USE OF LABORATORY SOIL COLUMNS TO OPTIMIZE IN SITU BIOTRANSFORMATION OF TETRACHLOROETHENE

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INTRODUCTION

Tetrachloroethene (perchloroethylene, PCE) is a major groundwater contaminant at many U.S. Department of Energy (DOE) sites, often at concentrations of several milligrams per liter (Davis et al. 1988). At the Savannah River Integrated Demonstration (SRID) site, PCE, together with trichloroethene (TCE), has been detected in both soil and groundwater (Eddy et al. 1991). Biological degradation of the combination of PCE and TCE may prove difficult in the subsurface gas injection scheme at the SRID. The injection of methane into the subsurface at SRID will provide aerobic, methanotrophic conditions that are favorable for TCE degradation. However, PCE is degraded by a reductive dechlorination process under anaerobic conditions (Freedman & Gossett 1989; DiStefano et al. 1991) but has proven recalcitrant under aerobic, methanotrophic conditions (Fogel et al. 1986). The research reported herein includes three main objectives: (1) to evaluate the potential for anaerobic dechlorination of PCE at high mg/L concentrations; (2) to evaluate the potential for PCE removal by coupling anaerobic PCE dechlorination with aerobic, methanotrophic degradation of the anaerobic products; and (3) to simulate the subsurface environment of the SRID gas injection test site in the laboratory and evaluate the fate of PCE in this laboratory simulation.

To accomplish these objectives, two types of experiments were conducted: (1) saturated sand column experiments inoculated with either anaerobic digester sludge or soil from local waste disposal sites; and (2) soil column experiments packed with soil from the SRID site and purged with either 3% CH_4 in air or N_2. This technical note describes the experimental methods used in and the results obtained from these ongoing experiments.

METHODS

Saturated Sand Column. A 70-cm by 5-cm-diameter glass column was packed with coarse sand and equipped with five intermediate sampling ports. For the first set of column experiments, the sand column was inoculated with anaerobic

digester sludge obtained from the primary digester at the local sewage treatment plant. The sludge was diluted to 10% in the revised anaerobic mineral medium (RAMM) of Shelton and Tiedje (1984) supplemented with 1 g/L sodium acetate and 50 mg/L yeast extract. The diluted sludge was applied to the column using a peristaltic pump in an upward flow mode. The supplemented RAMM was fed to the column for three weeks in order to establish biological activity.

After biological activity was established in the column (based on a steady production of CH_4 containing off-gas), a flow of PCE in RAMM was initiated. Influent PCE concentrations ranged from approximately 30 to 90 mg/L. A flowrate of 2 mL/hr (hydraulic retention time of 10 days) was maintained with a syringe pump. Effluent gas was collected over acidified water in a 40-mL vial.

The same column was used for experiments that attempted to combine anaerobic PCE dechlorination with methanotrophic degradation of the dechlorination products. These experiments involved the addition of methanotrophs of groundwater origin to the upper half of the sand column and the continuous injection of aerobic mineral medium at the column midpoint.

Soil Columns. Subsurface soil collected during drilling at the SRID site was packed into two 100-cm by 5-cm-diameter glass columns. The packing arrangement involved the addition of soil collected from the saturated zone at the SRID site to the lower one-fourth of the column and soil from the unsaturated zone to the remainder of the column. One column was purged with 3% CH_4 in air, and the other with N_2. PCE was applied to the soil columns as a vapor (approximately 2 mg/L) in the purge gas stream by allowing the purge gas to equilibrate with an aqueous solution of PCE to simulate the subsurface gas injection methods employed at the SRID site.

Analytical Methodology. Volatile chlorinated compounds in aqueous samples from the sand column were analyzed by headspace gas chromatography. In the SRID soil column experiments, PCE in the vapor phase was analyzed directly using an electron capture detector. Samples of sand column off-gas and SRID soil column inlet and outlet gas were analyzed for CH_4, CO_2, N_2, and O_2 using a gas chromatograph (GC) equipped with thermal conductivity and flame ionization detectors. Acetate concentrations were determined using a high-performance liquid chromatography system equipped with an ion-exclusion column and conductivity detector.

