

# Environmental Management Science Program

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## **Control of Biologically Active Degradation Zones by Vertical Heterogeneity: Applications in Fractured Media**

Frederick (Rick) S. Colwell  
Idaho National Engineering and Environmental Laboratory  
P.O. Box 1625  
Idaho Falls, Idaho 83415-2203  
Phone: 208-526-0097  
E-mail: [fxc@inel.gov](mailto:fxc@inel.gov)

Robert Smith  
Idaho National Engineering and Environmental Laboratory  
P.O. Box 1625  
Idaho Falls, Idaho 83415-2203  
Phone: 208-526-9345  
E-mail: [rqs@inel.gov](mailto:rqs@inel.gov)

James P. McKinley  
Pacific Northwest National Laboratory  
P.O. Box 999  
Richland, Washington 99352  
Phone: 509-375-6841  
E-mail: [jp\\_mckinley@pnl.gov](mailto:jp_mckinley@pnl.gov)

James K. Fredrickson  
Pacific Northwest National Laboratory  
P.O. Box 999, MSIN P7-50  
Richland, Washington 99352  
Phone: 509-376-7063  
E-mail: [Jim.Fredrickson@pnl.gov](mailto:Jim.Fredrickson@pnl.gov)

T. C. Onstott  
Princeton University  
Princeton, New Jersey 08544  
Phone: 609-258-1622  
E-mail: [tullis@geo.princeton.edu](mailto:tullis@geo.princeton.edu)

Anna-Louise Reysenbach  
Rutgers University  
Lipmann Hall  
Cook College  
New Brunswick, New Jersey 08903-0231  
Phone: 732-932-9763 ext 333  
E-mail: [alr@imcs.rutgers.edu](mailto:alr@imcs.rutgers.edu)

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Frederick (Rick) S. Colwell, Idaho National Engineering and Environmental Laboratory

Robert Smith, Idaho National Engineering and Environmental Laboratory

James P. McKinley, Pacific Northwest National Laboratory

James K. Fredrickson, Pacific Northwest National Laboratory

T. C. Onstott, Princeton University

Anna-Louise Reysenbach, Rutgers University

### **Research Objective**

The objective of this research is to determine the relationship between of biologically active contaminant degradation zones in a fractured, subsurface medium and vertical geological heterogeneities. The research is being performed on samples collected from the Test Area North (TAN) site at the Idaho National Engineering and Environmental Laboratory (INEEL) where a dissolved trichloroethylene (TCE) plume is migrating in the basalts and interbed sediments of the Eastern Snake River Plain (ESRP) aquifer. Results are leading to an enhanced understanding of the constraints placed on the activities and distribution of TCE-degrading organisms by the geochemical and hydrological environment. This understanding allows better decisions to be made regarding the use of remedial technologies such as natural attenuation and in-situ bioremediation at geologically complex waste sites. Through this research, investigations conducted by the DOE Subsurface Science Program at TAN have been extended in order to develop a mechanistic understanding of the coupled geomicrobial and hydrogeochemical processes that are necessary to predict field-scale intrinsic degradation rates of TCE. The research objective is being accomplished by characterizing paired cores and water samples from boreholes located in differing geochemical and flow environments within the plume. Analysis of these samples will allow the determination of the spatial correlation between microbial degradation and preferred flow paths for the contaminant and required electron donors and acceptors. A combination of traditional microbiological methods (e.g., enrichments) and molecular tools are being used to characterize the indigenous microbial communities.

### **Research Progress and Implications**

This report summarizes work conducted after 1.5 years of a three year project. As of May 1998, subsurface basalt and sediment cores had been collected for microbiological and chemical analyses from the saturated zone in three coreholes at TAN. Core materials were acquired from ca. 67 m below land surface (BLS) to 134 m BLS. One corehole (TAN-37) is located proximal (40 m) to the injection well and is in a zone that is heavily influenced by the waste (2000-3000 ppb dissolved TCE) while a second hole, TAN-33, is located more distally (305 m) where dissolved TCE is ca. 900 ppb. The third corehole, TAN-48, is even more distant (600 m) where the dissolved TCE is ca. 100 ppb. The aquifer consists of multiple basalt flows where dense fracture networks conduct the groundwater. At the upper and lower end of the range cored are two sedimentary interbeds. Microbiological analyses conducted on paired core samples include biomass and community structure by phospholipid fatty acid (PLFA) analyses; cultural enumerations of methanotrophs, propanotrophs, phenol-oxidizers, ammonia oxidizers, iron reducers, sulfate reducers, methanogens and fermenters; community-level physiological profiles (CLPP); 16s rRNA sequencing of polymerase chain reaction (PCR) products, denaturing gradient gel electrophoresis (DGGE) analysis of extracted DNA and acetate mineralization. Data from intentionally introduced tracers (microspheres and perfluorocarbons)

indicated several orders of magnitude reduction in potential contaminants was achieved by paring the cores. Comparison of the both whole communities and isolates from the core and drilling fluid corroborated the defensibility of the cores.

TCE concentrations in cores samples from TAN-33 were consistently low ( $< 6$  ppb) whereas higher values were detected in samples from TAN-37 (as high as 25 ppb). That these values for TCE in basalt are so low relative to the concentrations of TCE evident in groundwater at these locations is corroborated by evidence of low adsorption of TCE on basalt (Ingram et al. Surface Chemistry of Subsurface Basalt; OBER; Wolf-Broido Initiative). The TCE concentrations in TAN-33 cores peaked at 103 m; just above a zone of pervasive fractures that correspond to a perceived interflow zone. In TAN-37, the highest TCE values in the cores occurred at depths between 68 and 79 m, near the top of the aquifer at this location. These data are consistent with cross-hole seismic tomography conducted in TAN-37 and neighboring wells which suggest primary flowpaths may exist at ca. 74 m (see EMSP progress report submitted by E Majer entitled: "Subsurface High Resolution Definition of Subsurface Heterogeneity for Understanding the Biodynamics of Natural Field Systems: Advancing the Ability for Scaling to Field Conditions").

