## *1.0 INTRODUCTION*

Environmental Resources Management, Inc. (ERM) and its subcontractor, Terra Systems, Inc. (TSI) has completed an Anaerobic Microcosm Study to evaluate the potential for enhanced anaerobic bioremediation to remediate tetrachloroethene (PCE), trichloroethene (TCE) and their daughter products (cis-1,2-dichloroethene [cis-1,2-DCE], and vinyl chloride [VC]) in ground water underlying the Former Raytheon, Wayland, Massachusetts Facility (site).

Anaerobic biodegradation is a well-established methodology for the treatment of PCE, TCE, and other chlorinated volatile organic compounds (CVOC). In general, CVOCs can be reduced to carbon dioxide  $(CO_2)$  and methane (CH4) or other innocuous products such as ethene or ethane via reductive dechlorination or other biological processes.

As part of the treatability study soil and ground water samples were collected on May 19 and May 25, 2005. The soil/ground water sampling and subsequent treatability study were performed to determine:

- If biodegradation, or other abiotic processes (i.e., hydrolysis), of TCE is occurring naturally at the site;
- The process of biological reductive dechlorination or other processes);
- If the optimal biological microorganisms are present to facilitate biodegradation; and
- If the addition of amendments (nutrients and/or microorganisms) will enhance the biodegradation.

#### *2.0 SOIL AND GROUND WATER SAMPLING*

Ground water samples were collected using a low-flow sampling technique. Three quarts of soil were collected adjacent to the monitoring well MW-268M. Groundwater was collected from MW-268M. Both the soil and groundwater were shipped on ice to Terra Systems, Inc. in Wilmington, DE. Table 1 summarizes the ground water and soil analytical results at the time of the May 2005 sampling event.

The results of the soil/ground water analyses indicate that:

- The analytical data for CVOCs and natural attenuation parameters provide evidence of naturally occurring reductive dechlorination; and
- The optimal microorganism for naturally occurring reductive dechlorination, *Dehalococcoides ethenogenes* (DHE), was detected at a moderately high concentration of 25,600 16S rRNA genomes per gram in the soil sample collected as part of the sampling event. This is consistent with the ground water conditions being mostly anaerobic as required for DHE to thrive.

**Table 1.**

# **Concentrations of CVOCs, Dissolved Hydrocarbon Gases, Electron Acceptors, and Nutrients in MW-268M Ground Water and Numbers of**  *Dehalococcoides ethenogenes* **in Soil**



\*16s rRNA genome copies/g

#### *3.0 MICROCOSM STUDY*

TSI completed the laboratory portion of the Anaerobic Microcosm Study at its Wilmington, Delaware facility between June 2005 and September 2005. The study evaluated the potential for the addition of lactate, a soluble substrate, and a slow-release substrate (SRS, emulsified soy bean oil) to stimulate reductive dechlorination of TCE to ethene/ethane. Based upon the presence of cis-1,2-DCE, VC, and ethene and detection of the *Dehalococcoides ethenogenes,* it was thought that the site was limited by the availability of carbon to support anaerobic microbial growth and complete dechlorination of the TCE to ethene and ethane. The study procedures and results are presented in this section of the report.

#### *3.1 MICROCOSM PREPARATION*

Microcosms were prepared on June 2, 2005 using 1,000-milliliter (mL) amber bottles. Each microcosm was incubated for up to 112 days. Table 2 summarizes the individual microcosms prepared for this study, including the quantities of ground water, soil and amendments added to each microcosm. The microcosms were prepared and sampled in an anaerobic chamber containing 3% hydrogen, 5% carbon dioxide and 92% nitrogen to ensure anaerobic conditions were maintained. The microcosms were sealed with a Teflon-lined lid. The microcosms were incubated at 21°C throughout the study.

Control microcosms included a sterile control and an unamended control microcosm. The sterile control (also referred to as the poisoned control) was prepared using only MW-268M ground water and soil to account for potential abiotic losses of TCE and daughter producers from the microcosm. The sterile control microcosm was amended with 1,000 mg/L of mercuric chloride to reduce the potential for microorganism survival. The unamended microcosm was prepared using MW-268M ground water and soil. No substrates were added to the unamended control microcosm in order to evaluate whether organic compounds in the ground water or soil could support reductive dechlorination.

