Coupled Biogeochemical Process Evaluation for Conceptualizing Trichloroethylene Co-Metabolism

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North Wind, Inc.

ERSP
Fall PI Meeting

October 2006

Overview of a Multi-Disciplinary, Multi-Institutional Project

College of Oceanic & Atmospheric Sciences, Oregon State University	Overall PI and project management
Idaho National Laboratory	Microbiology, field techniques
University of Idaho	Microbiology
Berkeley Lab	Stable Isotope Geochemistry
North Wind	Hydrogeology, modeling, microbiology
CH2M•WG Idaho, LLC	Access and field support

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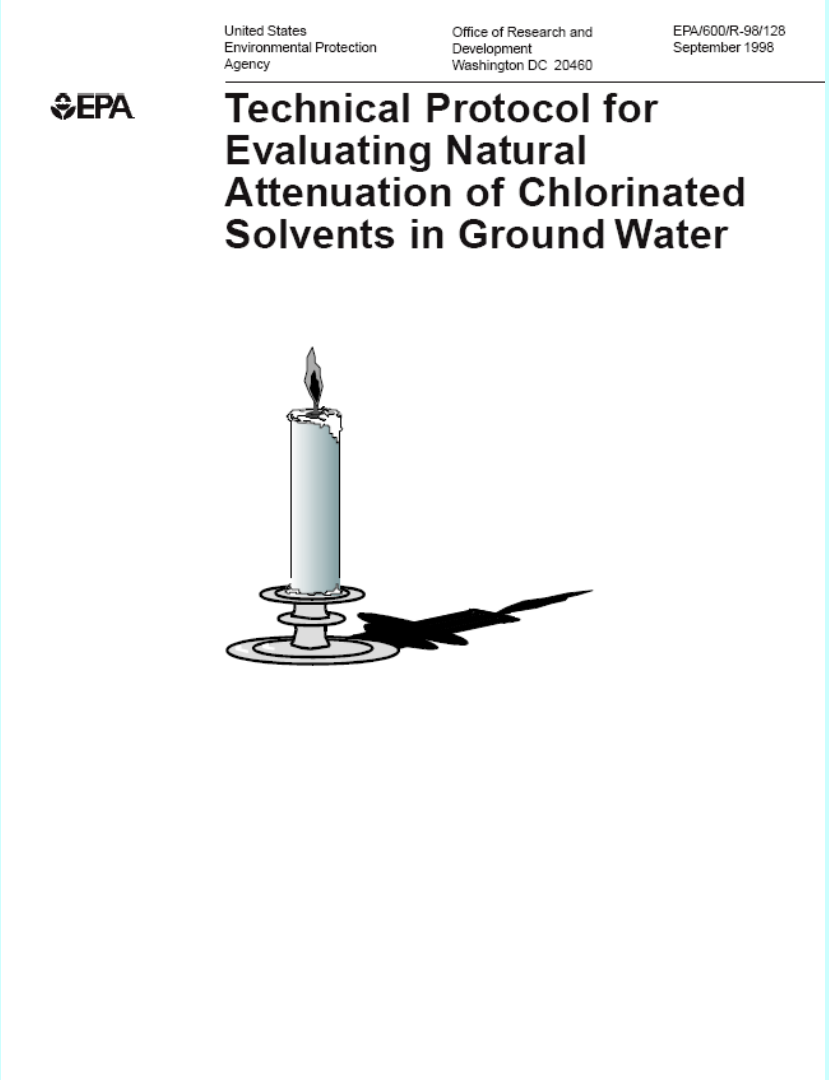
Background

- Monitored Natural Attenuation (MNA) - attenuation without active human intervention other than monitoring
- MNA is often less expensive than active remedies for contaminated groundwater
- EPA MNA protocol for chlorinated solvents is based on the paradigm that chloroethenes degrade under anaerobic, but not aerobic conditions, hence the presence of aerobic conditions often eliminates MNA from consideration as a remedy



