whey in the nutrient solution was 0.5%. The nutrient solution was prepared in four such barrels. On August 1, 2006 each injection well received 100 L of nutrient solution.

9. Analytical Methods

Gas chromatograph (Hewlett Packard 5890 Series II, Wilmington, DE) with FID detector and HP-1 column (Dimethyl Polysiloxane matrix, 30 m x 0.53 mm, Agilent Technologies, Wilmington, DE) was used to analyze chlorinated ethenes and methane. Hydrogen was used as the carrier gas. Injection temperature was 200 ºC and detector temperature was 300 ºC. Parameters were adjusted to obtain detectable peaks that could be distinguished from other compounds by elution time. Different isothermal temperatures were tested (80 ºC, 100 ºC, and 110 ºC). High column temperatures resulted in fast elutions but the peaks did not resolve very well from each other. Lower temperature allows good separation of the compounds, but took more time to finish the run. For example PCE elution time at 80 ºC was 4.1 min while at 110 ºC the PCE peak as detected after 2.2 min. At the end, a gas phase sample of 100 µL volume was taken with 100 µL Hamilton gas syringe, the column was set on an isothermal temperature of 100 ºC, and the run time was 5 min. The previous parameters were able to resolve chlorinated ethene peaks and methane. Gas flow rate was maintained at 1.5 mL/min. Detection limits were in the range of 1-10 µg/L for chlorinated ethenes; differences relate to the compounds and their Henry's constants. This analysis yields different headspace concentrations. Chlorinated ethene standards were prepared to determine elution times of different analytes of interest. A PCE standard was prepared once a month and run prior to each analysis to check the responses of the GC, and determine the relative elution times. The standard was prepared by injecting 10 µL of PCE liquid in a clean amber glass bottle of 4.2 L and allowing it to vaporize completely. The concentration of PCE in the standard bottle was 3.83 mg/L. At detection settings, elution times for chlorinated ethenes were as follow: PCE (2.7 min), TCE (1.6 min), DCE (1.1 min), VC (0.7 min) and Methane (0.6 min). Maintenance was done periodically to make sure that the gas chromatograph operation was uniform across the study. The injection septum (Thermogreen LB-2 / 11 mm diameter) was changed every 200 samples and inner glass column was cleaned every ~1000 samples. Methane and ethene were not resolved on this column at any temperature, and for that another GC was used. It was also difficult to create a calibration curve for vinyl chloride since the standard was prepared in methanol, and a large methanol peak masked the VC peak, even when water or sodium hydroxide was added to the standard.

10. Results and Discussion

Biostimulation Experiment

Water from MW-4D, MW-8S and MW-8D was used to study the extent of chlorinated ethene degradation upon stimulation of native microorganisms. In microcosms prepared with MW-4, methane was generated in the following treatments: SOME+YE, Glucose+ YE, Methanol, Methanol +YE, YE. About 50% of PCE decreased in SOME+YE treatment, and two thirds of PCE decreased in methanol and YE treatments. Trichloroethene (TCE) appeared after 6 weeks in SOME+YE, methanol+ YE and YE treatments. Unlike MW-4, addition of nutrients greatly enhanced reductive dechlorination of PCE in all vials except in SOME and methanol treatments. cis-Dichloroethene (c-DCE) was generated in the active microcosms in response to decrease of PCE concentrations during the second week of observation, but no further degradation of DCE was noted after 6 weeks. In general more frequent sampling events were needed to carefully monitor the variation of chlorinated ethene degradation. From this set of microcosms, wells had different responses to nutrient amendments. *Dehalococcoides* sp. that carries out complete degradation of PCE was either absent or not active under these conditions. Figure 3 shows microcosms results of MW-5 treated with nutrient solution with and without KB-1.

