Control of Biologically Active Degradation Zones By Vertical Heterogeneity: Applications In Fractured Media

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ABSTRACT

The objective of this research is to determine the relationship between active biological degradation zones and vertical heterogeneities in a fractured basalt aquifer. The study site is a trichloroethylene (TCE) plume at Test Area North (TAN) site at the Idaho National Engineering and Environmental Laboratory (INEEL). The research is being conducted by studying both subsurface cores and water and solids from a multi-level sampler (MLS) incubated in the same borehole. Microbiological analyses on the samples include traditional methods (e.g., enrichments) and molecular tools to determine the key communities which may be degrading TCE. Chemical measurements focus on species that are important in defining where the appropriate microbial communities are most active. The goal is an enhanced understanding of the constraints that the geochemical and hydrological environment places on the activities and distribution of TCE-degrading organisms. This understanding will aid selection of remedial technologies (e.g., natural attenuation, in situ bioremediation) at geologically complex waste sites and assist in conceptualizing the coupled geomicrobial and hydrogeochemical processes that are necessary to predict field-scale intrinsic degradation rates of TCE.

INTRODUCTION

Microbial ecology research has led to an understanding of the controls on microbial activity in the subsurface. Contrasts in permeability, organic matter content, and grain size can determine where microorganisms exist and their level of activity. For the TAN site, we hypothesized that hydrogeological properties (e.g., permeability) determine the size, diversity, and activities of microbial communities in fractured basalt by controlling the flux of aqueous constituents upon which these communities depend. Microorganisms would be most abundant, of highest diversity and of highest metabolic activity in high permeability zones (between basalt flow units).

TAN-37

Borehole characteristics
- 40 m from injection well, 2000-3000 ppb dissolved TCE

Microbiology
- Measurable biomass by PLFA (ca. 3 pmol/g)
- Higher levels of acetate mineralization; anaerobic activities > aerobic activities (low DO)
- Higher culturable biomass of key physiological groups: phenol oxidizers (< 10E5 cells/g) > propanotrophs (< 10E4 cells/g) >> methanotrophs (< 10E2 cells/g) >>> nitrifiers
- Interflow zones may contain higher numbers of microorganisms than within a basalt flow
- Extracted and amplified (eubacterial and archaeal primers) microbial DNA yielded higher levels of DNA than TAN-33; eubacterial PCR bands from all samples amplified (except control); 1 of 10 samples yielded an archaeal PCR band
- Cloning, sequencing of PCR bands indicate diverse microorganisms at the top of the aquifer including common soil types (Acinetobacter, Pseudomonas, and actinomycetes)
- DGGE microbial diversity profiles for 63.5 m (basalt; top of the aquifer) and 125.7 m (sediment interbed; below contaminated strata) were distinct, but shared 3 common banding patterns; both samples with 8 different 16S rDNA fragments; sequencing of one dominant band in both samples showed relationship to Acinetobacter (clone library developed for samples from 63.5 to 78.8 m is consistent). Acinetobacter spp. are nutritionally diverse and common in soils

Chemistry
- TCE in core up to 25 nmol/kg, highest at 68 to 79 m (top of aquifer); low TCE in basalt vs. groundwater, consistent with low TCE adsorption to basalt (see Ingram poster); consistent with cross-hole seismic tomography suggesting flowpaths at ca. 74 m (see E Majer poster)

Sample QA/QC
- Paired cores have reduced tracer concentrations; potential contaminants minimized
- Microbial analysis suggest cores and drilling fluids have distinct communities

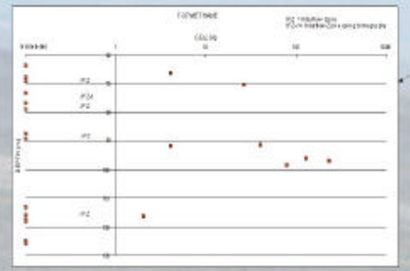
TAN-33

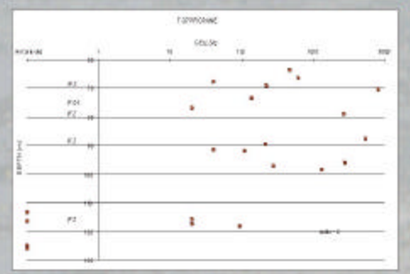
Borehole characteristics
- 305 m from injection well, 900 ppb dissolved TCE

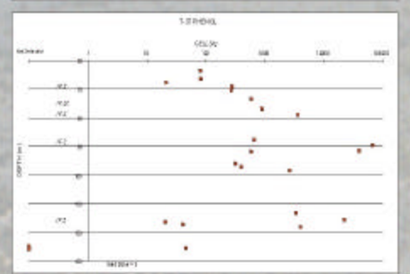
Microbiology
- Microbial biomass in basalt at the limit of detection
- Minimal acetate mineralization detected for selected samples
- Aerobic oligotrophic heterotrophs (0 to 10E4 cells/g); phenol-oxidizers (< 10E2 cells/g); methanotrophs (< 20 cells/g); DIRBs in some cores
- Extracted, amplified DNA yielded no visible PCR bands; invisible bands from 113.3 and 125.4 m were sequenced and were ca. 90% similar to eubacterial sequences

