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IMPACT OF COSOLVENT FLUSHING ON SUBSURFACE MICROBIAL ECOLOGY AT A FORMER DRY CLEANER SITE

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ABSTRACT: The Solvent Extraction Residual Biotreatment (SERB) technology was evaluated at a former dry cleaner site in Jacksonville, FL where an area of tetrachloroethene (PCE) contamination was identified. The SERB technology is a treatment train approach to complete site restoration, which combines an active in situ dense, nonaqueous-phase liquid (DNAPL) removal technology, cosolvent extraction, with an enhanced in situ bioremediation technology, reductive dechlorination. During the in situ cosolvent extraction test approximately 34 kL of 95% ethanol/5% water (v:v) were flushed through the contaminated zone, which removed approximately 60% of the estimated PCE mass. Approximately 2.72 kL of ethanol were left in the subsurface, which provided electron donor for enhancement of biological processes in the source zone and down-gradient areas. Results from ground water monitoring indicate reductive dechlorination transformations of tetrachloroethene have been enhanced following the introduction of ethanol to the system.

Subsurface samples were collected from several locations prior to and following the cosolvent extraction demonstration to evaluate changes in total biomass and microbial population through phospholipid fatty acid (PLFA) analysis. Total biomass estimates were low in most of the samples, with the majority of samples containing biomarkers representative for gram-negative bacteria. When normalized to background samples, total and gram-negative biomass was significantly higher (95% C.L.) in the one-year and two-year post-cosolvent flushing samples compared to the pre-treatment samples. Polymerase chain reaction (PCR) results from ground water collected from the source zone three years following the cosolvent flush were positive for the presence of *Dehalococcoides*, which are the only organisms shown to completely dechlorinate tetrachloroethene to ethene.

INTRODUCTION

Contamination of subsurface environments and ground water by chlorinated solvents, such as tetrachloroethene (PCE), is of significant concern due to its widespread use as a solvent and degreasing agent. Because of their unique chemical and physical properties, contamination by chlorinated solvents has produced numerous, technically challenging DNAPL remediation sites.

The Solvent Extraction Residual Biotreatment (SERB) technology is a treatment train approach for DNAPL remediation, which is focused toward complete site restoration. SERB combines cosolvent extraction with enhanced in situ bioremediation. The synergy of the process is based on the use of ethanol as both the cosolvent and as the electron donor for the reductive dechlorination of PCE. The cosolvent flushing process

Three injection wells (IW's) screened from 7.6 to 9.9 m bgs, surrounded by six recovery wells (RW's) screened from 7.9 to 9.6 m bgs, were used for implementation of the cosolvent flushing test (Jawitz et al., 2000). Monitoring wells (MW's) were screened from 7.9 to 9.6 m bgs and a series of 2.5-cm PVC wells (C's) were screened from 8.3 to 9.1 m bgs. All wells used for ground water monitoring are shown in Figure 1.

Cosolvent Flush. The pilot-scale field test of in situ alcohol flushing was conducted in August 1998 as described by Jawitz et al., 2000. The test zone was flushed with 34 kL (equivalent to 2 pore volumes) of a 95% ethanol/5% water mixture over a 3-day period. In the initial 10 hours the ethanol concentration was ramped from 0 to 95% to minimize density problems. Post-test hydraulic containment was conducted for approximately 10 days and was discontinued after the ethanol concentration in the treatment system influent dropped below the 10,000 mg/L (217 mM) termination criterion (LFR, 1998).

Core Material Collection and PLFA Analysis. Subsurface sediment samples were collected for direct molecular analysis (16S rDNA and PLFA) prior to the cosolvent flushing test and at intervals one and two years following the cosolvent flushing test. Samples were collected from approximately 7.9 to 9.4 m bgs to coincide with the zone targeted by the cosolvent flushing test.

Prior to the cosolvent flushing test, core materials were collected into sterile conical tubes and frozen by immersing into a liquid nitrogen canister for 2-5 min and stored frozen until analysis. Sterile spatulas were utilized to obtain samples of the undisturbed centers of the core. Between core sub-samples all materials used were sterilized with isopropyl alcohol. Core materials were collected from the areas indicated in Figure 2 (CL-1 to CL-7).

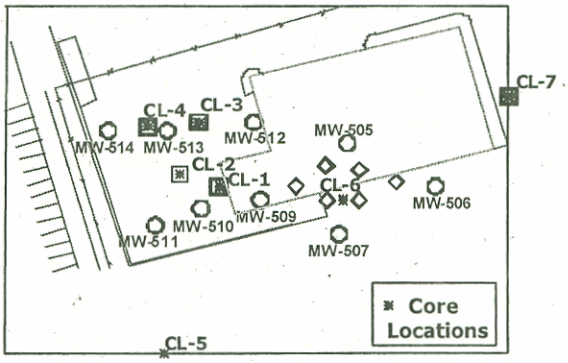

FIGURE 2. Locations of core samples collected for molecular analysis.