Cheese Whey

Cheese whey is a byproduct of the dairy industry and can be obtained inexpensively. Powdered whey is more costly, but easier to obtain, ship and store. First set of microcosms prepared with cheese whey was prepared to check if the lactate and vitamin B12 content in cheese whey was good for biostimulation and bioaugmentation studies. From here, several concentrations of cheese whey were tested (0.01%, 0.025%, 0.05%, 0.1% and0.25%) and compared to YE + SOME combinations used in previous studies. As expected, microcosms prepared without KB-1 bacteria were similar to control and PCE remained dominant in the system. On the other hand, PCE concentrations dropped almost 90% in 0.025% whey treatment and had a lag phase of 10 days, and were similar to YE+ SOME treatments. PCE concentrations also dropped in 0.05% and 0.01% whey treatments, but required a longer lag phase of 20 days (Figure 4). It was noticed that higher concentrations of cheese whey (above 0.05%) seemed to inhibit KB-1 bacterial culture and PCE remained through the study period. Meanwhile, 0.025% whey produced DCE when PCE concentrations dropped.

Groundwater Elevation

Precipitation affects the level of groundwater in the subsurface. Precipitation data was Variations in the data are due to rainfall events, but generally groundwater table in shallow zone was about 5 ft above that in the deep zone. In the pilot study area, the groundwater elevation is approximately 10 times higher in the shallow zones compared to deep zones as shown in Figure 3.60. The elevation grades between MW-8 and MW-12, and the general groundwater flow direction is to the east in both zones.

Chlorinated Ethenes in Pilot Study Area

Concentrations of PCE, TCE and DCE were plotted for three depths in deep and shallow wells. Deep well samples were collected at the following depths below top of casing: top (42 ft), mid (47 ft) and bottom (52 ft). Shallow wells sampled at the following depths: top (18 ft), mid (23 ft) and bottom (28 ft). Figures 5 to 7 represent chlorinated ethenes concentrations in deep zone of MW-8D, MW-9D and MW-10D respectively. In M-8D rapid decrease in PCE and increase in DCE concentrations were noticed