United States Environmental Protection Agency

Office of Research and Development Washington DC 20460

EPA/600/R-98/128 September 1998

EPA

Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water

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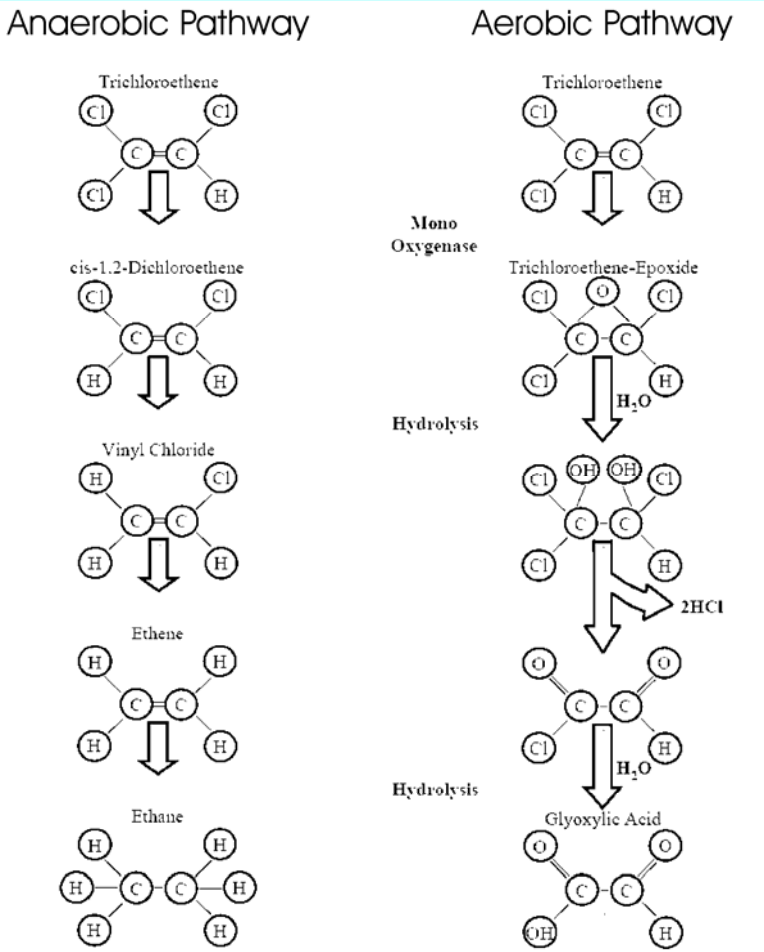
Chloroethene Degradation in Groundwater



Aerobic

- PCE: recalcitrant
- TCE: cometabolic oxidation
- DCE, vinyl chloride: direct utilization as carbon and energy source

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Cometabolism

- Fortuitous transformation of a substrate by an enzyme designed to react with a different substrate, without providing energy or carbon to the organism that produced the enzyme

- Requires the presence of a primary substrate (methane, toluene, phenols,….) and enzymes produced to react with the primary substrate that also react with a cometabolic substrate (TCE)

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Objectives

- Improve understanding of TCE degradation via cometabolism in aerobic groundwater
- Determine the effects of biogeochemistry (methane production and consumption) and hydrology (flow velocity) on TCE cometabolism
- Derive relationships between the expression levels of key genes related to TCE cometabolism, enzyme activity, and TCE transformation rates

