

# **Report for 2003AL9B: Characterizing the Biodegradation Rates of Chlorinated Compounds Under Natural and Anthropogenic Electron Donor Conditions**

- Articles in Refereed Scientific Journals:
  - Quezada, C.R., T.P. Clement, and K.K. Lee, 2004, Generalized Solution to Multi-Dimensional, Multi-Species Transport Equations Coupled With a First-Order Reaction Network Involving Distinct Retardation Factors, *Advances in Water Resources Journal*, Vol-27, pages 507-520.
  - Clement, T.P., Y.C. Kim, T.R. Gautam, and K.K. Lee, 2004, Experimental and Numerical Investigation of NAPL Dissolution Process in a Laboratory Scale Aquifer Model, submitted for publication in *Groundwater Monitoring and Remediation Journal*.
- Conference Proceedings:
  - Mendez-Sanchez, Naomi, T.P. Clement, and C.R. Lang, 2003, An Assessment of Microcosm Tests Used for Evaluating Chlorinated Solvent Bioremediation Model Parameters, in *Proceedings of the MODFLOW and More: Understanding Through Modeling*, Golden, Colorado, Vol-2, pages 814-818.
  - Quezada, C.R., C.M. Hansen, T.P. Clement, N.L. Jones, and K.K. Lee, 2003, ART3D An Analytical Model for Predicting 3-Dimensional Reactive Transport, in *Proceedings of the MODFLOW and More: Understanding Through Modeling*, Golden, Colorado, Vol-1, pages 275-279.

Report Follows

# **CHARACTERIZING THE TRANSPORT AND BIODEGRADATION PATTERNS OF CHLORINATED COMPOUNDS UNDER VARIOUS ENVIRONMENTAL CONDITIONS**

PI: Dr. Prabhakar Clement  
Co-PI: Dr. Clifford Lange  
Department of Civil Engineering  
Auburn University

## **A. PROBLEM STATEMENT AND OBJECTIVES**

Due to their adverse effect to both human health and the environment, many researchers have studied the patterns of PCE and TCE degradation in laboratory-scale microcosm tests. Microcosms are small microbial habitats that simulate microbial degradation under various conditions observed in the natural environment. Currently, microcosm experiments are the best way to provide convincing evidence for demonstrating natural attenuation and predicting effects of bioremediation activities. Often this is accomplished by quantifying the disappearance of the target compound while tracking the evolution of various degradation byproducts within the controlled experimental system. While microcosms play a vital role in understanding the fate of chlorinated solvents in the subsurface environment, no standardized protocols for conducting these tests are used. Instead, investigators utilize their own techniques, making comparisons of studies problematic. Clearly, a consistent methodology for conducting microcosm studies is needed. A common procedure for conducting microcosm experiments involves the use of small serum bottles as microcosm reactors. This allows investigators to set up large numbers of replicates while using only a small amount of contaminated soil and thus generating small quantities of waste. However, the small sample size may exacerbate the effects of heterogeneities in the aquifer/soil material. To initiate a test, serum bottles are filled with a salt (buffer) medium, water, soil, substrate (electron donors), and target compound(s). Usually the salts medium is formulated to mimic conditions present at the site in question (i.e., pH, ionic strength, alkalinity, etc.) Different amounts and combinations of soil, substrate, and target compound(s) have been used which makes interpretation of the results difficult.

In addition to performing microcosm test, laboratory scale column experiments coupled with modeling can also provide a greater insight into understanding the overall fate and transport PCE and TCE species in realistic groundwater aquifers.

The overall objective of this work is to completed laboratory and modeling investigation of chlorinated solvent biodegradation patterns and various environmental conditions. Specific tasks completed as a part of this research project include development of a standardized protocol for conducting microcosm studies, standardizing the methods for performing PCE and TCE fate and transport experiments in soil columns, and to explore modeling methods for predicting the PCE/TCE degradation reactions that are coupled to biological degradation processes.

## **B. RESEARCH METHODOLOGY FOR LABORATORY WORK**

For conducting the microcosm experiments, soil samples were obtained at three different locations from a Super Fund site. The site is located at the north of Baton Rouge, LA, approximately 1.5 km away from the Mississippi River. This waste site was opened in 1969 to accept different chemical wastes and its operations ended in 1980. Large quantities of dense non-aqueous phase liquids (DNAPLs) have been identified in this site; the DNAPL waste products originated from various chlorinated solvent manufacturing plants, and from other refineries. The soil samples were collected within one meter of the surface and were transferred into a glass container after removing roots, leaves, and dead vegetation. Analysis of the soil was performed as soon as it arrived to the laboratory. Afterwards, the soil was stored in a refrigerator.

