

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

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ABSTRACT

The microbial ecology of a basalt aquifer at Test Area North (TAN), Idaho National Engineering and Environmental Laboratory (INEEL) was characterized along two transects with respect to a contaminant plume: longitudinally along the plume axis and vertically within three coreholes. The objective of this research is to determine the relationship between biologically active contaminant degradation zones in a fractured, subsurface medium and vertical geological heterogeneities. The source of the plume was an injection well which originally contained sewage, chlorinated hydrocarbons, and radionuclides. The aquifer consists of multiple basalt flows where dense fracture networks conduct the groundwater. Core was collected for microbiological analyses from the saturated zone in the coreholes from ca. 220' to 440' below land surface. One corehole (TAN-37) was located proximal (ca. 30 m) to the injection well and is influenced heavily by the waste; the second hole (TAN-33) was located more distally (300 m) where the major contaminant is thought to be dissolved trichloroethylene (TCE) (ca. 500 ppb); and, the third corehole (TAN-48) was located yet further (ca. 1 km) from the source with lower concentrations of TCE (< 200 ppb). Microbial analyses of cores included 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 fermentors; sequencing of polymerase chain reaction (PCR) products derived from 16S rDNA extracted from the samples and acetate mineralization. Polymerase chain reaction-most probable number (PCR-MPN) analyses for methanotrophs were performed on selected groundwater samples to corroborate culture-based determinations of these microorganisms. Data from intentionally introduced tracers (microspheres and perfluorocarbons) and indigenous microbial tracers indicated several orders of magnitude reduction in potential contaminants was achieved by paring the core. Microbial biomass in the basalt from the distal two coreholes (TAN-33 and 48) was at the limit of detection for most assays; minimal acetate mineralization was detected for selected samples. On the other hand, basalt from near the injection well (TAN-37) showed low but measurable biomass by PLFA (ca. 3 pmol/g), much higher levels of acetate mineralization and positive enrichments for nearly all physiological types of microorganisms in all samples. These data were also corroborated by molecular determinations of the microorganisms in the cores which indicated that TAN-37 had much higher levels of amplifiable DNA than TAN-33. Cloning and sequencing of TAN-37 PCR bands indicated 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). Estimates of numbers of free-living methanotrophs as derived independently from PCR-MPN and culture-based methods were comparable within a given well and highest in TAN-37. In TAN-37, considerable vertical variability existed in the microbiology of the cores. Correlations between vertical distributions of hydraulic conductivity and biological and geochemical characteristics were performed. Vertically-averaged data from the longitudinal transect indicate that bacteria associated with basalt may be present in low numbers in pristine areas of the aquifer while a substantial stimulation of similar organisms may result from organic contamination. The variability in microbiological parameters along a vertical axis within a single corehole can rival that seen on a longitudinal gradient although the predictability of this vertical variation is more elusive. The inability to predict vertical variation of microbial characteristics may be related to the lack of a significant hydraulic gradient at the TAN site which may be a more significant factor in determining microbial distribution than differences in permeability.

INTRODUCTION

Subsurface microbiology 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 with an extended TCE plume, 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). To evaluate this hypothesis we characterized microbial communities in three vertical sections along the axis of the plume.

RESULTS:

TAN-37 (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 (< 10⁶ cells/g) > propanotrophs (< 10⁶ cells/g) >> methanotrophs (< 10⁶ cells/g) >> nitrifiers
- 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); *Acinetobacter* were also identified from DGGE profiles from 63.5 m (basalt; top of the aquifer)

Chemistry:

- TCE in core up to 25 ppb; 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)
- Recently deposited calcite is consistent with anaerobic conditions which allowed co-precipitation of Mn

TAN-33 (305 m from injection well, 900 ppb dissolved TCE)

Microbiology:

- Microbial biomass at the limit of detection
- Minimal acetate mineralization detected for selected samples
- Aerobic oligotrophic heterotrophs (0 to 10⁶ cells/g); phenol-oxidizers (< 10⁶ 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
- Multi-level sampler studies of dissolved gases, TCE, and culturable microorganisms suggest few differences through an interval that transitions interflow zones