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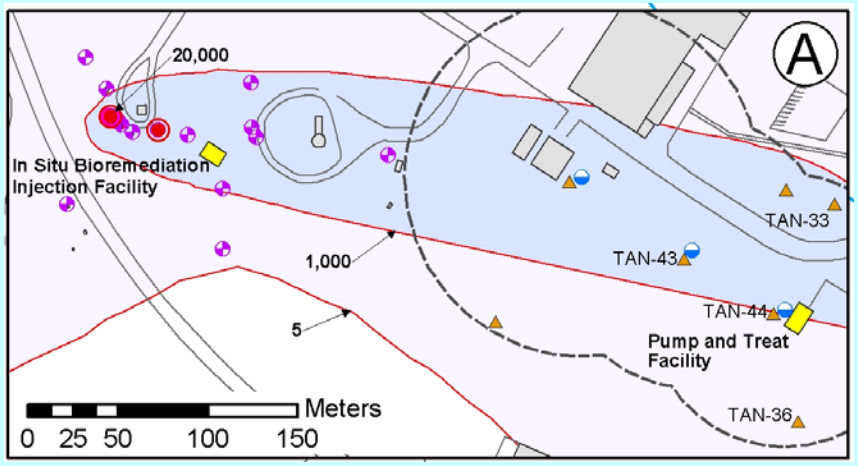
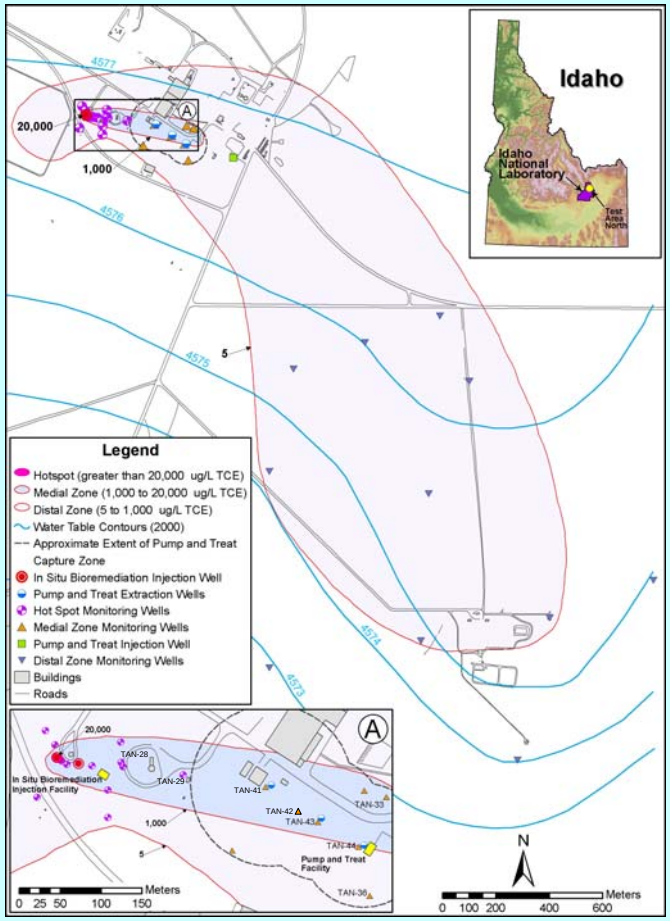
TCE in Aerobic Groundwater at Test Area North