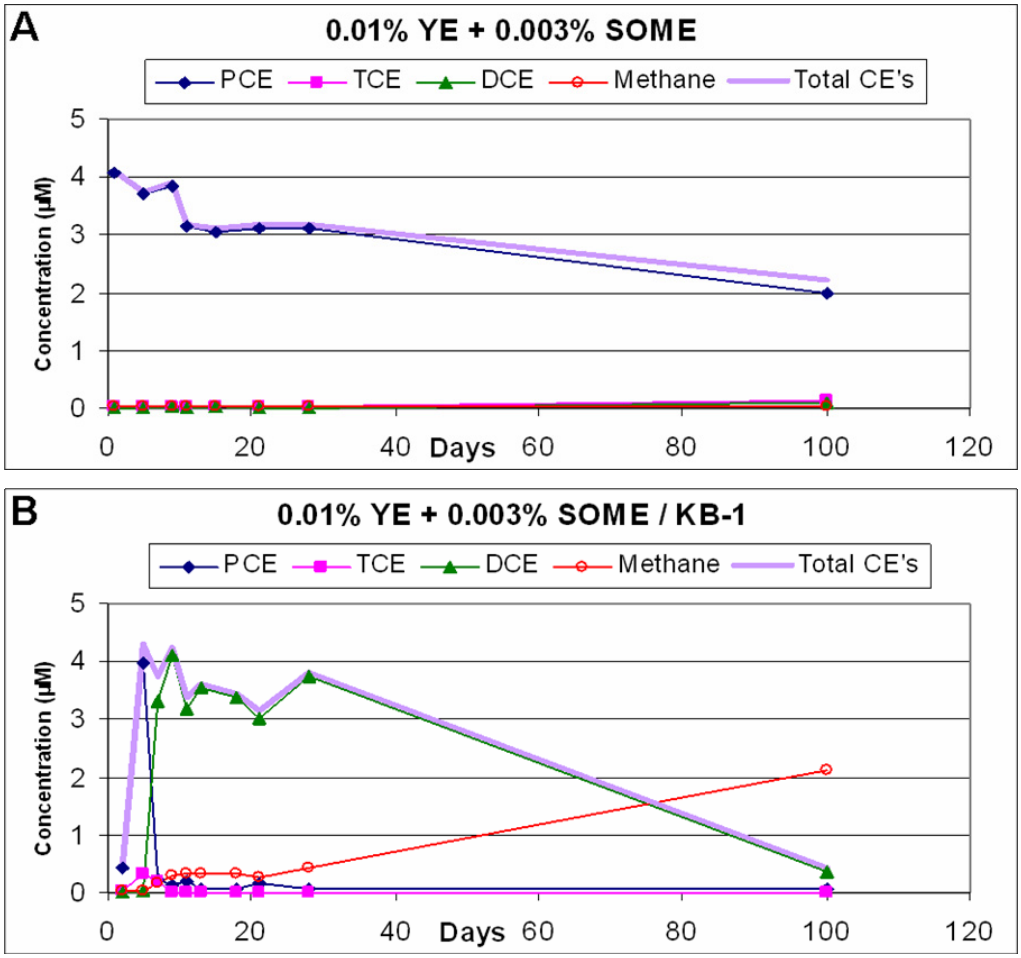

Figure 3. Microcosm data of MW-5D showing (A) biostimulation and (B) bioaugmentation with KB-1. Vials amended with 0.01% Yeast extract (YE)+ 0.003% Soy oil methyl ester (SOME).

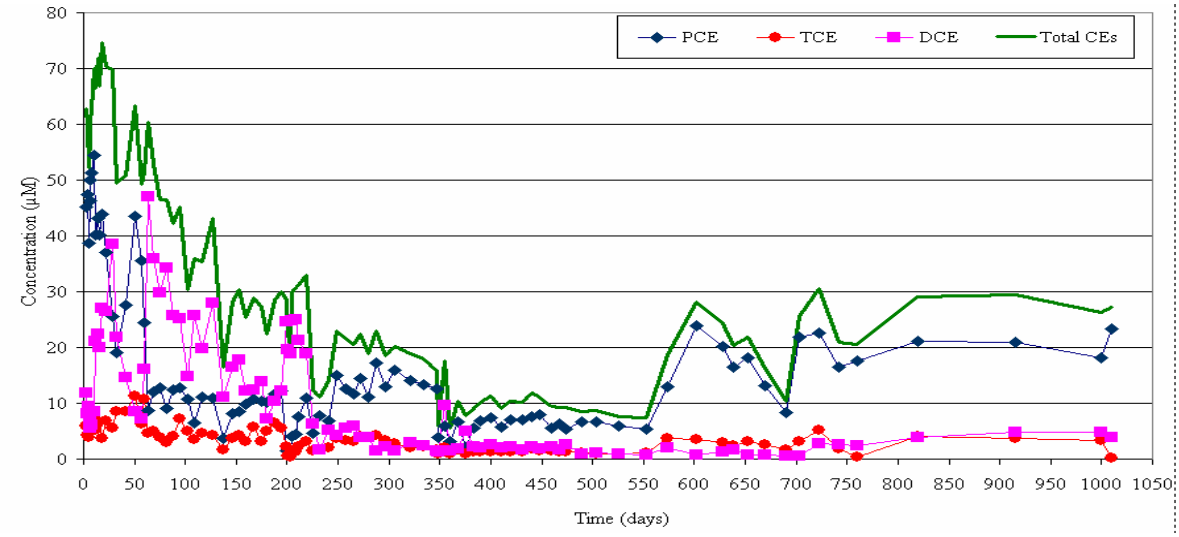

Figure 4. Cheese whey microcosms showing PCE degradation inhibition at concentrations greater than 0.05%.