# **Table 2. Microcosm Amendments**



Sodium lactate was added a dosage of 500 mg carbon per liter of groundwater (mg C/L) as an electron donor and fermentable substrate to support the generation of hydrogen. The SRS was added at a higher loading of 2,000 mg/L because it is typically biodegraded more slowly than a soluble substrate such as lactate. The SRS also contains sodium lactate as a fast-acting substrate to rapidly generate anaerobic conditions. Yeast extract was added to both substrate-amended treatments as a source of trace elements. Both substrate-amended treatments received 50 mg/L nitrogen and 5 mg/L phosphorus from ammonium chloride and disodium phosphate as nutrients.

The bicarbonate and resazurin were added to all treatments. The bicarbonate was added to buffer acid generated from biodegradation of the organic substrate and dechlorination of the TCE and daughter products. A solution of 1 mg/L resazurin was added to each microcosm as a visual indicator of oxidation-reduction potential (ORP). The microcosms remain clear when conditions are anaerobic and reducing, which is necessary for reductive dechlorination to occur. A pink color is observed when the microcosm is under aerobic, oxidizing conditions. Resazurin does not affect the biodegradation process and would not be added as part of a full-scale implementation. All of the microcosms became clear soon after they were set up indicating that there was sufficient organic matter in the soil and groundwater to support anaerobic conditions.

#### *3.2 MICROCOSM SAMPLING AND ANALYSIS*

Samples were collected from each microcosm for analysis after 0, 14, 28, 43, 56, and 84 days. On Day 84 of the study, samples were collected before and after inoculating the microcosm C (sodium lactate) with dechlorinating enrichment. The dechlorinating culture was grown on sodium lactate and a Reduced Anaerobic Mineral Media (RAMM) containing inorganic nutrients, vitamins and trace minerals and TCE prior to inoculation. Additional substrate was added to treatment C (sodium lactate) and treatment D (SRS) to ensure that the availability of carbon did not limit the extent of reductive dechlorination. Treatments C (sodium lactate) and D (SRS) were also sampled on Day 98 and 112.

Duplicate samples were collected from the microcosms within an anaerobic glove box to maintain anaerobic conditions. Samples were collected for CVOC and light hydrocarbon analyses. Two aliquots (2 to 9 mL) of the sample to be analyzed by TSI for CVOCs in general accordance with SW-846 Method 8021B and for light hydrocarbons (ethene, ethane, and methane) in general accordance with a modified SW 846 Method 8015 was transferred directly into a 20-mL headspace vial containing 1 mL of a 25% sodium chloride solution adjusted to pH 2.0 with phosphoric acid and enough distilled water to bring the entire volume of sample and sodium chloride solution to 10 mL.

The microcosms contained 27 mL headspace initially. As samples were removed, the volume of headspace increased depending upon the volume of sample removed. The concentrations of the contaminants in the liquid and gas phases were calculated based upon the estimated volume of each phase and the Henry's Law constant for each constituent and the incubation temperature (21  $\textdegree$ C). Graphs were prepared based upon the total moles of the contaminants in each phase. The data was expressed in

moles so that one unit of PCE is equivalent to 1 unit of TCE, cis-1,2-DCE, VC, ethene, or ethane.

## *4.0 RESULTS AND DISCUSSION*

## *4.1 METABOLIC ACTIVITY*

Metabolic activity refers to the level of biodegradation occurring, and has been evaluated in this study by measuring dissolved methane concentrations. The presence of methane in a microcosm is an indication that microorganisms are present and actively biodegrading the organic substrate. Increases in methane concentrations following addition of an organic substrate to a microcosm indicate that the growth of anaerobic microorganisms can be stimulated. Methane is produced when other electron acceptors (e.g., oxygen, nitrate, sulfate, iron) have been utilized, and reductive dechlorination occurs most readily under these methanogenic conditions.