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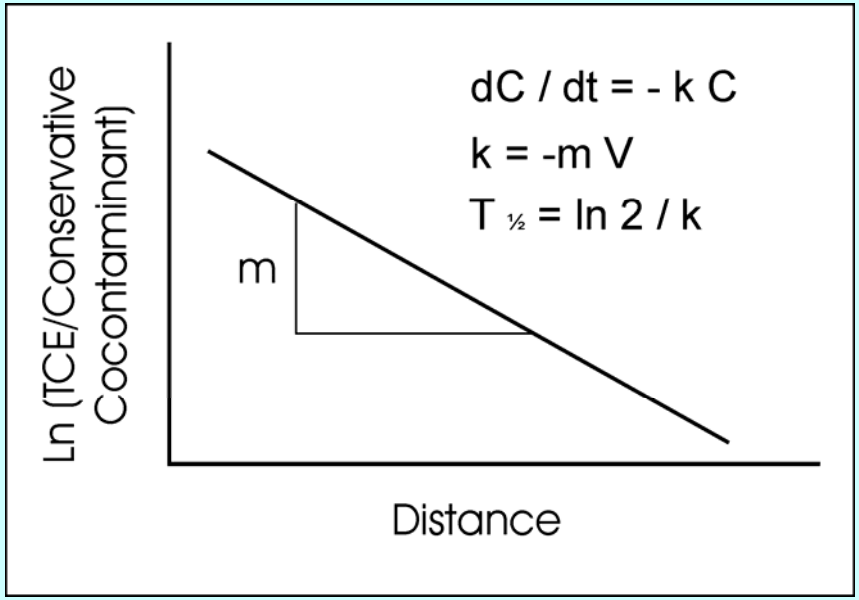
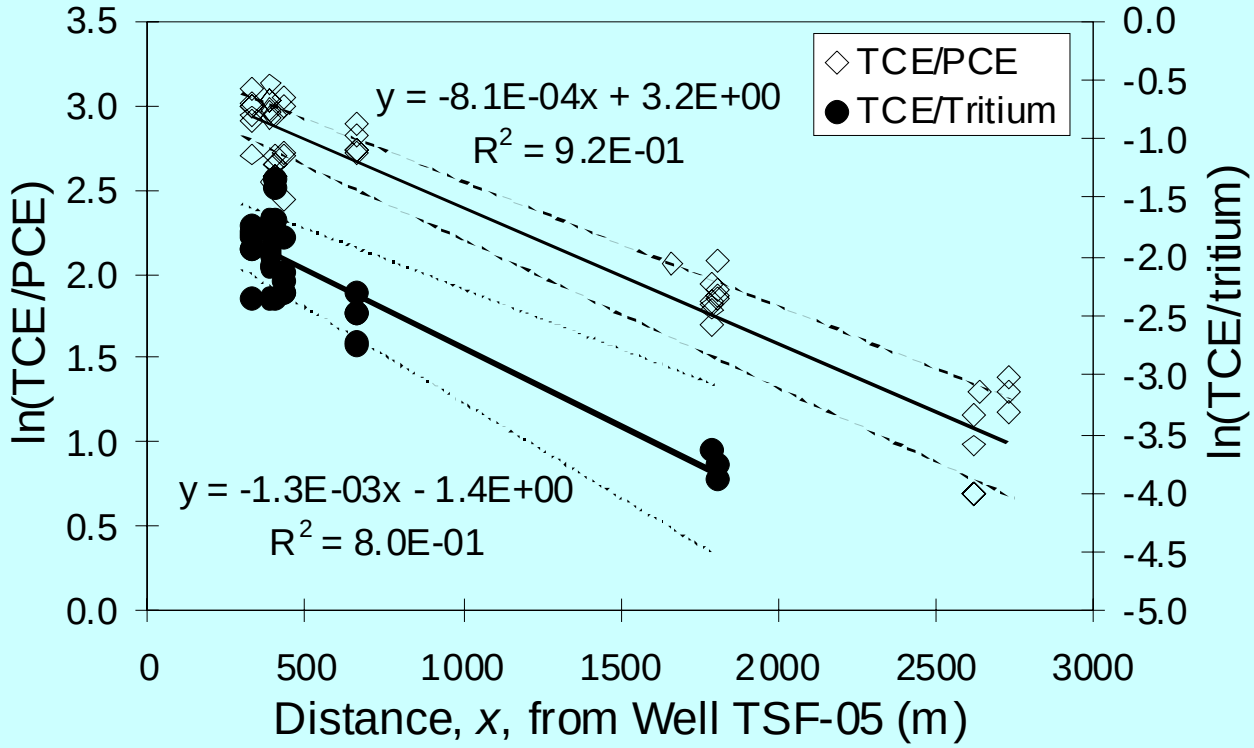
Evidence for TCE Attenuation in the TAN Plume

- Tritium and PCE are conservative at TAN
- If TCE, tritium, and PCE had the same source function and transport properties, then TCE/PCE and TCE/^{3}H would be constant along the flowpath
- If TCE degrades, then ratios would decline with distance downgradient

Tracer Corrected Method



TCE Degradation at TAN



Estimated aerobic TCE degradation half-life: 8-17 years (95% confidence, PCE & ^3H)

