METHANOTROPH COMMUNITY PROFILES AS A PREDICTOR OF TCE DEGRADATION POTENTIAL

Elizabeth A. Richardson[1], Charles H. Jagoe[1], Travis C. Glenn[1,2], J Vaun McArthur[1]

[1] University of Georgia - Savannah River Ecology Lab, Aiken, SC

[2] University of South Carolina, Columbia, SC

Abstract

Trichloroethylene (TCE) is a primary soil and groundwater contaminant on the USDOE Savannah River Site near Aiken, SC. In 2000, 67% of the monitoring wells exceeded the limits for organic content as outlined by the federal primary drinking water standards. The contaminated groundwater is surfacing in Four Mile Creek, a tributary to the Savannah River. The surface waters have both TCE and the degradation product vinyl chloride. Vinyl chloride is indicative of natural attenuation. Methanotrophic bacteria produce methane monooxygen ases (MMO) capable of cometabolizing TCE. Soil sampled within the riparian zone of Four Mile Creek has both Type I and Type II methanotrophs as determined by DNA extraction and screening with methanotroph specific primers in PCR. Based on the profile of the methanotrophic community, can the soil's potential for TCE degradation be predicted? Microcosms were prepared using the selected soils, spiked with TCE, and the TCE concentration was measured over a two week period. Meanwhile, DNA extraction and methanotroph screening verified the presence of methanotrophs in 3 of the selected soils. Soils "B" and "D" contained both Type I and Type II methanotrophs and were most similar in community profile. Soil "A" was positive for Type II methanotrophs and soil "C" was negative for both Type I and II. Comparison of TCE degradation patterns reflected the same similarities and differences; soils "B" and "D" showed similar degradation patterns while soils "A" and "C" were each different from the others. The preliminary results suggest differences in methanotroph community as demonstrated by DGGE did correspond to differences in TCE degrad ation patterns.

Introduction

The Savannah River Site (SRS) is a U.S. Department of Energy facility. The SRS covers approximately 803 square kilometers and was established in 1950 to promote national defense efforts by producing plutonium, tritium, and other nuclear materials. The site served in this capacity for over 40 years. Subsequently, the mission of the SRS was redirected toward waste management and remediation.

The specific area of interest for this study is the C-area burning rubble pit (CBRP) and the nearby wetlands along Four Mile Creek. C-area is home to one of the site's decommissioned, heavy water reactors. The burning rubble pit was originally used for waste reduction by open burning. Paper, plastics, rubber, oils, and organic solvents were discarded and burned in the pit. Eventually the p it's use was limited to disposal of inert rubble. The pit was finally covered by a 0.61 meter layer of native soil to prevent leaching due to rainwater. The contents of the pit and discharges from the reactor seepage basins are the sources of the various contaminants that have been identified in the soil and groundwater.

The burning rubble pit measures approximately 7.6 by 106.7 meter s and has a depth of 2.9 to 3.7 meters. A plume of contamination extends from the pit area toward Four Mile Creek and includes the Twin Lakes wetland area. The contamination in the vadose zone is estimated to cover an area of some 808.3 square meters to a depth of about 18.3 meters. The highest concentration of TCE was 286ug/kg measured at a depth of 9.1 meters. Due to the volatility of TCE, the contamination in the vadose zone p rovides a continuing source of groundwater contaminants. The concentration of PCE, a metabolite of TCE, which is also seeping into the groundwater at levels exceeding regulatory requirements, is an indication that some degradation of TCE is taking place. Contaminated groundwater containing TCE, PCE, and vinyl chloride(another metabolite) is in turn seeping into the surface water of Four Mile Creek.

