Coupled Biogeochemical Processes for Evaluating TCE Cometabolism

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Abstract

Two tasks within a larger project investigating cometabolism of trichloroethene (TCE) in aerobic groundwater have the objectives of detecting and quantifying cometabolic activity by assaying the responsible enzymes, and simulating TCE transport in groundwater using a realistic description of cometabolic kinetics. Here we focus on progress in the enzyme activity assessment. Chlorinated solvent plumes occur in complex geologic settings at numerous government and private sites across the country. One such site is Test Area North (TAN) at the Idaho National Lab, where a plume of groundwater contaminated with TCE extends about 3 km from a former waste disposal well and lies at a depth of about 60 to 120 m below ground surface in a fractured rock aquifer (Figure 1). Aerobic conditions in most of the plume inhibit anaerobic reductive dechlorination, but previous studies indicate that cometabolic degradation of TCE occurs at an environmentally significant rate. The goal of our study is to evaluate the activity of cometabolic organisms in this plume using multiple innovative technologies. Cometabolism by organisms that have either the soluble methane monooxygenase (sMMO) or toluene oxygenase enzyme was evaluated using a suite of enzyme activity probes (Figure 2), which indicate if a targeted enzyme is not only present but active in a microorganism. The number of cells likely to have cometabolic capability was measured using fluorescent in situ hybridization (FISH) and gene expression.

Native planktonic microorganisms from the Snake River Plain Aquifer (SRPA) were sampled from multiple wells in the medial zone of the TAN plume (Figure 1), where TCE concentrations currently range from 100-300 mg L^{-1}. Total cell abundance was similar at all wells sampled, $2.41 \pm 1.2 \times 10^6$ cells per mL of groundwater. Polymerase chain reaction (PCR) for the mmoX of the sMMO gene and each of the toluene oxygenase genes resulted in positive amplifications in every groundwater sample assayed, clearly indicating that the indigenous microbial community has the genetic potential to produce these enzymes. sMMO enzyme activity was variable, but the enzyme activity probe showed a significant response relative to controls at all wells evaluated. Toluene oxygenase activity determined by enzyme probes was also variable, with 5-20% of the total cells in any given groundwater sample having a positive response. FISH results for the sMMO enzyme supported the enzyme probe results, with higher percentages of active cells detected using FISH corresponding with higher enzyme activity probe results. These data provide direct evidence of the genetic potential for TCE cometabolism and that significant aerobic cometabolic activity is occurring in the medial zone of the TAN TCE plume. The results of these analyses suggest that MNA could be a viable remedial strategy for the medial zone. Future work will include characterization of attached microorganisms that colonized solid substrates in a medial zone well, and incorporating cometabolic kinetics into a flow and transport model for this plume.

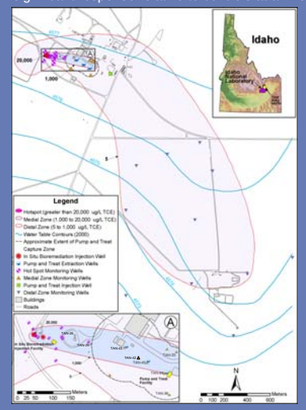

Figure 1. The TCE plume is divided into 3 zones based on historical TCE concentrations; hotspot (>20,000 ug/L); medial zone (1,000-20,000 ug/L); and distal zone (<1,000 ug/L). Wells sampled within the medial zone of the plume are labeled on the figure inset.

Enzyme Activity Probes

Enzyme Activity Probe Preliminary Analysis in Three Medial Zone Wells: January 2006

- To test the potential for direct evidence of cometabolic activity, enzyme activity probes (Figure 2) were applied to samples collected from 3 medial zone wells (TAN-43, TAN-33, and TAN-36; Figure 1) in January 2006.
- Total cell abundance was $2.41 \pm 1.2 \times 10^6$ cells per mL of groundwater.
- sMMO activity was present in all 3 wells (the respective wells were 33, 28, 35 intensity/min).
- Toluene activity was tested only in groundwater from TAN-43, and positive evidence was present (3HPA=11.23 cells/mL; PA=19.21 cells/mL; CINN=5.00 cells/mL).