Figure 1 and Appendix I present the dissolved methane concentrations for each microcosm with MW-268M ground water and soil. Methane concentrations in the microcosms at the beginning of the study ranged from 0.027 to 0.037 mg/L. Methane was detected in the sterile and unamended control microcosms, but the maximum concentration of 0.037 mg/L was low relative to the substrate-amended microcosms and the methane concentrations declined over time with no evidence that additional methane was formed in these treatments. In the substrateamended microcosms, maximum methane levels were 55 mg/L in the microcosm amended with SRS . Somewhat lower levels of methane were found in the lactate-amended treatment with a maximum of 19 mg/L.

The results of the microcosm study indicate:

- As expected, sterile and unamended control microcosms contained low methane concentrations throughout the study period with low gas production relative to amended microcosms. It should be noted that methane concentrations decreased between week 0 and week 6 in the poisoned control and unamended treatments due to volatilization into the headspace in the microcosm;
- Growth of indigenous microorganisms can be stimulated through the addition of an organic substrate. This is based on increases in methane concentrations observed in non-bioaugmented microcosms relative to the control microcosms; and

• Increased methane concentrations in each substrate-amended microcosm occurred by Day 42.

## *4.2 Dissolved Metal Production*

Samples were collected from the all four microcosms at the end of the study to be analyzed for dissolved arsenic, dissolved iron, and dissolved manganese. Table 3 summarizes the results of the initial metals analyses and the concentrations of these metals after incubation for 16 weeks. Arsenic levels were slightly elevated in the poisoned control, unamended controls, and lactate-enriched treatments compared to the initial concentrations. Arsenic levels increased substantially in the SRS enriched treatment to 165 μg/L. Dissolved iron and manganese levels increased in all treatments after incubation for 16 weeks under anaerobic conditions compared to the initial concentrations. The greatest iron and manganese reduction occurred with the SRS with the production of 520 mg/L of dissolved iron and 6.4 mg/L manganese.

#### **Table 3.**

# **Concentrations (mg/L) of Dissolved Metals Initially and after 16 Weeks Incubation**



## *4.3 Contaminant Removal*

Appendix I presents the analytical results for the microcosms containing MW-268M ground water and soil. Figures presenting the results for the various microcosms are summarized as follows:



CVOC concentrations presented on the figures are expressed in total micromoles (μMoles) units. This was done so that each CVOC is expressed on an equivalent mass basis for comparison purposes. The micromolar concentrations are calculated by dividing the concentration in  $\mu$ g/L by the molecular weight of the CVOC (PCE = 165.8 g/mol; TCE = 131.4 g/mol; cis-1,2-DCE = 97 g/mol; VC = 62.5 g/mol; ethene = 28  $g/mol$ ; and ethane = 30 g/mol). 1,1-DCE and trans-1,2-DCE were present at low concentrations and were not included in the graphs.

The following summarizes the results for microcosms containing MW-268M ground water and soil:

- Initial TCE concentrations in the microcosms ranged from 1.4 mg/L to 2.25 mg/L. Due to an instrument malfunction, only one initial sample from Treatment D SRS was analyzed. Initial cis-1,2-DCE concentrations ranged from 3.4 mg/L to 5.4 mg/L. Initial VC concentrations ranged from 0.13 mg/L to 0.20 mg/L. The variability in the initial TCE, cis-1,2-DCE and VC levels may be a result of using different bottles of groundwater to prepare the treatments, losses of cis-1,2-DCE and VC during the set-up of the microcosms, or analytical variability. No ethene or ethane were detected in the initial samples;
- Over the 84-day incubation period for the sterile control microcosm, there was no evidence for reductive dechlorination of the TCE to daughter products or microbial activity based upon accumulation of methane. Losses of 78 % of the TCE, 86.5 % of the cis-1,2-DCE and more than 99 % of the VC were observed which might be attributed to TCE, cis-1,2-DCE and VC partitioning into the headspace in the microcosm or adsorption onto the glass bottle, glass beads. Similar abiotic losses have been observed in other microcosm studies.
- There was no detectable conversion of TCE to cis-1,2-DCE or VC in the unamended control microcosm B. However, this microcosm did show a 80 % loss of TCE, 90 % loss of cis-DCE and >99% of VC from the microcosm; these losses were similar to those experienced from the abiotic control and thought to be for the same reasons;
- The microcosm C amended with SRS showed almost complete conversion of the initial TCE to cis-1,2-DCE by Day 28, but little VC was produced, although the VC present initially was degraded or otherwise lost. No detectable levels of ethene were measured during the first 84 days of incubation. These results suggest that the addition of lactate alone was not able to achieve complete