Trichloroethylene

- Organic solvent and common industrial pollutant, not a naturally occurring compound
- Environmental fate and transport
  - Adsorbs to soil
  - Partitions into sediments of lakes and streams
  - Several groups of bacteria are capable of degrading TCE, including methanotrophs, sulfate reducers, and nitrate reducers
  - Highly volatile- evaporates from soil or water surfaces
  - Volatized TCE is photodegradated
- Human health and environmental concerns
  - Liver and kidney toxicity
  - Respiratory and reproductive effects
  - Carcinogenic metabolites- vinyl chloride
  - Cometabolic injuries to the bacteria capable of degrading TCE by inhibition or inactivation of the enzyme or its active site
  - Disruption of bacterial metabolism from a non-growth substrate (TCE)
  - Fish exposed to TCE lost normal schooling behavior, swimming ina spiral pattern near the water surface
- Remediation techniques used at CBRP
  - Air sparging- uses injection and extraction wells to force air below the groundwater, TCE volatizes in the air and both are drawn out of the soil
  - Soil vapor extraction- wells are used to increase the soil/air interface and increase volatilization
  - Monitored natural attenuation- naturally occurring microorganisms used to degrade pollutants
  - Phytoremediation- utilizes plants to uptake contaminants from the soil and ground water

Study site

Soil Moisture Content

Materials and Methods

Soil Samples

- Collected from 4 locations along the bank of Four Mile Creek , characterized for moisture content and particle size. A portion from each location was frozen at –80C for DNA analysis
- 9 microcosms (50 g of soil with 100 ml of distilled water in a 2 50 ml bottle with Cheminert mininert sampling valve) were assembled for each location, 3 replicates o f 3 treatments, all microcosms were spiked with 200uL of TCE solution
  1. unamended soil
  2. 2% methane amended soil
  3. autoclaved soil (abiotic control)
- Microcosms were incubated for 15 days with contents sampled by r emoving 0.50ml aliquots with a 1.0 ml Hamilton syringe on days 0,3,6,9, and 15 for GC analysis
- Following the sampling on day 15, all microcosms were sacrificed and the contents frozen at –80C for DNA analysis

GC Analysis:

- Analysis was performed on a Hewlett-Packard 5890 Series II Plus gas chromatograph interfaced with a HP 5972 Mass Selective Detector.
  - Samples were introduced to the GC using a HP 7694 Headspace Sampler
  - heated at 90C for 30 minutes
  - Helium was used as a carrier gas at a constant flow rate of 1.5ml/min.
- Analytical separation on a HP-PLOT Q capillary column (3m x 0.32mm ID, 20um film thickness)
  - 80C initial temperature for 2 min. followed by a linear increase of 10C/min to 200C, and held for 8.50 minutes
  - injector temperature and GC/MS interface maintained at 250C
- Amount of target compound was calculated by comparing compound target ionpeak areas and peak heights obtained by 1.0 ml headspace sampler injections with that of standards made from dilutions of a Supelco (Belfonte, PA) VOC Custom Mix solution (light VOC's, 25ppm each) using HPChem integration software.
  - Detection limits: 0.5ppb to 500ppb
  - VOC component values normalized to 9.75g (standard mass) of material

DNA Analysis:

- DNA was extracted using the Bio101 FastPrep DNA for soil kit w ith procedure modifications for humic soils
- Elutions were screened for methanotroph Type I and II bacteriawith PCR
  - Type I primer pair: MethT1bR, MethT1dF (Integrated DNA Technologies, Inc.)
  - Type I positive control: ATCC 33009 *Methylococcus capsulatus* (Bath)
  - Type II primer pair: MethT2R, 27F
  - Type II positive control: ATCC 49242 *Methylosinus trichosporium* (OB3b)
- Positive samples underwent a subsequent nested PCR in preparatio n for DGGE
  - Nested primer pair: GC358F, 517R
- PCR product quantification was determined with a 1% agarose gelstained with ethidium bromide and imaged on a Stratagene EagleEye imaging system.
- Denaturing Gradient Gel Electrophoresis was performed to separate different species within the methanotroph types
  - Methanotroph Type I run conditions: 6.5% polyacrylamide, denaturing gradient 20-70%, 130 volts, 4:30 hours, 60C
  - Methanotroph Type II run conditions: 6.5% polyacrylamide, denatu ring gradient 30-60%, 130 volts, 4:30 hours, 60C