Microbial biomass in the basalt from the corehole at intermediate distance from the injection well (TAN-33) was at the limit of detection for most assays; minimal acetate mineralization was detected for select samples. Aerobic heterotrophs were cultured on oligotrophic liquid media (0 to  $10E4$  cells/g) and phenol-oxidizers ( $< 10E2$  cells/g), methanotrophs ( $< 20$  cells/g), and dissimilatory iron reducing bacteria were present in some core samples. On the other hand, basalt from TAN-37, proximal to the injection well, showed measurable biomass by PLFA (ca. 3 pmol/g) and much higher levels of acetate mineralization. Higher numbers of the different physiological types of microorganisms were enriched in all samples from TAN-37 relative to those from TAN-33. The following order of prevalence was discernible in enumerations of specific microbial types in cores from TAN-37: phenol oxidizers ( $< 10E5$  cells/g)  $>$  propanotrophs ( $< 10E4$  cells/g)  $\gg$  methanotrophs ( $< 10E2$  cells/g)  $\gg$  nitrifiers. Furthermore, evidence from TAN-37 cores suggests that samples from interflow zones (strata that represent the boundary of two adjacent basalt flows) may contain higher numbers of microorganisms than samples obtained from within a single basalt flow. If true, this is consistent with the hypothesis that the interflow zones which are highly fractured and contain basalt rubble are the preferred flow paths for contaminants and that these zones also support a more robust microbial community. Comparison of the data from TAN-33 and TAN-37 indicate that bacteria associated with basalt may be present in low numbers in pristine areas of the aquifer while a substantial stimulation of similar types of organisms may result from organic contamination.

Microbial DNA was extracted from selected TAN-33 and TAN-37 samples and amplified using PCR with eubacterial and archaeal primers. TAN-33 samples did not yield visible PCR bands but when spiked with as few as  $10E4$  cells/g samples PCR bands were apparent. Invisible PCR bands from two of the TAN-33 samples along with a full procedural blank (processed without basalt) were cloned and a subset of the clones was submitted to Amplified Ribosomal DNA Restriction Analysis (ARDRA). Six ARDRA types (all eubacterial) were identified from the 113.3 m sample and three ARDRA types (all eubacterial) were identified from the 125.4 m sample. Basic Local Alignment Search Tool (BLAST) was used to compare the sequences of these eubacterial clones to that of known microorganisms. Most of the sequences that resulted from these clones were approximately 90% similar to known eubacterial sequences. Extractions from selected TAN-37 samples indicate much higher levels of DNA than in TAN-33 samples. Eubacterial PCR bands were recovered from all of the samples that were amplified except the combusted basalt control sample. Only one of the ten samples yielded an archaea PCR band. Cloning and sequencing of these PCR bands indicate a diversity of microorganisms in the samples from the top of the aquifer in TAN-37 including types that are common in soil environments (e.g., *Acinetobacter*, *Pseudomonas*, and actinomycetes).

Preliminary determination of microbial diversity in TAN-37 samples from 63.5 m (basalt; top of the aquifer) and 125.7 m (sediment interbed; below of contaminated strata) were obtained using DGGE. The diversity profiles were different for the two samples, although they did share three common banding patterns. Both samples had about eight different bands, representative of eight putative different 16S rDNA fragments. Initial recovery and sequencing of one of the dominant bands common to both samples revealed that this product was most closely related to members of

Acinetobacter. This genus is consistent with the results obtained from the clone library developed for TAN-37 samples that came from 63.5 to 78.8 m depths. Most Acinetobacter spp. are nutritionally diverse and common inhabitants of soils.

This EMSP research will help address EM-40 needs in the cleanup of the waste plume in the groundwater at TAN by: 1) determining the specific vertical location of contaminants in the ESRP aquifer, 2) establishing the presence and distribution of naturally occurring microbial communities that are capable of contaminant degradation, and 3) determining the abiotic conditions (chemical and physical) under which these microorganisms are active in the degradation of the TCE. Ultimately, by acquiring data to allow estimates of the natural rates of waste remediation in the aquifer at TAN, this EMSP research will assist EM-40 and regulatory agencies that have oversight of the cleanup activities, in determining where aggressive remediation must be conducted and where it is likely that natural attenuation of the contaminants will occur.

### **Planned Activities**

Multi-level sampler (MLS) for discrete static sampling of aquifer microbiological and chemical characteristics will be retrieved from TAN-33 (Jun 98).

Initiate MLS study in TAN-37 (Aug 98).

Complete microbiological and chemical analyses on samples from TAN-48 (most distal corehole) (Sep 98).

Complete microbiological and chemical analyses of MLS samples from TAN-33 (Oct 98).

MLS retrieved from TAN-37 (Apr 99).

TAN-33 MLS data analysis completed allowing: 1) comparison of MLS data to that obtained from cores at the same vertical location in the borehole, 2) estimation of importance of attached vs. unattached microbial communities in the context of contaminant degradation, and 3) verification of distinctive microbial communities according to geohydrological environment (May 99).

Complete microbiological and chemical analyses of MLS samples from TAN-37 (Aug 99).

Deduction of the natural rates of waste remediation in the aquifer at TAN (Sep 99).