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LABORATORY STUDIES USING EDIBLE OILS TO SUPPORT REDUCTIVE DECHLORINATION

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Abstract: In laboratory microcosm tests, single additions of corn oil, beef tallow, melted corn oil margarine, coconut oil, corn oil, soybean oil, or partially hydrogenated soybean oil supported complete reductive dechlorination of perchloroethene (PCE) or trichloroethene (TCE) to ethene when a dechlorinating population was present. TOC production from the edible oils continued for up to 200 days. More than 250 mL of gas were produced in some microcosms. TCE concentrations as high as 236 mg/L were degraded. Coconut oil was added to a soil column that had been bioaugmented with a dechlorinating enrichment culture to evaluate edible oil-based TCE dechlorination in a flow-through system. Ethene and ethane were the only products found in the column effluent during the 88 days following the oil addition. The 10 mL of coconut oil (1,500 mg/kg) was projected to support TCE degradation for over two years. The edible oils are much cheaper than soluble substrates such as sodium lactate. Operation and maintenance costs for an edible oil based reductive dechlorination system should also be less than for a soluble substrate. The edible oil will need to be added only periodically. This technology should be applicable for source zone treatment.

INTRODUCTION

Some anaerobic microbial populations are capable of completely degrading chlorinated solvents such as perchloroethene (PCE), trichloroethene (TCE), or carbon tetrachloride to less chlorinated intermediates and innocuous products such as ethene, ethane, or methane (Lee et al. 1998). This process, known as reductive dechlorination, has been applied at several sites to degrade these chlorinated solvents. Recirculation based systems with a series of extraction and injection wells or trenches have generally been used to treat a source area or downgradient portion of a plume. Soluble substrates such as benzoate, methanol, and acetate have been added to the recirculated groundwater to generate anaerobic conditions in the groundwater and to provide the hydrogen necessary for reductive dechlorination. Bioaugmentation with a dechlorinating enrichment or isolate has been recently demonstrated for sites where the native microbial population is not capable of completely degrading the contaminants (Dybas et al. 1998).

Recirculation based systems suffer from several drawbacks including the relatively high costs for many of the soluble substrates, the frequent additions of substrate, and the need for on-going operation and maintenance program to maintain the recirculation system (Lee et al. 1998). Biofouling of the injection

wells is a particular problem with recirculating reductive dechlorination systems that may result in decreased recirculation rates.

One approach that avoids the need for a recirculation system would be to use a long-lasting substrate that is injected only periodically into the subsurface. A polylactate ester, Hydrogen Release Compound from Regenesis, San Clemente, CA, has been developed as a slow-release source for hydrogen (Koenigsberg and Farone, 1999). Dybas et al. (1997) demonstrated that corn oil, solid food shortening, and hydrogenated cottonseed oil beads, would support carbon tetrachloride degradation by *Pseudomonas stutzeri* strain KC. Other edible oils should also support reductive dechlorination. Mackie et al. 1991 reported that long and short chain fatty acids can be beta-oxidized to acetate by certain anaerobic organisms including the H₂-producing syntrophs.

MATERIALS AND METHODS

Microcosm Studies. A single addition of 6 mL of corn oil, beef tallow, melted corn oil margarine, and coconut oil were added to duplicate 280 mL serum bottles containing 50% by volume soil from the Victoria, TX site and 50% by volume water. The soil had been used in column studies fed benzoate or molasses and PCE for almost two years (Lee et al. 1997). The columns had been bioaugmented with a dechlorinating enrichment from another area of the site. A sterile control microcosm treatment (autoclaved for one hour on two successive days) amended with corn oil was also run. The microcosms were prepared in an anaerobic chamber with an atmosphere containing a mixture of 5% hydrogen, 5% carbon dioxide, and 90% nitrogen. Each microcosm was dosed with TCE on days 0, 2 and 71. Samples from the bottles were removed periodically over 200 days to be analyzed for volatiles by gas chromatography-mass spectrometry, end product gases (ethene, ethane, and methane) by head-space gas chromatography, and total organic carbon (TOC) by a Total Organic Carbon Analyzer using the methods described by Odom et al. (1995). The microcosms were incubated in the anaerobic chamber at 22 °C. Gas production was estimated by inserting a needle through the rubber septa and collecting the excess gas in the attached syringe.