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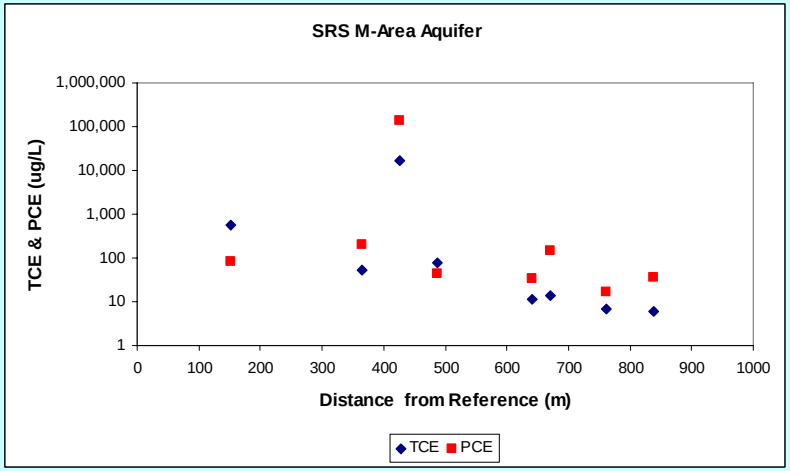
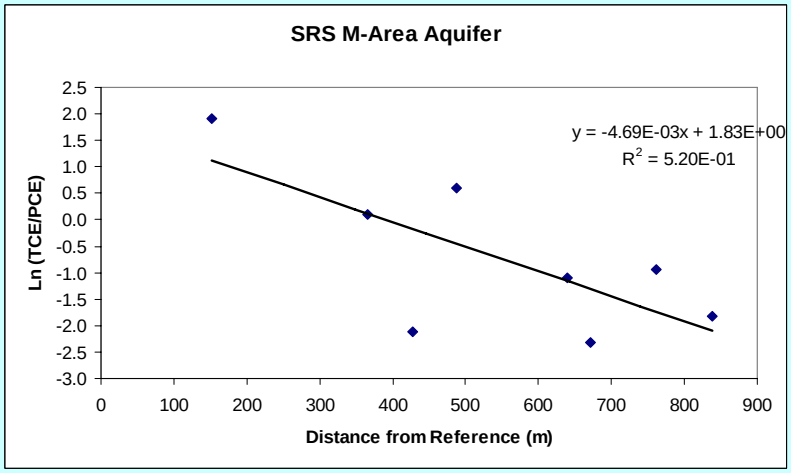
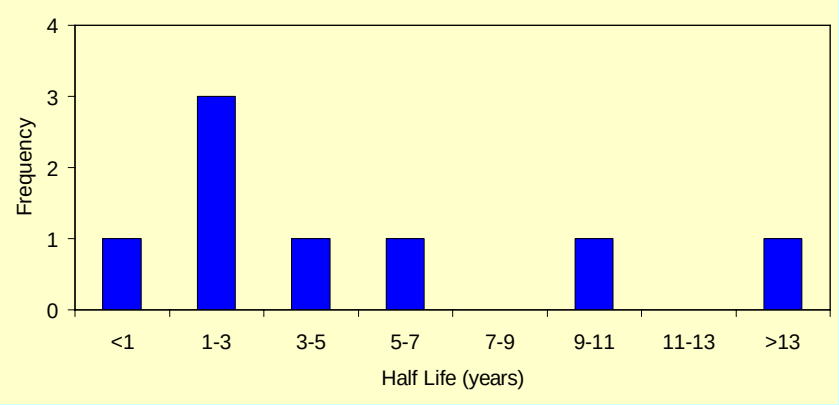
Evidence for TCE Degradation in Other Aerobic Groundwater





- Screened 127 TCE plumes across the DOE complex
- TCE was preferentially attenuated in 8 of 9 plumes examined



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Critique of the TCM Approach

ADVANTAGES

- Uses existing data
- Provides information on kinetics
- May reveal degradation trend that is not apparent in concentration data alone

DISADVANTAGES

- Lack of conservative co-contaminants limits application (< 10% of plumes screened)
- Co-contaminants are often hazardous => MNA not feasible
- No information on attenuation mechanisms
- Data are often noisy, low R^2
- Assumptions difficult to verify

Need to Develop Additional Techniques that Provide Information on Attenuation Mechanisms and Kinetics

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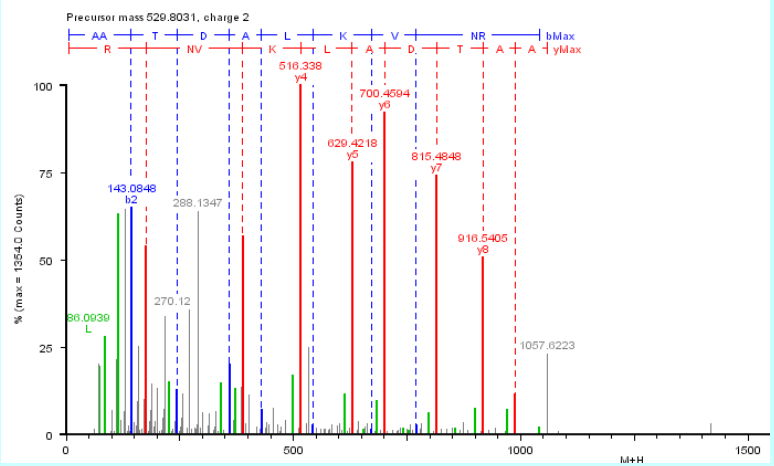
Microbiological Approach-I