Enzyme Activity Probe Analysis in Eight Medial Zone Wells: June 2006

- Total biomass in groundwater within the medial zone was not significantly different ($p<0.05$; Table 1).
- Enzyme activity detected using sMMO decreased with distance from the hot spot of the plume, except TAN-42.
- Enzyme activity detected using the toluene probes was variable with at least one probe showing activity in all of the wells. Total activity ranged from 5 to 25% of the total cells with the highest toluene oxygenase activity at the downgradient edge of the medial zone (TAN-44 and TAN-33). PA showed significantly higher activity throughout the medial zone, when compared to both 3HPA and CINN.

Figure 3. Example micrographs following application of enzyme activity probes to TAN groundwater. **A:** Micrograph of total cells DAPI-stained. **B: Positive Response-** Transformation of non-fluorescent probes into a strongly fluorescent product if the appropriate enzyme is present and active. **C: Negative Response-** No fluorescent transformation if the appropriate enzyme is not present or is present but not active.

Molecular Technology	Implications
Enzyme Activity Probe	Direct measurement of enzyme activity.
PCR (polymerase chain reaction)	Measurement of potential; i.e. detection of DNA, gene for toluene or methane oxygenase.
FISH (fluorescent in situ hybridization)	Measurement of the number of cells likely to have cometabolic activity.

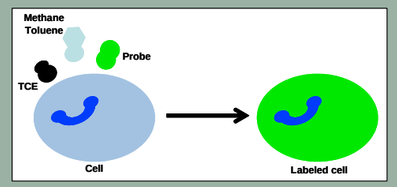

Figure 2. Enzyme activity probes are substrate surrogates that are transformed by the enzyme of interest into colored or fluorescent products, when the enzyme is active. This technology takes advantage of the substrate non-specificity of the degradative enzymes.

Table 1. Enzyme probe analysis in eight medial zone wells, June 2006.

Well Location	DAPI (cells/mL)	sMMO probe Coumarin (intensity/min)	Toluene probes (% positive cells)			
			3HPA	PA	Cinn	Total
TAN-28	6.07E+05	37.72	0.56	6.12	1.35	8.03
TAN-29	5.68E+05	37.89	4.75	7.90	0.72	13.37
TAN-41	4.86E+05	32.67	0.69	10.21	3.44	14.34
TAN-42	7.79E+05	27.38	1.03	6.15	1.07	8.25
TAN-43	1.30E+06	32.49	0.11	4.30	0.88	5.29
TAN-44	6.53E+05	31.16	6.66	12.33	0.43	19.42
TAN-33	4.03E+05	24.25	10.54	10.14	4.49	25.17
TAN-36	4.75E+05	18.10	6.88	7.05	0.89	14.82

DAPI = 4,6-diamindino-phenylindole; sMMO = soluble methane monooxygenase; 3HPA = 3 hydroxy-phenylacetylene; PA = phenylacetylene; CINN = trans-cinnamonitrile

Medial Zone Enzyme Activity Probe Results

- Significant aerobic cometabolic activity is occurring in the medial zone of the TAN plume.
- The sMMO oxygenase pathway appears to be the dominant biological pathway in the medial zone, since methane is more abundant (> 100 ug/L) and oxygen is somewhat limited (<1 mg/L).
- In the distal zone and aerobic fringe regions of the medial zone (where TCE is <250 ug/L), other oxygenase pathways (such as toluene) appear to be the dominant biological pathway, since groundwater becomes saturated with oxygen and methane is limited (<100 ug/L).
- It appears that from TAN-28 to the distal edge of the plume, aerobic cometabolic degradation of TCE is an active component of the biological community present in the groundwater system.

PCR

An example PCR gel providing supportive indirect evidence that the genes are also present and detectable using DNA technology.