Corn oil, soybean oil, and partially hydrogenated soybean oil were also tested as substrates to support reductive dechlorination in microcosm studies for three additional sites. The solids content of the microcosms ranged from 10 to 50 percent by volume site soil with the remaining volume being site groundwater. Some of the treatments were bioaugmented with the Pinellas Dechlorinating Enrichment (PDE) or the Dover Landfill enrichment (DLFE) two weeks after the studies were begun to evaluate whether bioaugmentation could stimulate complete dechlorination. The PDE was isolated from a site in Pinellas, FL (Harkness et al. 1999). The DLFE was isolated from a landfill at the Dover Air Force Base. The non-replicated microcosms were prepared, incubated, and sampled in an anaerobic chamber with 3% hydrogen, 5% carbon dioxide, and 92% nitrogen at 22 °C. These microcosm studies were analyzed for volatiles and metabolic gases by head-space gas chromatography in a modification of EPA

Methods 8021B and 8015. The microcosm studies lasted for between 84 and 189 days.

Column Study. An additional study involved the introduction of coconut oil to a soil column to evaluate edible oil-based reductive dechlorination in a flow-through system. The 7.6 cm diameter by 76 cm long steel Shelby tube held an intact core from Dover Air Force Base weighing 6 kg. The soil column had been bioaugmented with the PDE. Complete dechlorination of up to 90 μM TCE and 20 μM DCE to ethene had been observed when the column was fed lactate. Ten mL of coconut oil was added to the bottom of the up-flow column. Groundwater or tap water spiked with about 76 μM TCE (10 mg/L) was fed onto the column using a Ranin Rabbit® pump. The influent and effluent from the column were maintained at 4 °C to minimize changes in the substrate or chlorinated ethene concentrations between sampling events. The retention time on the column varied between 6 and 11 days.

RESULTS

Microcosm Tests. All of the oils and molasses supported complete reductive dechlorination of TCE to ethene over a period of greater than 49 days. Table 1 summarizes the results of the microcosm studies for four sites where edible oils have been tested as substrates.

Figure 1 shows the average concentrations of TCE and daughter products in μM concentrations, and methane and TOC concentrations in mg/L for a representative treatment from Site 1 (Victoria, TX) amended with corn oil. The TCE was degraded to a mixture of ethene and ethane over the 49 day incubation period. The autoclaved control treatment fed corn oil showed the production of TOC and ethene following the second TCE spike; autoclaving had not killed all of the microbes. Dissolved TOC production was highest initially with the maximum TOC of 670 mg/L recorded for the treatment with beef tallow at Day 29. TOC production continued in all oil based treatments over a period of 200 days (Figure 2) with coconut oil sustaining the longest TOC release. Dechlorination continued with the second spike of TCE on day 71. In excess of 50 mL of gas were produced in some of these microcosms; the septa was displaced in some microcosms. The high quantity of gas generated by the oils indicates that the oils may lead to plugging problems if gas bubbles accumulate in situ.

In the site 2 microcosms, the treatment with corn oil only showed dechlorination of TCE to DCE with no production of VC or ethene over the 84 day study. When the PDE was bioaugmented into a microcosm fed corn oil, TCE was dechlorinated completely to ethene. More than 290 mL of gas was produced with the treatment with the PDE and corn oil.

Treatments were prepared for the site 3 with both the till and the deeper, fractured bedrock soils and groundwaters. The bedrock samples were broken into small particles to place it into the 560 mL serum bottles and provide additional surface area. Groundwater from the same zone as the soil samples were added to the microcosms. The till groundwater contained an average of 215 μM TCE (28

mg/L) and 50 μ M DCE (4.8 mg/L). The bedrock groundwater contained an average of 1,800 μ M TCE (236 mg/L) and 78 μ M DCE (7.5 mg/L). Both of the till microcosm treatments fed soybean oil and partially hydrogenated soybean oil were bioaugmented and showed complete conversion of the TCE and DCE to ethene. More than 200 mL of gas was generated in the till microcosms. The bedrock treatments with the soybean oil and partially hydrogenated soybean oil promoted the dechlorination of TCE to DCE. Some VC was produced in the treatment with soybean oil, but ethene or ethane was not generated. In the PDE-bioaugmented bedrock treatments with both the soybean oil and partially hydrogenated soybean oil, a mixture of VC and ethene were detected after 189 days. The Pinellas dechlorinating enrichment was able to biodegrade the very high concentrations of TCE. However, complete dechlorination to ethene was not observed possibly due to a substrate limitation or the need for a second inoculation with the PDE.