TAN-48 (1000 m from injection well, <200 ppb dissolved TCE)

Microbiology:

- Microbial biomass at the limit of detection
- Minimal acetate mineralization detected

Chemistry:

- TCE in core consistently low (< 6 ppb); peak at 103 m, just above fractures/interflow zone
- Near background Snake River Plain aquifer chemistries

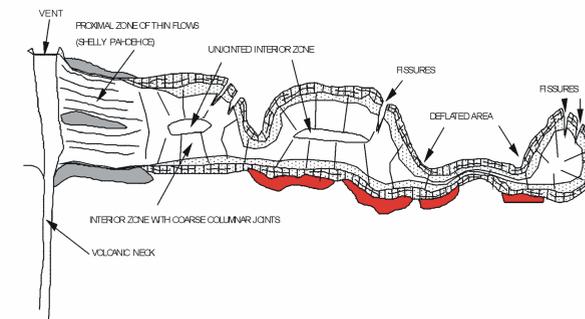


Figure 1. Typical structure of a basalt flow in the Eastern Snake River Plain with vertical exaggeration (modified from Knutson et al. 1990).

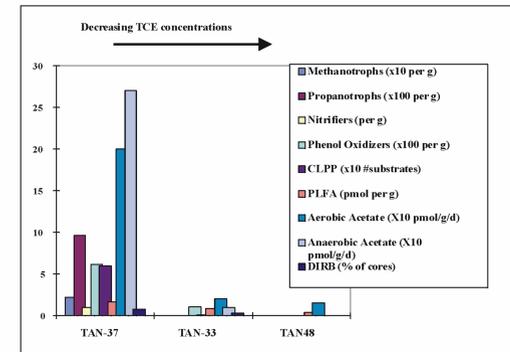
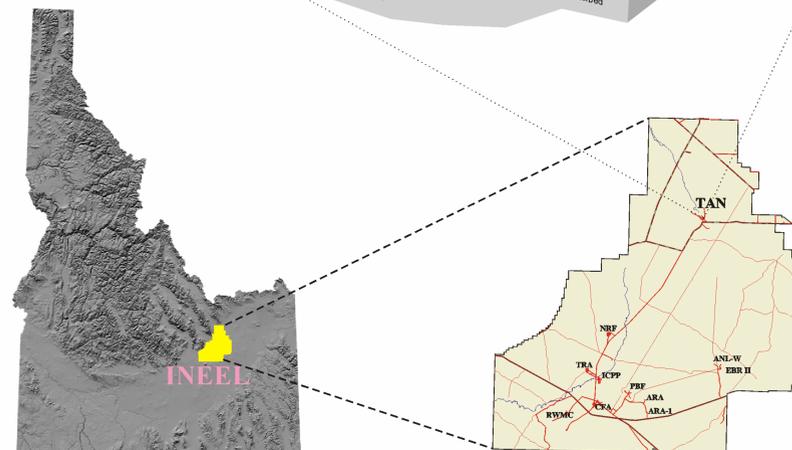
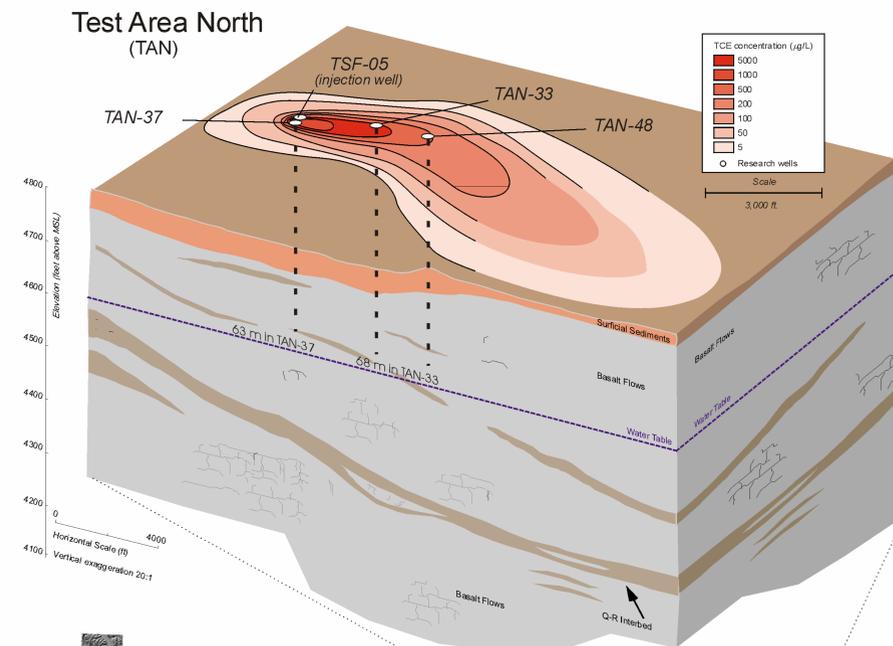