Lane	Sample
1	TAN-28
2	TAN-29
3	TAN-33
4	TAN-36
5	TAN-41
6	TAN-42
7	TAN-43
8	TAN-44
9	Negative control
10	Positive control
11	Positive control
12	100 bp ladder

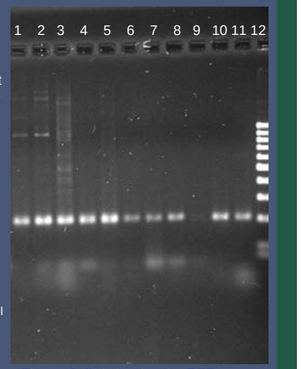

Figure 4. Gel showing the PCR-amplified fragments (206 bp) produced when using the PHE primers on DNA extracted from TAN groundwater.

FISH

FISH provides a secondary measure of activity based on either (a) expression of mRNA or (b) 16S rDNA and can provide evidence in support of enzyme probes. When applied simultaneously, these technologies provide information about a range of activity states for the cells of interest. Activity is a relative term and is based on the metric evaluated. The use of both techniques provides a more comprehensive and complete look at the activity and, in particular, the sMMO enzyme in situ.

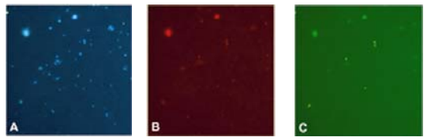

Figure 5. Micrographs of groundwater taken from a TAN well stained with (A) DAPI, (B) Cy3, and (C) FAM-labeled methanotrophic oligonucleotide probes. B represents hybridization with a universal methanotrophic probe, while C shows a fraction of the same cells hybridizing with a sMMO-specific probe.

Geochemistry

Table 2. Concentrations detected in samples collected from TAN medial zone wells, June 2006.

Well Location	TCE (ug/L)	Methane (ug/L)	Dissolved Oxygen (mg/L)	pH
TAN-28	1061	2791	0.17	7.01
TAN-29	364	372	0.33	7.03
TAN-41	259	1624	0.95	7.01
TAN-42	365	320	8.13	7.04
TAN-43	213	175	6.98	7.03
TAN-44	234	110	6.91	7.15
TAN-33	237	134	12.03	7.25
TAN-36	175	121	9.32	7.18

- TCE concentrations in June 2006 (Table 2) and variability in concentrations over time (Figure 6) decrease with distance from the hot spot of the plume.
- Methane concentrations decrease with distance from the hot spot of the plume.
- Dissolved oxygen increases with distance from the hot spot of the plume. The plume is anaerobic from the hot spot downgradient to around TAN-41. The remainder of the medial and distal zone is aerobic.
- No change in pH, in relation to distance, is observed.

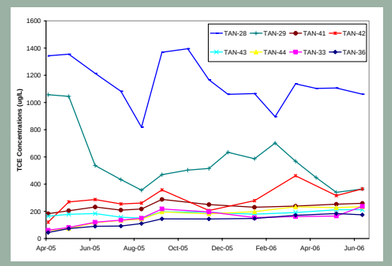

Figure 6. TCE concentrations over time at TAN medial zone wells.

Accomplishments

- **Enzyme activity probe** analysis results showed the presence and activity of organisms responsible for in situ aerobic cometabolic activity.
- **PCR and FISH** support the enzyme activity results and showed a significant number of cells likely to have cometabolic capability.
- Overall, the data provide **direct evidence** of the genetic potential for TCE cometabolism and that significant aerobic cometabolic activity is occurring in the medial zone of the TAN TCE plume.

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Current & Future Work

- Currently, basalt chips are incubating, for attachment of indigenous microbial cells, in two TAN wells. One well is located in the medial zone and the other is located outside of the TCE plume (March 2006 through September 2006).
- In October 2006, the substrate from these columns will be used as inoculums for in situ reactors to be constructed and deployed in these same wells for in situ evaluations.
- These studies and future work on this project will provide a better understanding of the biological and geochemical in situ processes, especially natural attenuation of contaminants.
- Data will be used to determine the long-term fate of contaminants in the subsurface (absorption vs. degradation vs. dilution).