At one year (July, 1999) and two year (July, 2000) time intervals following the cosolvent flushing test core materials were collected from the same locations using a direct-push Simco rig (Figure 2). These materials were collected into polyethylene sleeves that were sawed into sections and then capped. Materials used to handle these cores were sterilized with isopropyl alcohol between core sections. Cores were immersed into a liquid nitrogen canister for 5 min and stored frozen until analysis.

Selected samples were sent to Microbial Insights, Inc. for 16S rDNA and phospholipid fatty acid (PLFA) analysis. PLFA analysis was conducted by extraction of lipids from the soil samples using a modified Bligh and Dyer method (White et al., 1979), separation into lipid classes, and analysis by gas chromatography/mass spectrometry (GC/MS).

Groundwater Sampling and Analysis. Ground water samples were collected prior to the implementation of the cosolvent extraction technology to provide background conditions. Following the cosolvent extraction test, ground water samples were collected and

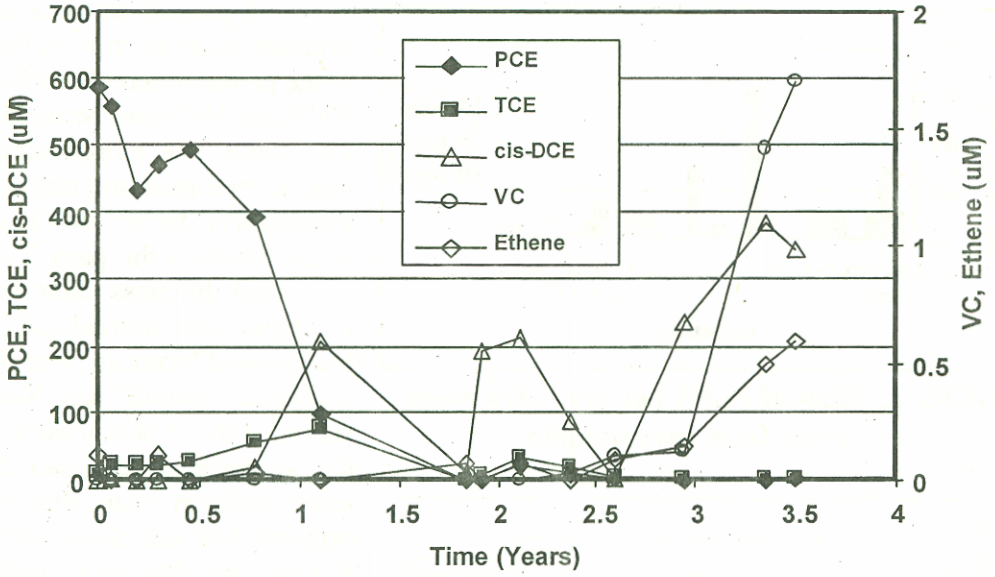

FIGURE 3. Concentration changes of chlorinated ethenes over time at well C-1.

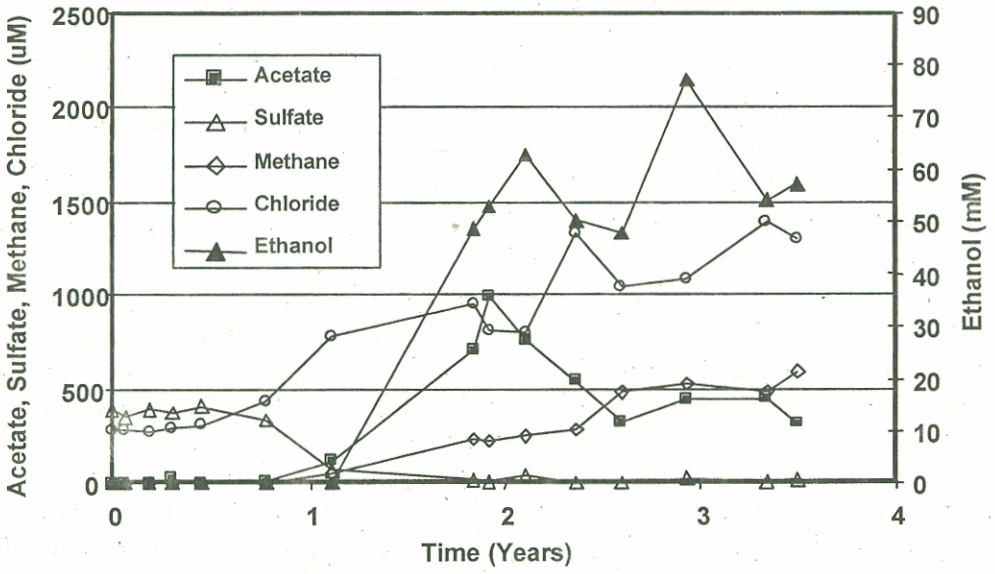

FIGURE 4. Changes in ground water parameter concentrations over time at well C-1.