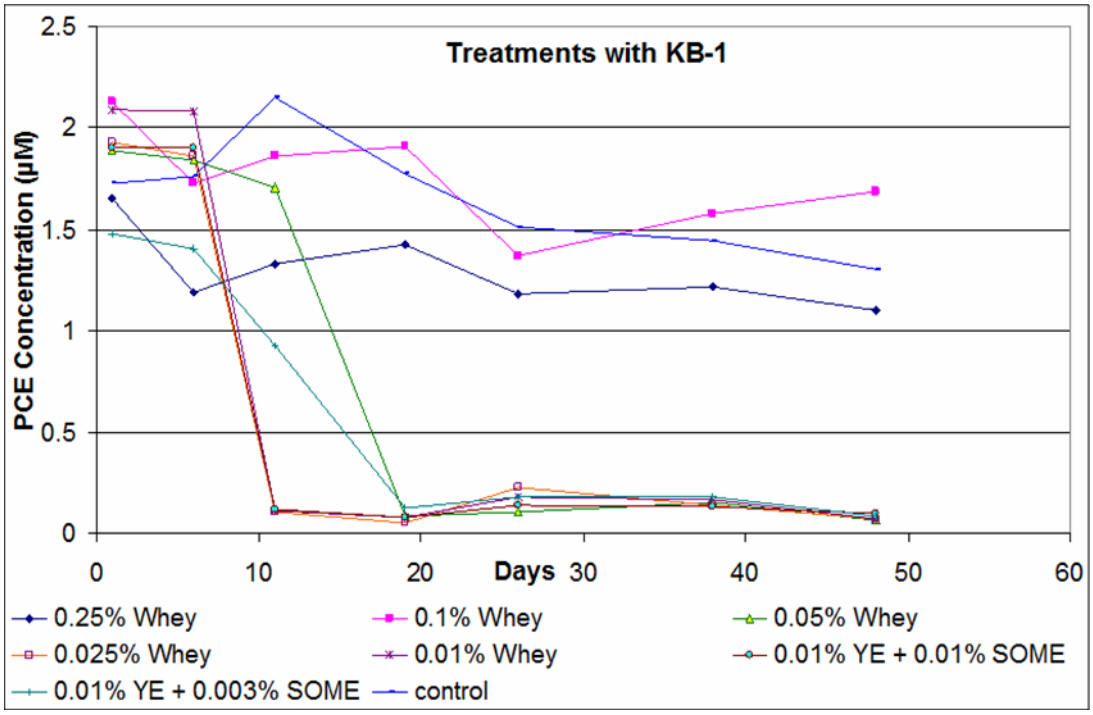

Figure 5. Mean concentration of chlorinated ethenes in MW-8D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (October 13, 2005); nutrients and bromide on day 197 (March 3, 2006); nutrients on day 348 (August 1, 2006).

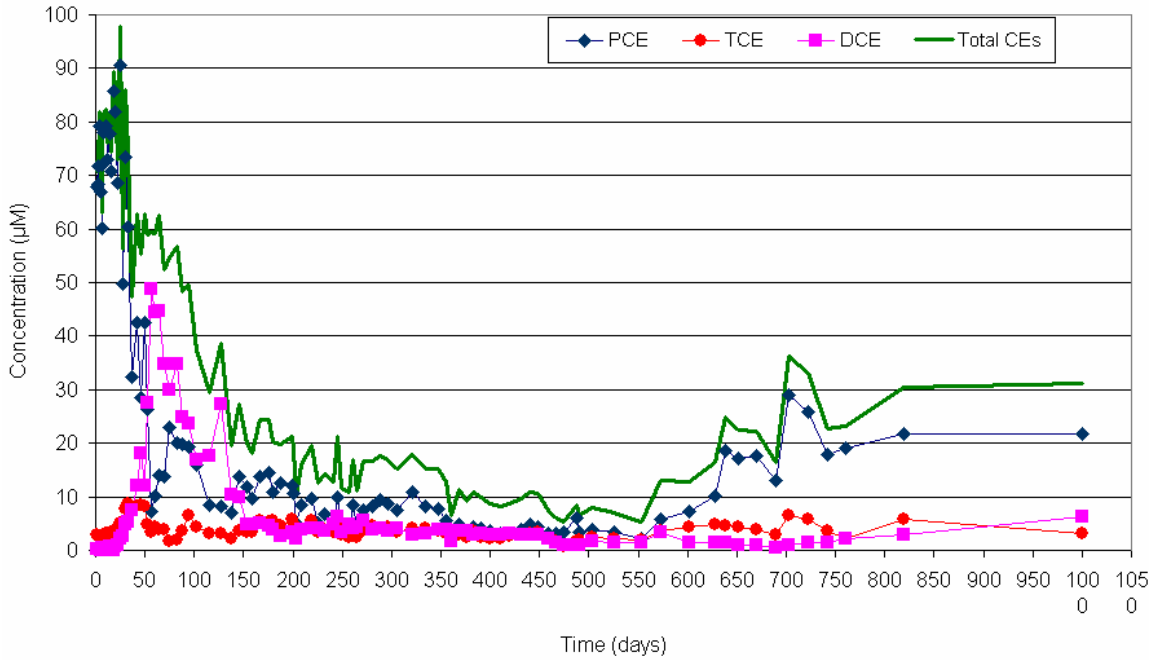

Figure 6. Mean concentration of chlorinated ethenes in MW-9D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (October 13, 2005); nutrients and bromide on day 197 (March 3, 2006); nutrients on day 348 (August 1, 2006).