In the Site 4 microcosm studies, soybean oil alone promoted the essentially complete conversion of a mixture of PCE, TCE, cDCE, and VC to ethene. A dechlorinating enrichment had previously developed at this site and bioaugmentation with the Pinellas Dechlorinating Enrichment or the Dover Landfill Enrichment was not necessary. The maximum methane concentration observed in this study was 34 mg/L. Methane concentrations in excess of the aqueous solubility of about 25 mg/L at 22 °C were generated under the high pressures generated in the bottles.

Column Studies. Groundwater without substrate (TOC = 3-10 mg/L) plus TCE was fed onto the column. Ethene and low concentrations of ethane (<1.7 μ M) were the only products found in the column effluent during the 88 days following the oil addition (Figure 3). The dissolved TOC in the column effluent ranged between 40 and 120 mg/L and the maximum methane concentration was 3.6 mg/L. One dose of oil supported complete dechlorination for almost 90 days in the flow-through system. A mass balance analysis of the column influent and effluent showed that less than 1% of the organic carbon present in the added coconut oil was used for TCE degradation, sulfate reduction, and methane production. Just under 9% of the added organic carbon was released from the column as dissolved organic carbon with over 90% unaccounted for. Assuming the unaccounted for organic carbon remained in the column and could be used for reductive dechlorination, the 10 mL of coconut oil (1.5 mg/kg) was projected to support TCE degradation for over two years.

DISCUSSION

The microcosm studies showed that edible oils including corn oil, beef tallow, corn oil margarine, coconut oil, soybean oil, and partially hydrogenated soybean oil can support reductive dechlorination at several sites. At one site, the native microbial population was able to dechlorinate TCE to ethene with just the addition of soybean oil. At the other four sites investigated, bioaugmentation with an dechlorinating enrichment allowed for complete dechlorination to occur. Very

high concentrations of TCE were degraded at one site suggesting that this technology has potential applications for treatment of source areas with residual dense non-aqueous phase liquids. The column study showed that edible oil should be effective in aquifers.

The edible oils are much cheaper than soluble substrates such as sodium lactate. Operation and maintenance costs for an edible oil based reductive dechlorination system should also be less than for a soluble substrate since the edible oil will need to be added only periodically. The maintenance of a recirculation system of extraction and injection wells for the soluble substrate may also be avoided with an edible oil based system. However, the vegetable oils may cause excessive gas production in some soils potentially leading to gas bubbles partially plugging the subsurface.

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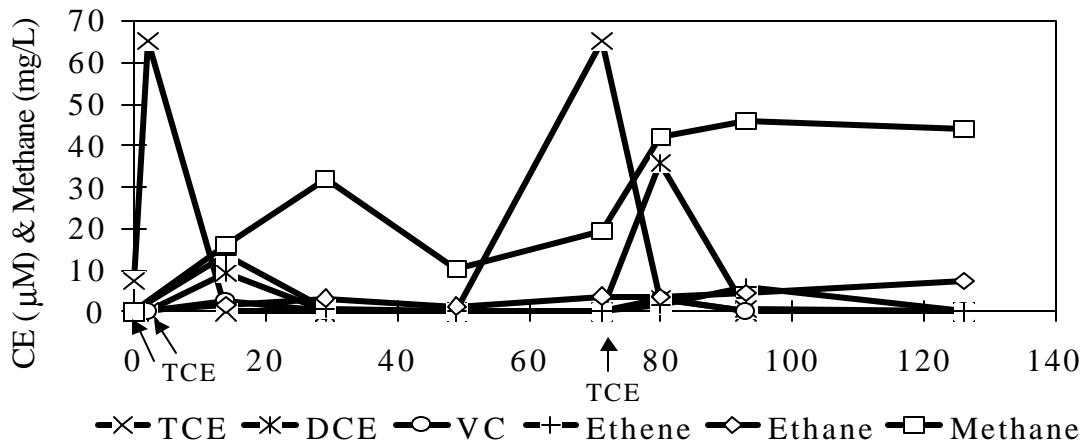