reductive dechlorination of TCE to cis-1,2-DCE, VC, and finally ethene in a timely manner. However with bioaugmentation and additional substrate on day 84, cis-1,2-DCE was further degraded to VC and ethene. At the end of the 112 day study, TCE concentrations had been reduced by 100%, cis-1,2-DCE by 99.5% and VC by >99% ; and

- The microcosm D amended with SRS achieved almost complete reduction of TCE to cis-1,2-DCE within 56 days. Cis-1,2-DCE persisted over the remaining 56 days of incubation with no detectable production of VC, ethene, or ethane even with the addition of more SRS on day 84.
- Bioaugmentation increased the rate of complete reductive dechlorination of TCE to ethene in ground water at the Former Raytheon Wayland Facility. Although the soil contained a substantial population of *Dehalococcoides ethenogenes*, the indigenous population was not able to complete the transformation of TCE to ethene under the conditions employed in the microcosm test. Sodium lactate is a potential organic substrate candidate for ground water based on the corresponding bioaugmented microcosms showing almost complete conversion of TCE to VC and ethene.

## *5.0 CONCLUSIONS AND RECOMMENDATIONS*

Conclusions of the bioremediation evaluation based on the results presented in this report are as follows:

- The growth of indigenous microorganisms at the Former Raytheon Wayland Facility can be stimulated through the addition of an organic substrate. This is supported by the observed increase in metabolic activity including production of methane, dissolved iron, and dissolved manganese and conversion of TCE to cis-1,2-DCE through the addition of only SRS. This is consistent with the DHE analyses of the ground water, which showed that the native microbial population contains organisms capable of complete dechlorination. However, based upon the microcosm study, the native dechlorinators were not able to completely dechlorinate the TCE and cis-1,2-DCE during the sixteen week study;
- Bioaugmentation was necessary to achieve complete reductive dechlorination of TCE to ethene. Microcosms amended with only SRS were able to convert TCE to cis-1,2-DCE, but were not able to convert cis-1,2-DCE to VC and ethene during the sixteen-week study. Microcosms bioaugmented after 84 days, achieved almost complete conversion of cis-1,2-DCE to VC and ethene;
- Based upon this study, sodium lactate is a potential organic substrate to be considered for full-scale implementation. It will require as frequent replenishment; and
- The dechlorinating enrichments containing DHE should be considered for full-scale implementation. The microcosm bioaugmented with the dechlorinating enrichment achieved nearly complete reductive dechlorination of TCE to ethane.

The results of this study indicate that enhanced anaerobic bioremediation is a viable remedial alternative for the Former Raytheon Wayland Facility to address the chlorinated VOC plume. Remedial performance can be evaluated and modified as part of the field-scale pilot test. It is recommended that injection of the lactate be conducted and monitored for at least three months prior to bioaugmentation to determine if the substrate can be effectively distributed and perhaps lead to the growth of the dechlorinating population.

**FIGURES**



**Figure 1. Methane Concentrations**



**Figure 2. A Poisoned Control Chlorinated Ethenes**



#### **Figure 3. Treatment B Unamended Control Chlorinated Ethenes**



#### **Figure 4. Treatment C Lactate Enriched Chlorinated Ethenes**



#### **Figure 5. Treatment D SRS Enriched Chlorinated Ethenes**

# **APPENDIX I**

# **CONCENTRATIONS OF CHLORINATED ETHENES AND LIGHT HYDROCARBON GASES**