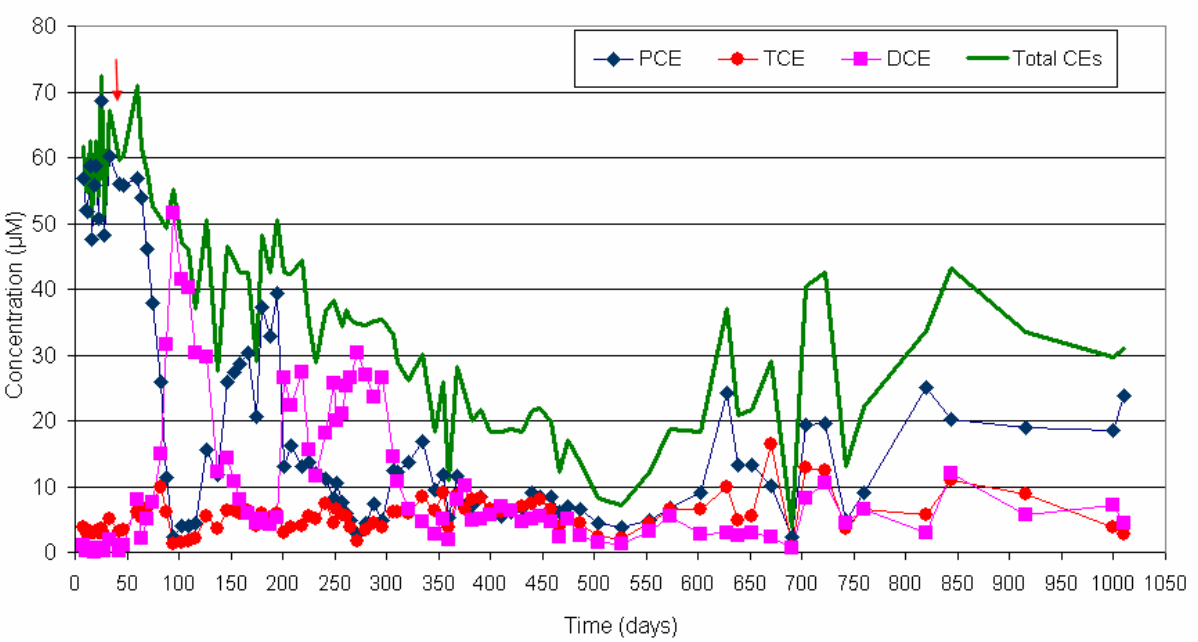

Figure 7. Mean concentration of chlorinated ethenes in MW-10D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (October 13, 2005); nutrients and bromide on day 197 (March 3, 2006); nutrients on day 348 (August 1, 2006).

after first injection event in day 0. After second nutrient injection with KB-1 PCE remained low and DCE concentrations decreased. PCE concentrations then increased to about 10 µM before the third nutrient injection in day 197, and decreased rapidly after nutrient addition accompanied by DCE generation which soon decreased to lower levels and remain low for long time (750 days). Similar trend was also followed after last nutrient injection on day 348. Monitoring the well further we noticed that PCE concentrations are rebounding, but did not reach initial concentrations. This is perhaps due to decrease in microbial biomass and reducing conditions at the site.

MW-9D showed a dramatic response due to nutrient and KB-1 addition. PCE concentrations dropped from about 80 µM before KB-1 to about 10 µM after third nutrient feeding. DCE was generated after PCE decrease then decreased to low levels until about 750 days when concentrations start to rebound to about 7 µM for DCE after 1000 days and around 23 µM for PCE after the same time (Figure 3.78). In MW-10, PCE and DCE concentrations were fluctuating and not reflecting what is happening in MW-9D indicating that this well is receiving water from another path that can create that rebound of PCE concentration to about 40 µM before the nutrient injection in day 197. Total CEs concentrations decreased to 10 µM after 500 days and remained steady until they started to rise again.