FIGURE 1. Chlorinated ethenes and methane concentrations for corn oil treatment of site 1 microcosms

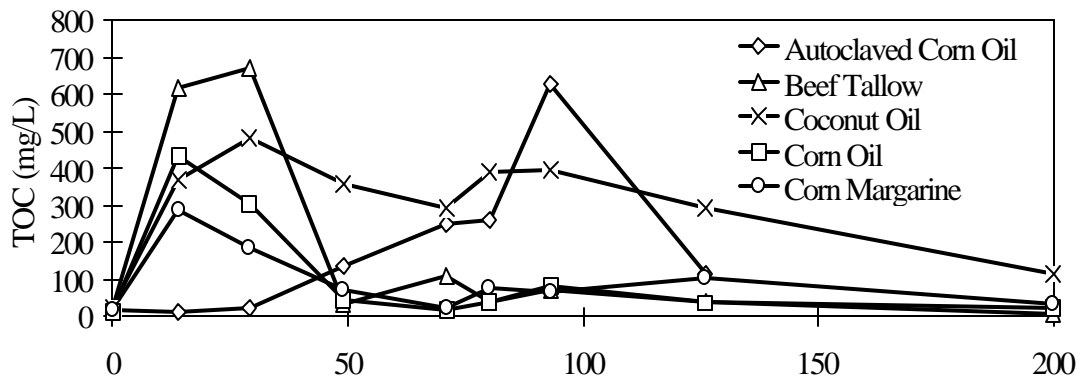


FIGURE 2. TOC concentrations for site 1 microcosms

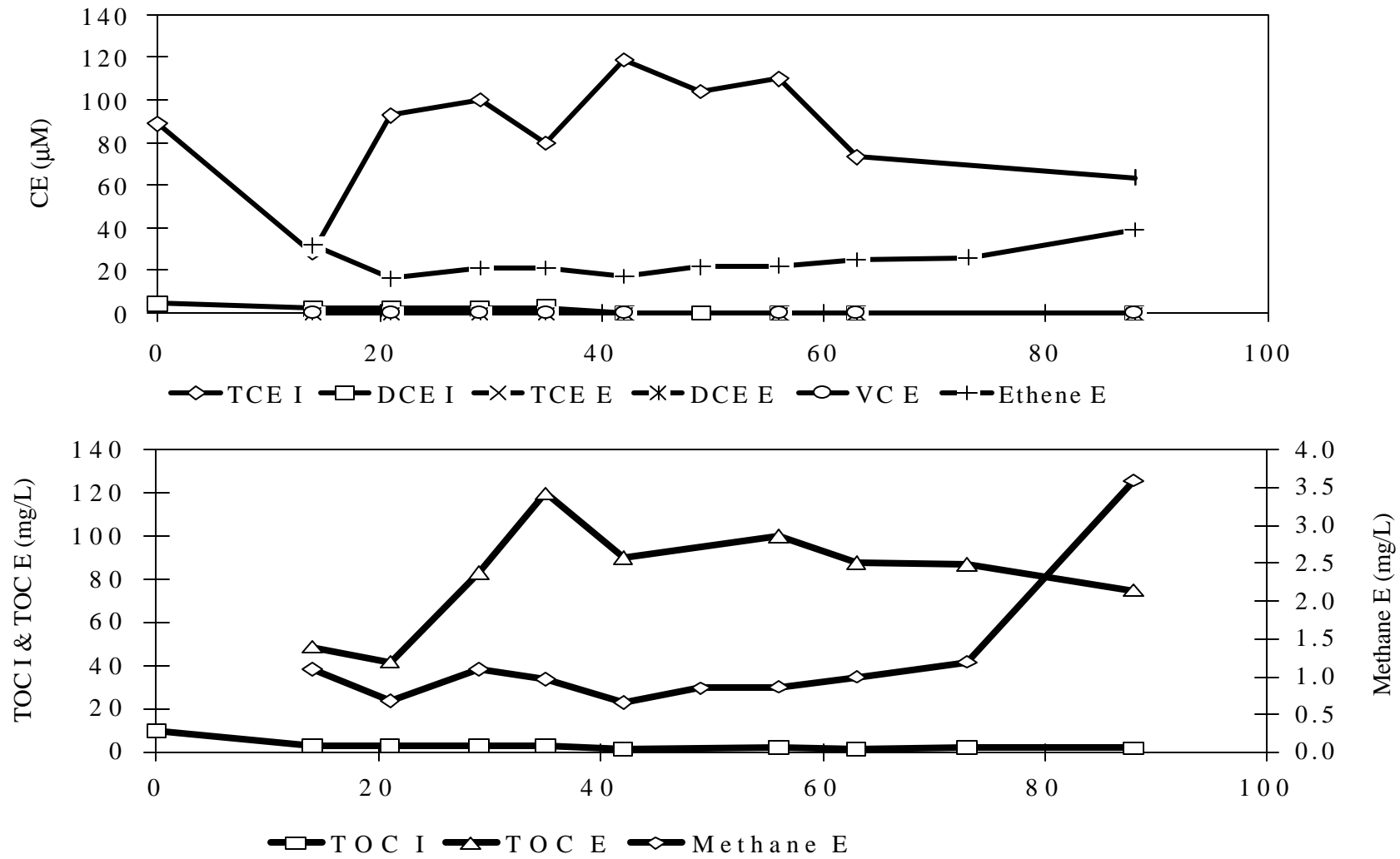


FIGURE 3. Column study results (I = Influent; E = Effluent)

TABLE 1. Summary of microcosm studies with edible oils

Treatment	Initial TCE μM	Initial cDCE μM	Final TCE μM	Final DCE μM	Final VC μM	Maximum Ethene μM	Maximum Ethane μM	Maximum Methane mg/L	Quantity Gas mL	Maximum TOC mg/L	
Site 1 50% Soil 280 mL Microcosm Bottles Study 49 Days Long											
6 mL Corn Oil Autoclaved	94	<0.8	2.1	34	<1.2	10	5.5	29	>13	630	
6 mL Corn Oil	65	<0.8	<0.6	<0.8	<1.2	13	1.4	32	>25	440	
6 mL Beef Tallow	60	<0.8	<0.6	<0.8	<1.2	10	11	150	>51	670	
6 mL Corn Oil Margarine	105	<0.8	<0.6	<0.8	<1.2	20	4.2	56	>30	290	
6 ml Coconut Oil	31	<0.8	<0.6	<0.8	<1.2	4.3	4.3	46	>25	480	
Site 2 50% Soil 560 mL Microcosm Bottles Study 84 Days Long											
2.8 mL Corn Oil	7.1	<0.1	<0.05	6.9	<0.1	<0.2	<0.2	>200	>106		