RESULTS

Saturated Sand Column. Initially, the sand column was inoculated with anaerobic digester sludge and remained anaerobic along its entire length. At an influent PCE concentration of 75 mg/L, PCE was reduced below detection within the first 15 cm of the column (Figure 1) after a two-month adaptation period. The dechlorination products were TCE and both the *cis*- and *trans*-isomers of 1,2-dichloroethene (DCE). No further dechlorination was observed beyond

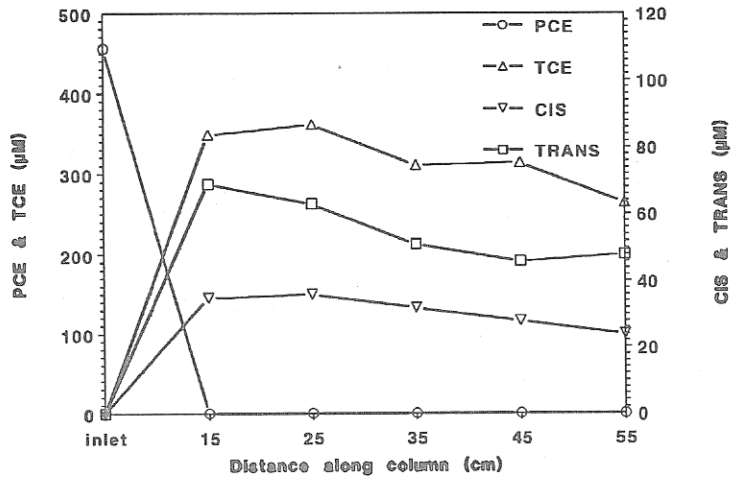

FIGURE 1. Dechlorination of 75 mg/L influent PCE in the saturated, anaerobic sand column inoculated with anaerobic digester sludge.

the first sampling port, and no vinyl chloride (VC) was detected. The reduction in concentration of *cis*- and *trans*-1,2-DCE was mainly due to the expected approximately 50% dilution. However, cometabolic degradation was confirmed by the detection of both *cis*- and *trans*-1,2-DCE epoxide.

The anaerobic column remained actively methanogenic whether or not PCE was present. Acetate was used rapidly, and an average of 15 mL of off-gas was collected each day, with a typical composition of 40% CH_4, 50% CO_2, and 10% N_2.

When the influent PCE concentration to the anaerobic column was reduced to 35 mg/L, PCE was again dechlorinated within the first 15 cm of the column. This reduced influent concentration resulted in the first appearance of VC in the column (Figure 2). Although not quantified, VC was estimated from the GC response to be the major volatile compound present.

When methanotrophs were added to the column and oxygenated mineral medium (Wittenbury et al. 1970) was supplied at the 35-cm port at approximately the same flowrate as the influent anaerobic medium, the concentrations of all four dechlorination products were reduced (Figure 3). The reduction in concentration of *cis*- and *trans*-1,2-DCE was mainly due to the expected approximately 50% dilution. However, cometabolic degradation was confirmed by the detection of both *cis*- and *trans*-1,2-DCE epoxide (Figure 4) which were identified by mass spectral analysis (Arvin 1991). Further experiments in which the anaerobic and aerobic processes are separated in different columns are presently in progress.

Soil Columns. Soil columns designed to simulate the SRID subsurface gas injection process at the water table have been in operation for several months. Both the CH_4/air and the N_2 columns are being purged at a rate of approximately

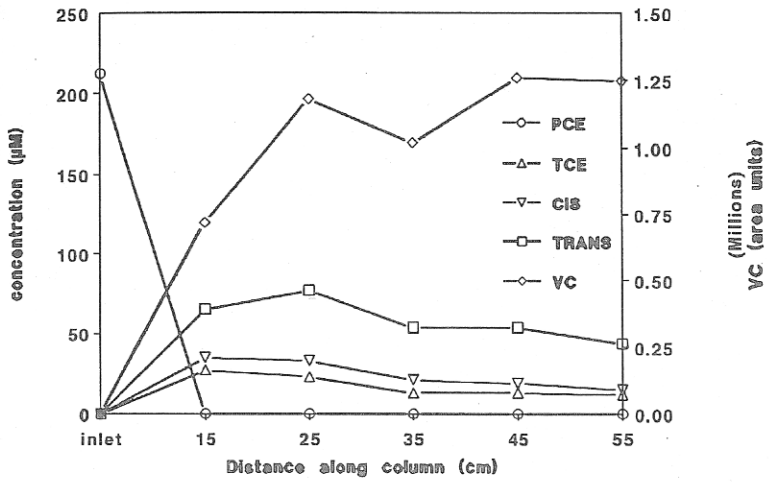

FIGURE 2. Dechlorination of 35 mg/L influent PCE in the saturated, anaerobic sand column inoculated with anaerobic digester sludge.

5 mL/min, which is the slowest gas flowrate that can be maintained in the columns. The result is an unsteady flow with an average gas retention time of about 2.5 hours (discounting the obvious channeling that occurs as the gas flows upward through the columns).