Results and Discussion

The presence of methanotroph type I bacteria was indicated in soils "B" and "D." The Jaccard index shows that the two soil samples were more similar (42%) to each other than to the positive control (8%-20%.) Methanotroph type II bacteria were indicated in soils "A", "B", and "D." Among the type II bacteria, soils "B" and "D" had the greatest similarity at 71%. The "A" soil showed the least similarity to the positive control at 8% and only 11-12% similarity to the other two soil samples. Based on this information, a prediction might be made that microcosms with "B" and "D" soils would result in a similar degradation pattern and noticeably different from the "A" microcosms.

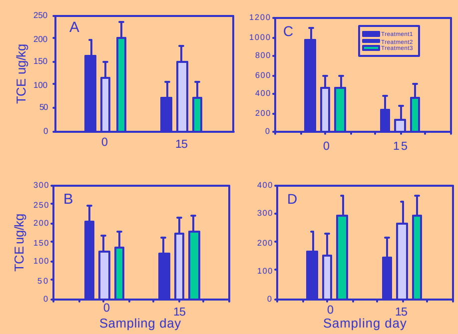
The GC/MS analysis of the microcosm samples revealed only 2 soil/treatment conditions that had significant TCE reduction. The third treatment (abiotic control) for soil "A" reduced TCE ($p=0.0114$.) This result was unexpected since the abiotic controls had been autoclaved to destroy microorganisms. In addition, each set of "A" microcosms revealed the presence of chloroform after 3 to 6 days of incubation. There were no MMO pathway degradation products identified in the samples. An abiotic pathway may be the explanation for this reduction in TCE concentration. Isolated measurements of chloroform also appeared in soil "B" treatment 2, day 6 and soil "D" treatment 2, day 9.

The first treatment (unamended soil) for soil "C" also indicated reduced TCE concentrations ($p=0.0034$.) However, no degradation products were measured and no methanotrophs were present in this soil.

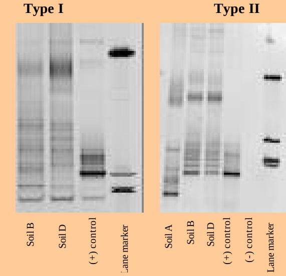
The "B" and "D" microcosms were similar in that they did not show significant amounts of TCE reduction and did not produce chloroform.

Monitored natural attenuation (MNA) is more cost effective and less environmentally disruptive than other methods of remediation. Bacteria have been found to be effective with low to moderate concentrations of contaminants. High or very low concentrations as well as NAPL form of contamination are not effectively degraded by MNA. Successful biodegradation of a chlorinated solvent depends on a complex combination of factors that may not be present at a given site, so MNA is best employed as a part of a comprehensive remediation plan.

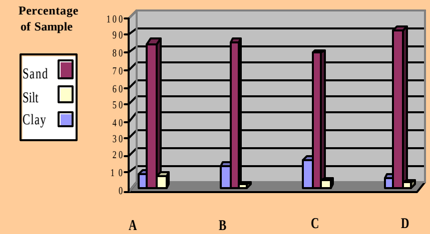
TCE concentration variations within microcosm and treatment



DGGE banding patterns for Type I and II Methanotrophs



Comparison of Soil Particle Size Between Sample Locations



Conclusions

The methanotroph community profiles indicated similarities and differences between the soils sampled. The most similar soils, "B" and "D", also demonstrated the most similar response to TCE. Whereas the more dissimilar soils, "A" and "C", showed TCE patterns differen t from each other and from "B" and "D."

The ability to predict the microbial response to TCE exposure will facilitate decision-making regarding contaminated sites and the most effective and cost-efficient remediation strategies to employ. Ongoing efforts to improve the microcosm procedures and accuracy of detecting degradation products may potentially refine the prediction process.

The University of Georgia

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