Figure 2. Mean values for microbiological properties measured in core materials collected along the horizontal transect through the TAN TCE plume.



Correlation with aq. [TCE]		Coefficient of Variation	
methanotrophs	0.998	methanotrophs	173%
propanotrophs	0.998	propanotrophs	173%
nitrifiers	0.998	nitrifiers	173%
phenol oxidizers	0.999	phenol oxidizers	169%
CLPP	0.998	CLPP	169%
PLFA biomass	0.936	PLFA biomass	62%
aerobic acetate	0.999	aerobic acetate	134%
anaerobic acetate	0.999	anaerobic acetate	164%
DIRB	0.965	DIRB	115%

Table 1. Large scale horizontal variations and correlations for microbiological factors measured in TAN core samples. The horizontal factor used is aqueous TCE concentration.

Correlations with:		
	Flow Zones	[TCE], rock
PLFA	0.438	-0.080
CLPP	-0.182	0.105
phenol oxidizers	-0.405	0.477
methanotrophs	0.359	0.205
aerobic acetate	0.289	-0.155
anaerobic acetate	0.113	-0.208

Coefficients of Variation	
PLFA	100%
CLPP	194%
phenol oxidizers	79%
methanotrophs	177%
aerobic acetate	99%
anaerobic acetate	124%

Table 2. Large scale vertical variations and correlations for microbiological factors measured in TAN core samples. The vertical factors used are hydraulic flow zones and TCE concentration in basalt.

Coefficient of Variation		Correlation with Flow Zones	
PLFA	55%	DEPTH	-0.383
Propane	206%	PLFA	-0.009
Methane	247%	Propane	0.445
Phenol	251%	Methane	0.263
NH3	480%	Phenol	0.181
CLPP	32%	NH3	-0.187
aerobic Ac	19%	CLPP	0.410
anaerobic Ac	49%	aerobic Ac	0.045
CFU	280%	anaerobic Ac	0.438
		CFU	-0.169

Table 3. Large scale vertical variations and correlations for microbiological factors measured in TAN-37 core samples. The vertical factor used is hydraulic flow zones.

CONCLUSIONS:

- Large multidimensional variability in distribution of bacteria in fractured rock
- Horizontal variation associates with contaminants; bacteria present in low numbers in more pristine areas (TAN-48 and TAN-33), in higher numbers where past organic contamination occurred (TAN-37)
- Vertical variation not easy to explain; interflow zones are preferred flow paths for contaminants and may support a more robust microbial community; permeability differences are accentuated where hydraulic gradient is high (TAN-37)

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

FUTURE:

- Determine importance of attached vs. unattached communities in the context of contaminant degradation
- Determine which microbial communities are responsible for natural attenuation of TCE in the dissolved plume and the rates at which they degrade the TCE under oligotrophic conditions

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