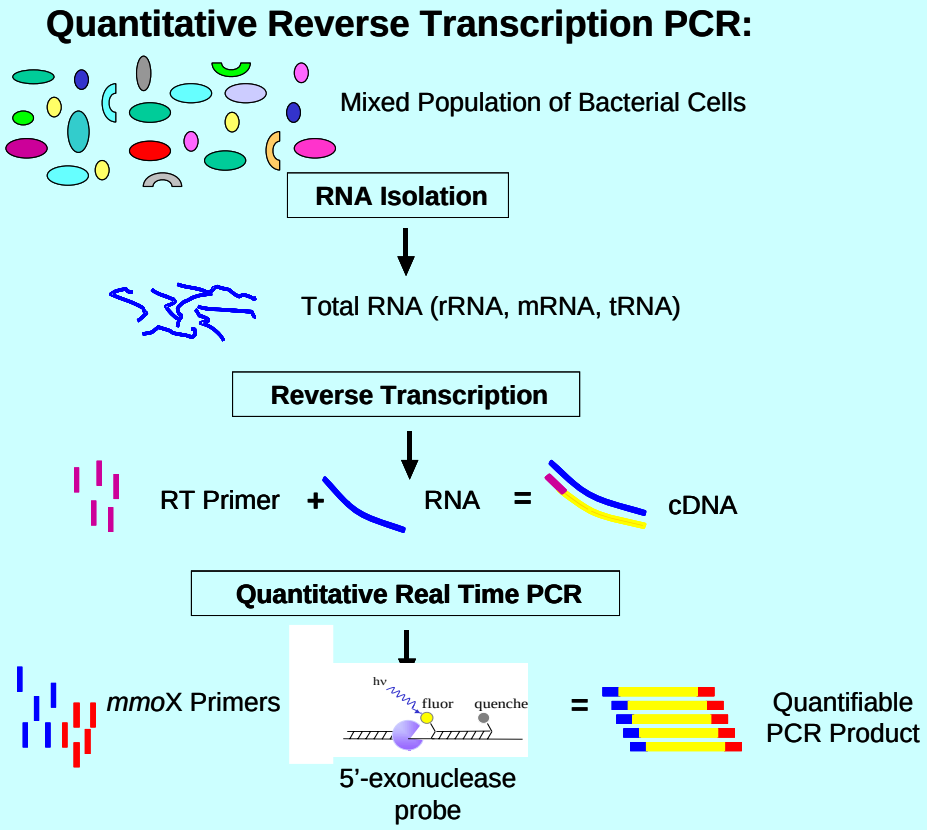
- Does the microbial community have the *genetic capability* to produce sMMO?
- Determine the presence of the functional gene, mmoX, that encodes for the sMMO enzyme, and the amount of sMMO expressed
- Quantitative Polymerase Chain Reaction (pPCR), MS/MS
- Applied to lab cultures and natural biofilms

- University of Idaho
  - Ron Crawford
  - Andrzej Paszczynski
  - Ravindra Paidisetti



Microbial Approach-II

- Does the microbial community *express* its ability to produce sMMO?
- RNA based techniques
- INL
  - Debra Newby
  - Joni Barnes
  - Lynn Petzke



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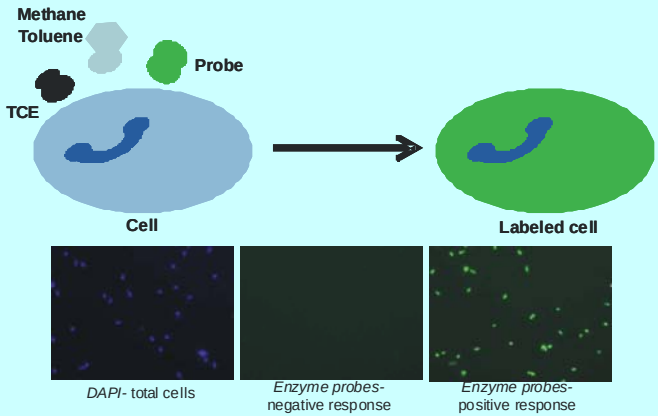
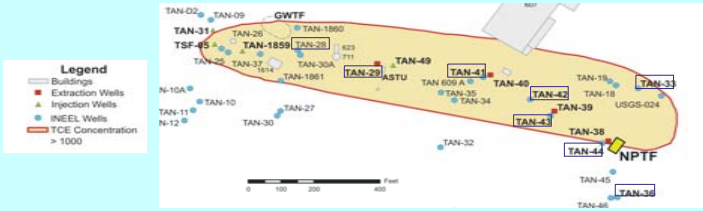
Microbial Approach-III