Shallow wells in general had lower PCE concentrations than deep wells (< 10 µM) except for MW-10S that had a higher value of 17 µM. MW-8S was not affected much with nutrient addition and concentrations remain similar across the study. In MW-9S, PCE concentrations decreased following bacterial injection then rebounded. This was also the case when nutrients were added on day 348. After 1000 days PCE concentrations in this well were close to the starting point at around 6 µM. In MW-10S first response was detected after 230 days when PCE concentrations decreased and DCE increased. Now DCE has gone back to background values while PCE concentrations are reaching initial values.

11. Impact Downstream from the Point of Injection

The results that are presented for MW-9D, MW-10D, and MW-12D show that the nutrients and KB-1 move downstream with the water that is flowing to the east. For MW-10D, the concentrations of DCE, which are more than 20 µM between 200 and 300 days, are larger than those that are observed for MW-9D. Similarly, there are concentrations of DCE for MW-12D that exceed 30 µM between 350 and 450 days. This and other aspects of the data for MW-10D and MW-12D provide evidence that there is microbial action downstream of the injection point as one would expect. The observed times when the DCE concentration reaches 20 µM are about 20 days for MW-8D, 50 days for MW-9D, 80 days for MW-10D, and 300 days for MW-12D. The impact of KB-1 on the DCE concentration and CE concentration has a greater time lag; if we examine when the CE concentration is less than 30 µM, the values are about 130 days for MW-8D and MW-9D, 310 days for MW-10D, and 570 days for MW-12D. The KB-1 movement in the aquifer is slower than the nutrient movement, but there is evidence that both move downstream and have an impact downstream. The expectation is that the nutrients and the KB-1 are dispersed with time to locations that are north and south of MW-10D and MW-12D as well as at the well location.

12. Conclusions

Laboratory Studies

Microcosms experiments indicate that SOME, glucose, lactate, yeast extract, and cheese whey are good electron donors for chlorinated ethene biodegradation. Biostimulation of native microbes results in PCE conversion to DCE, while bioaugmentation with KB-1 bacterial culture was necessary to promote complete degradation of PCE to the end products methane and ethene. Cheese whey provided a good source of carbon and vitamins and can be used in biodegradation studies, though, inhibition of bacterial activity with high cheese whey concentrations was observed. This was due to acid formation and below optimal pH for growth. Therefore, use of cheese whey should be monitored in terms of concentration and perhaps frequency of addition. Adding small and frequent increments would work better than one bulk application that has a high chance of acidifying the aquifer. It seems that the complex nature of cheese whey would support microbial growth for a long time. It can be added monthly as recommended previously (Moretti, 2005 and AFCEE, 2004). Use of cheese whey will reduce cost of treatment, but this may not apply if pH control is needed. The soil has low buffering capacity at the pilot study area and therefore, careful optimal concentrations of cheese whey should be applied for remediation. Nutrients should be added to maintain KB-1 activity for long period; otherwise the microbial activity may be affected.

Field Studies

Soil tests at the pilot study area revealed that the subsurface is silty clay in the shallow zone grading to more silty sand soil in the deep zone. Tracer studies show that groundwater flow direction is toward the east in shallow and deep zones. Groundwater elevation is influenced by precipitation events. The hydraulic gradient is higher in the shallow zone than the deep zone and this may support the fact that ground water velocity is higher in the shallow zone than the deep zone. Two tracer studies, which were done during the bioremediation study, found that groundwater velocity decreased from 2004 to 2006 due to biomass growth near the injection sites. The results show that KB-1 was successfully established in this location as noted from the decrease in DCE and the total chlorinated ethenes concentrations. the rates of chlorinated ethene degradation in the field is slower than that of microcosms due to difference in temperature of groundwater of about 19 ºC and the incubation temperature of microcosms at 23 ºC that result in faster microbial growth and enhanced activity. When nutrient feeding was stopped CEs concentrations remained low for a long time after the last nutrient feeding, suggesting that substrates used may have provided a long term hydrogen source at the site. The biomass formed earlier would provide a source of organic substrates that can be used to sustain active dehalorespiring organisms and reduce biomass volume. This can also be noted when the final tracer study was conducted, which shows that ground water velocity is approaching the initial state.

13. Acknowledgments

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