In addition to this field soil samples, we also collected several soil samples near a creek in the downtown Montgomery area closer to the location where Capitol city plume was expected to present. However, these samples were found to lot less microbially active than the Louisiana superfund site plume. As stated before, the Louisiana site was heavily contaminated chlorinated solvent contaminants and the soil microbes are well exposed to the contaminants. Therefore, the overall activity of the superfund site soil was much higher than the Montgomery soil. Hence, in this study primarily used the Louisiana soil in all our test microcosms.

### Microcosm Experiments

Small glass serum vials (160 mL) were used as microcosms and were filled with 40.0±0.2 g of the collected soil. A total of 9 bottles were prepared per soil. Table 1 presents the design matrix for the microcosms prepared using the soil obtained at the Super Fund site.

Site	Treatment
I	A – TCE, nutrients, glucose
	B – nutrients, glucose
	C –TCE, nutrients, biocide, glucose
II	A – TCE, nutrients, glucose
	B – nutrients, glucose
	C –TCE, nutrients, biocide, glucose
III	A – TCE, nutrients, glucose
	B – nutrients, glucose
	C –TCE, nutrients, biocide, glucose

**Table 1. Design matrix for the microcosms using the Super Fund site soil.**

Four microcosms from each soil were filled with 150 mL of a spring water-TCE solution (treatment A). A stock solution was prepared by adding 30 mL TCE (99.9+%, spectrophotometer grade, Aldrich Chemical Company, Inc.) in order to saturate the solution. Afterwards, dilutions were performed in order to obtain a final solution with an initial concentration of 30 ppm of TCE. The following salts were added to this solution (g/L): 0.053 KH<sub>2</sub>PO<sub>4</sub>, 0.1068 K<sub>2</sub>HPO<sub>4</sub>, 1.0 NH<sub>4</sub>Cl, 2.0 Na<sub>2</sub>SO<sub>4</sub>, 2.0 KNO<sub>3</sub>, and 0.2 MgSO<sub>4</sub> • 7H<sub>2</sub>O. Additionally, 2.0 g/L α – D (+) glucose was added as the electron donor in these experiments. The same solution was added to the rest of the bottles, which were used as controls in each soil (treatment B and C). TCE was not added to microcosm in treatment B. In treatment C, 200 mg/L of NaN<sub>3</sub> were added in order to observe the abiotic degradation of TCE in the system. The vials were closed with aluminum caps and PTFE lined rubber septa. After

making sure the microcosms were securely sealed and air bubbles were not present, they were mixed by handshaking for one minute. The microcosms were kept in a shaker at 150 rpm in the dark at ambient temperature (21 °C) through the duration of the experiments.

### Soil Column Transport Experiments

A Kontes Chromaflex glass column (ID = 4.8cm, L = 30cm) was dry packed with Ottawa Sand Standard, mesh size 20-30. The column was packed by adding small amounts of sand at a time and tapping the sides of the column to ensure compaction. The dry and wet weight of the column was measured to calculate porosity. Once packed and weighed, the column was connected to a Masterflex Consol Drive pump and was flushed with deionized water for over 24 hours to ensure complete saturation and removal of air bubbles. After each experiment the column was emptied, cleaned and the sand was replaced. The weight of sand added to the column was weighed and the same weight was used for all experiments.

The inflow solution was prepared in a large 2.5L glass bottle. Stock solutions of PCE and TCE were initially prepared by adding 30mL of solvent to deionized water to create a saturated solution. Dilutions of this saturated solution were then made to achieve a target inflow concentration of approximately 60ppm. Uranine (fluorescein sodium salt C<sub>20</sub>H<sub>10</sub>Na<sub>2</sub>O<sub>5</sub> – Fisher Chemical) was used as a tracer. This solid salt was weighed and dissolved in deionized water to achieve a target inflow concentration of 25ppm for all column experiments. Sodium azide (NaN<sub>3</sub>) from Fisher Chemical was used as a biocide in some of the experiments. The solid was weighed and dissolved in deionized water to reach an initial inflow concentration of approximately 200ppm for all column experiments.