- Are enzymes involved in TCE cometabolism *active* in the microbial community?
- Enzyme Activity Probes to assay for sMMO and other cometabolic enzymes
- Fluorescent In Situ Hybridization (FISH)
- North Wind
  - Hope Lee
  - Dana Dettmers

What is an enzyme activity probe?

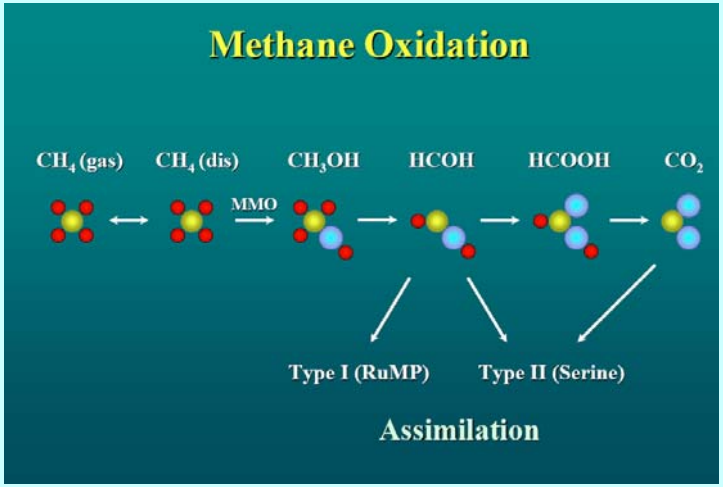


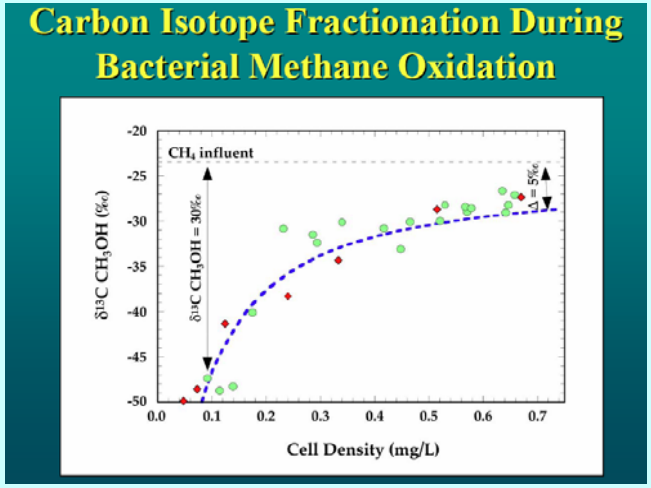
Well Location	DAPI (cells/mL)	sMMO (l/min)	TCE (µg/L)	Methane (µg/L)	Dissolved Oxygen (mg/L)
TAN-28	6.07E+05	37.72	1061	2791	< 1
TAN-29	5.68E+05	37.89	364	372	< 1
TAN-41	4.86E+05	32.67	259	1624	< 1
TAN-42	7.79E+05	27.38	365	320	8.13
TAN-43	1.30E+06	32.49	213	175	6.98
TAN-44	6.53E+05	31.16	234	110	6.91
TAN-33	4.03E+05	24.25	237	134	12.03
TAN-36	4.75E+05	18.10	175	121	9.32



Stable Isotope Geochemical Approach

- Use changes in $^{13}C/^{12}C$ in chloroethenes, methane, and DIC to make inferences about TCE transformation pathways, methane oxidation rate, and to determine the fate of carbon
- Lawrence Berkeley National Laboratory
  - Mark Conrad
  - Eoin Brodie