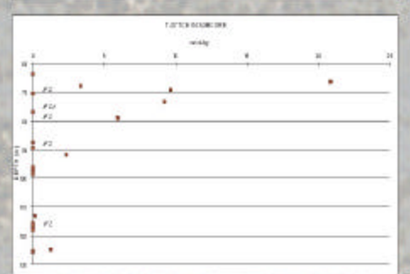
Chemistry
- TCE in core consistently low (< 6 ppb); peak at 103 m, just above fractures/interflow zone

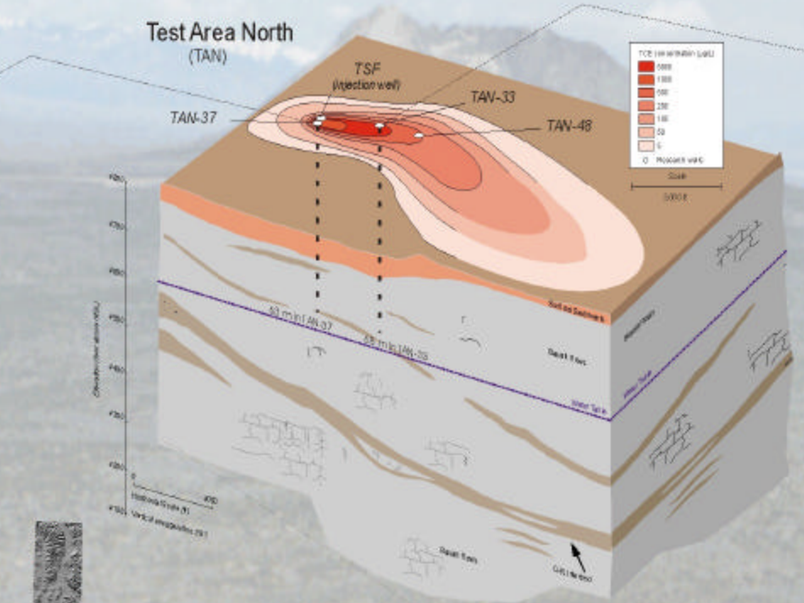
MLS
- Dissolved gases, TCE and culturable microorganisms suggest few differences throughout the sampled interval though it ranges across massive basalt and interflow zones
- Stable isotope ratios for carbon decrease at ca. 103 m; corresponds to ca. 10% rise in DIC
- Numbers and diversity of communities in MLS basalts are higher than in the native core

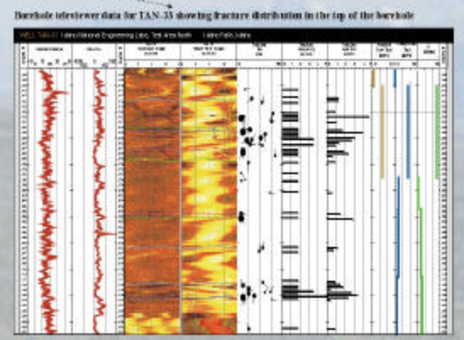


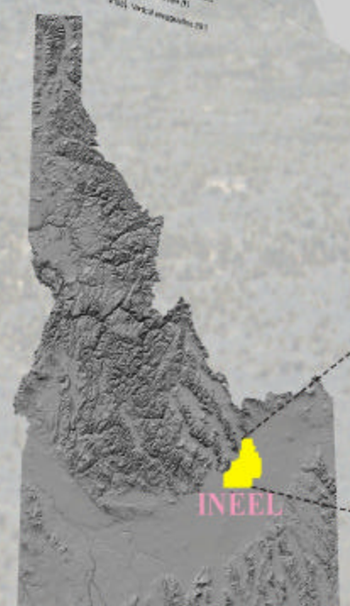












CONCLUSIONS

- Bacteria associated with basalt may be present in low numbers in relatively pristine areas (TAN-33) while a substantial stimulation of similar types of organisms may result from organic contamination (TAN-37).
- In TAN-37, fractured interflow zones are the preferred flow paths for contaminants and appear to support a more robust microbial community.
- Low gradient at TAN-33 suggests that permeability differences alone are not enough to control microbial community distribution, activities. Proximity of TAN-37 to active pumping related to cleanup imposes a forced gradient upon the basalts and accentuates permeability differences.
- Microbial communities that colonize the MLS appear to be distinctive from those present in fresh core.

SUPPORT OF EM-40 CLEANUP NEEDS

- Determine specific vertical location of contaminants in the aquifer
- Establish presence and distribution of microbial communities capable of contaminant degradation
- Determine abiotic conditions under which microorganisms degrade TCE

PLANNED ACTIVITIES

- Complete microbiological and chemical analyses on cores (TAN-48) and MLS (TAN-33)
- MLS studies in TAN-37; compare MLS data to core data from same borehole location
- Determine importance of attached vs. unattached communities in the context of contaminant degradation o Verify distinctive microbial communities according to geohydrological environment
- Deduce natural rates of waste remediation in the aquifer

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