PLFA Evaluation. Core samples collected from the treatment zone depth at two background locations, one injection/extraction treatment area location, and four down-gradient locations were sent to Microbial Insights, Inc. for PLFA extraction and analysis. Recovery of PLFA was low from all of the samples and averaged about 25 pmols/g dry sediment (Figure 5).

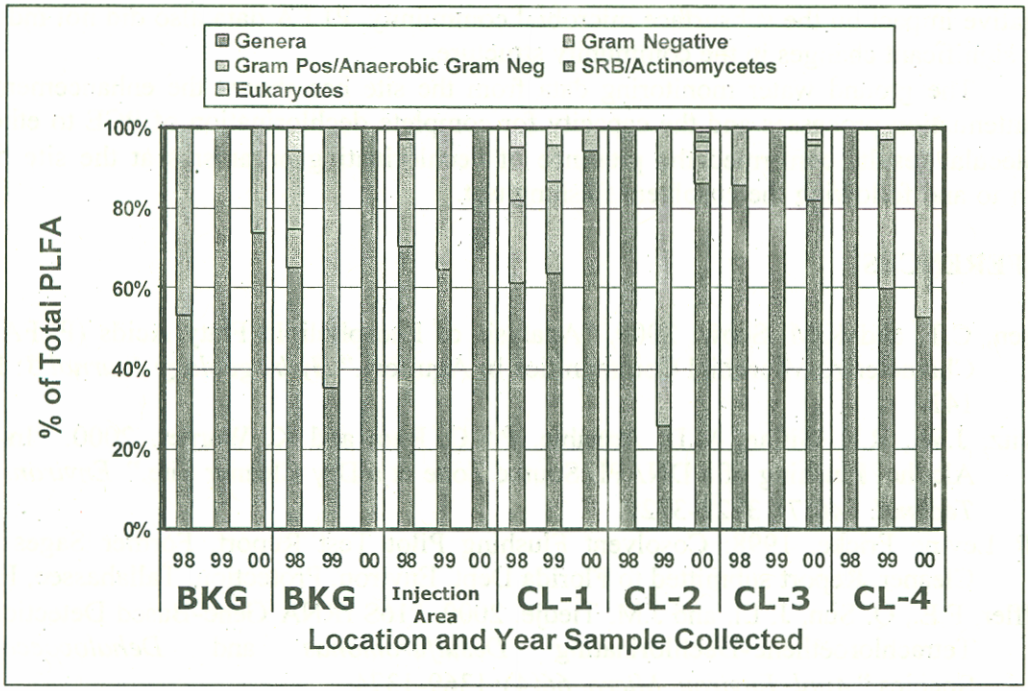

FIGURE 7. General classification of PLFA by microorganism groups.

primers, which yielded a positive signal with the MW-510 sample. Microcosm studies conducted with the MW-510 materials confirmed the results obtained with the molecular approach (Löffler et al., 2000). MW-510 microcosms were the only aquifer material-based microcosms that indicated the presence of a hydrogenothrophic PCE-dechlorinating population.

Extraction of DNA from soil samples from the injection/extraction location, four down-gradient locations, and two background locations collected both prior to and following the cosolvent flushing test was attempted with numerous methods by Microbial Insights, Inc. Due to the high amount of inhibitors and low amounts of total biomass these attempts failed to produce reproducible results. Analysis of the extracted DNA by PCR using primers specific for *Dehalococcoides ethenogenes* did not indicate its presence in any of these samples. These results were most likely impacted by PCR inhibition so should be viewed with caution.

Ground water samples were collected from the injection/extraction area (RW-3), a down-gradient location (C-2), and a background location (C-7) for analysis using the Gene-Trac™ *Dehalococcoides* analysis. *Dehalococcoides* was not detected in the background sample or in the down-gradient sample, but was positive for three out of three primers in the injection/extraction area sample.

CONCLUSIONS

Biomass estimates, as indicated by total PLFA concentrations, were low for all samples. Statistical analysis of the normalized biomass data showed an increase in biomass between 1998 and 1999 and between 1998 and 2000. The cosolvent flushing process, which entails exposure to high ethanol concentrations, did not appear to have a