Treatments of the columns to date have included replacement of the liquid phase in the saturated zone of each column with (1) distilled water, (2) SRID

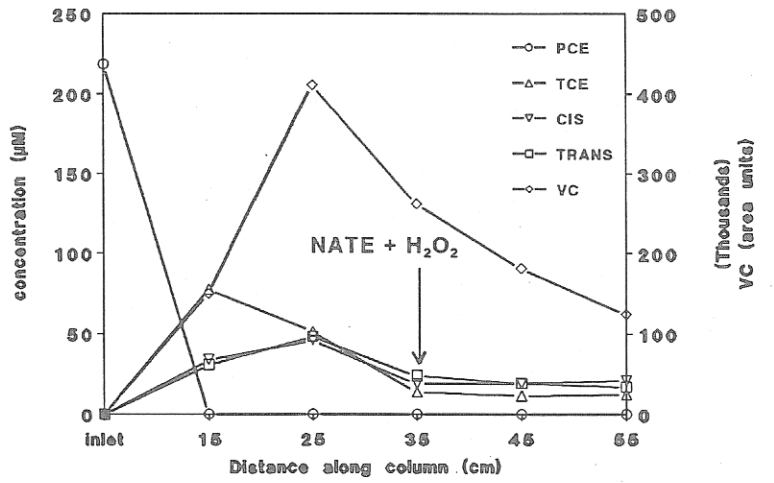

FIGURE 3. Changes in PCE dechlorination products in the aerobic zone of the saturated sand column after the addition of methanotrophs, NATE medium, and H_2O_2 to the upper half of the column.

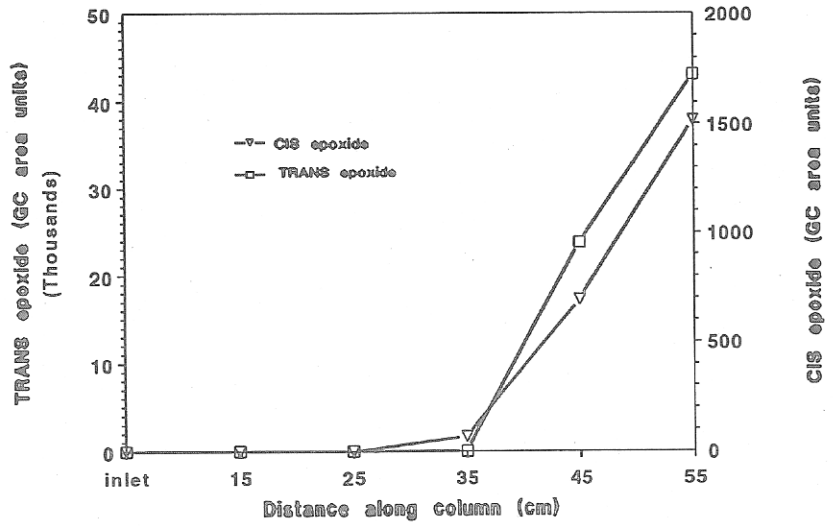

FIGURE 4. Formation of *cis*- and *trans*-1,2-DCE epoxides by methanotrophs in the aerobic zone of the saturated sand column.

groundwater, and (3) SRID groundwater supplemented with NATE medium. Each treatment regime has been monitored for 2 to 4 weeks. Monitoring of PCE in the influent and effluent gas to date has shown no indication of PCE degradation in either column under any of the treatment regimes. After groundwater from the SRID site was added to the columns, the use of CH_4 through the CH_4/air column was observed. Most probable number analyses of soil from the CH_4/air purged column confirm that the growth of methanotrophs has been stimulated.

These experiments are still in progress. Studies with DCE, known to be degradable by methanotrophs, and with the sequential addition of an electron donor (acetate) and a known PCE-degrading anaerobic inoculum to the columns are currently being conducted. Also, larger columns with online analytical capabilities are being designed to permit investigation of the effect of substantially longer column residence times on PCE degradation.

DISCUSSION

The saturated sand column experimental results indicate that high concentrations of PCE that may be encountered during an in situ remediation of groundwater can be rapidly dechlorinated anaerobically, in the presence of necessary nutrients and an electron donor, to a mixture of TCE, *cis*- and/or *trans*-DCE, and, under some conditions, VC. We have shown that the potential exists for combining an aerobic biological process in situ with anaerobic dechlorination to degrade the anaerobic dechlorination products. Further work is needed to demonstrate the maximum removal rates that can be expected.

The column tests designed to simulate the SRID in situ process conditions have thus far shown that no biodegradation of PCE occurs under the treatment regimes employed. Additional treatment regimes will be evaluated to identify conditions under which PCE may be degradable within the operational bounds of the SRID gas injection process. Treatment methods to produce these conditions might then be applied to the SRID project to maximize PCE removal.

ACKNOWLEDGMENTS

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