2.8 mL Corn Oil + PDE	14	<0.1	<0.05	<0.06	<0.1	32	<0.2	>290	>153		
Site 3 10% Soil 560 mL Microcosm Bottles Study 84-189 Days Long											
Bedrock 2.8 mL Soybean Oil	1990	80	8.6	1010	24	<18	<17	<0.5	0		
Bedrock 2.8 mL Partially Hydrogenated Soybean Oil	2060	76	<4	1390	<8	<18	<17	<0.5	0		
Bedrock 2.8 mL Soybean Oil + PDE	1450	82	4	<1.2	610	270	<8	8	2.2		
Bedrock 2.8 mL Partially Hydrogenated Soybean Oil + PDE	1700	72	2.4	<4	1000	190	<17	0.6	1.2		
Till 2.8 mL Soybean Oil + PDE	240	58	<0.2	<0.3	<0.4	420	<3	155	>255		
Till 2.8 mL Partially Hydrogenated Soybean Oil + PDE	190	42	<0.2	<0.3	<0.4	200	<3	67	>200		
Site 4 50% Soil 560 mL Microcosm Bottles Study 84 Days Long											
Treatment	Initial PCE μM	Initial TCE μM	Initial cDCE μM	Initial VC μM	Initial Ethene μM	Final PCE μM	Final TCE μM	Final DCE μM	Final VC μM	Maximum Ethene μM	Maximum Methane mg/L
2.8 mL Soybean Oil	29	35	29	14	2.5	<0.06	<0.08	<0.1	0.4	47	0.7
2.8 mL Soybean Oil + PDE	28	48	38	20	2.2	<0.04	<0.05	0.1	<0.1	85	34
2.8 mL Soybean Oil + DLFE	45	47	38	<0.4	<0.9	<0.04	<0.05	<0.06	<0.1	34	21