Column experiments were defined as either pulse or step. Pulse experiments involved pumping a known volume of spiked solution and then continuing the flow with deionized water. This type of experiment

allowed observation of the tail of the breakthrough curve. Step experiments involved continuously pumping a known volume of spiked solution and running the column until reaching the equilibrium concentration in the breakthrough curve. A flushing rate of approximately once per hour was desired. This was achieved by calculating the volume of water in the column using the porosity and adjusting the flow rate as required. This ideal pumping rate was manipulated slightly to achieve a flow rate that accommodated the sample size required (i.e. 4ml/min).

## **Analytical Procedures**

The concentration of the chlorinated ethenes was measured at times days 0, 3, 7, 14, every two weeks for two months, and every four weeks after the third month. A 10 mL gas tight syringe was used to extract 6 mL of water solution from the microcosms. This sample was filter using a 0.22  $\mu\text{m}$  nylon syringe filter (Osmonics Laboratory Products). One milliliter of the sample obtained was placed in a 10 mL test tube to which hexane was added in a sample to solvent ratio of 40:60 to make the extractions. The test tube was shaken by hand for 30 seconds. Afterwards, part of the organic phase was placed in a 2 mL auto sampler amber glass vial and analyzed by gas chromatography.

An Agilent 6890 gas chromatograph was used to quantify the concentrations of the target compounds and degradation byproducts. The method used a split injection port (split ratio 3:1) using a helium carrier gas at a temperature of 250°C (482°F) and an inlet pressure of 34.13 kPa (4.95 psi). The capillary column used was an HP-1 methyl siloxane 30 m by 0.32 mm by 1.8  $\mu\text{m}$ . The carrier velocity was 20 cm/sec (flow rate of 2 cc/min). A micro electron capture detector (ECD) was used with a temperature of 340°C and an argon methane (5%) makeup flow of 30 mL/min. The oven was ramped from 40°C to 170°C at a rate of 10°C/min. and then ramped to a final temperature of 300°C at 40°C/min

Standards were prepared by dissolving PCE, TCE, or DCE in hexane in order to make a 250 ppm standard solution. The solutions were used to make sequential dilutions (150 – 0.05 ppm) of the standard; again hexane was used as the solvent. The remaining 5 mL of sample was used to measure the oxidation-reduction potential (ORP) and pH immediately after filtering the sample. An ATI Orion expandable ion analyzer (EA 940) was used to measure both the ORP and pH. Tukey's honestly significant difference test ( $\alpha = 0.05$ ) was performed in order to determine statistical differences between the soils and treatments used in the experiments.

## **C. RESEARCH FINDINGS AND SIGNIFICANCE**

The laboratory work completed as a part of this project is the first step in completing a set of experiments for the standardization of microcosm tests. Results obtained are satisfactory as ORP decrease to levels optimal for the reductive dechlorination of chlorinated compounds. TCE levels decreased by approximately 10 ppm after one week of experiments. Biodegradation byproducts concentration had increased from zero to 1.6 ppm for 1,1-DCE. Also, other unidentified compounds have been observed in the microcosms, and their quantification is currently being pursued. Future experiments for the standardization of microcosms experiments will focus on the variation of the soil to water ratio, as well as the use of microcosms with headspace.

Column tests on TCE and PCE carried out in this study found that there was minimal retardation or adsorption onto sand at a flow rate of 4mL/min. Full recovery of both TCE and PCE was obtained in the final experiments. It was also found that due to the relative insolubility of PCE, concentration levels of PCE after complete breakthrough were always lower than TCE concentrations. The protocol developed during this study needs to be followed to ensure accurate results. A glass column should be used with fittings that are non-reactive with chlorinated solvents (e.g. PTFE), and all tubing should be made from viton. Solutions and samples should be kept covered from light at all times to reduce photo degradation. The inflow solution should also be very slowly stirred to keep the solution homogeneous. This technique can now be used to observe these same characteristics with mediums other than sand, and the results from these further experiments will complement the microcosm study currently underway.

The modeling work supported by this project allowed us to develop a novel modeling method for predicting the fate and transport of PCE and TCE species in bioreactive groundwater aquifers. In addition, the funding also allowed us to team-up with an overseas research partner to study a PCE-DNAPL dissolution experiment using a numerical reactive transport model. The modeling results were submitted for journal publications.