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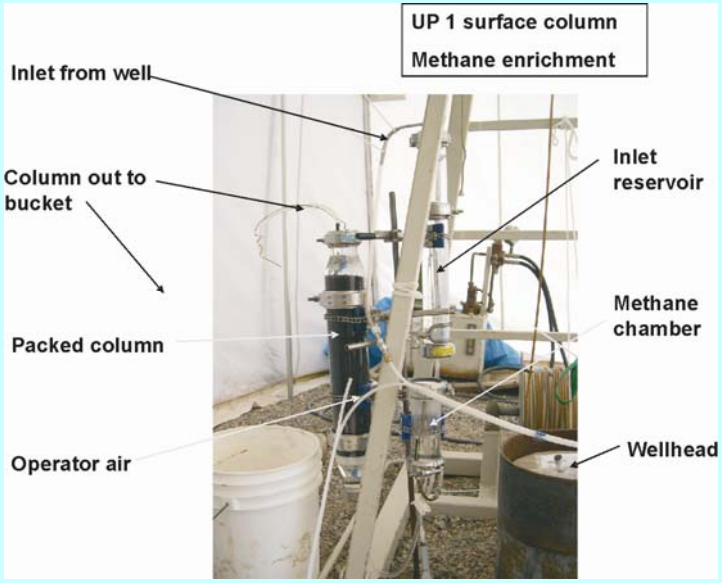
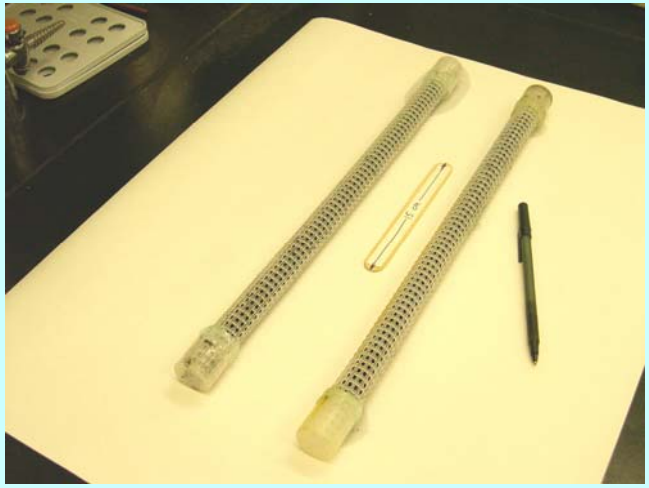
Combined Field and Lab Studies

- Focus on methanotrophs at TAN
- Year 1: Develop / prove techniques using pure culture (OB3b), mixed culture derived from TAN, and biofilm from the Snake River Plain Aquifer
- Years 2 and 3: Characterize the microbial community in the TAN TCE plume





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Collection of a Representative Microbial Community

- Flow Through In Situ Reactors - FTISR
- Packed with crushed basalt
- Placed into well in TAN medial zone
- Flushed with groundwater for ~ 6-8 months at 0.1 and 1 m/day

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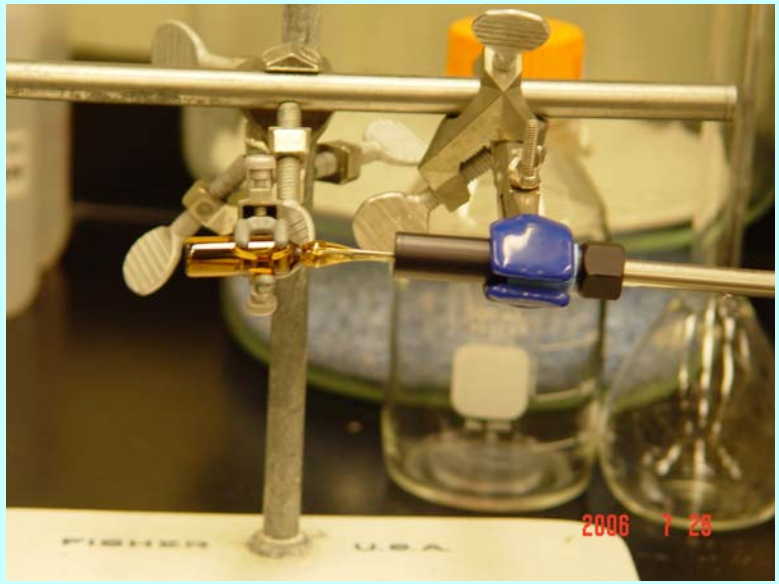
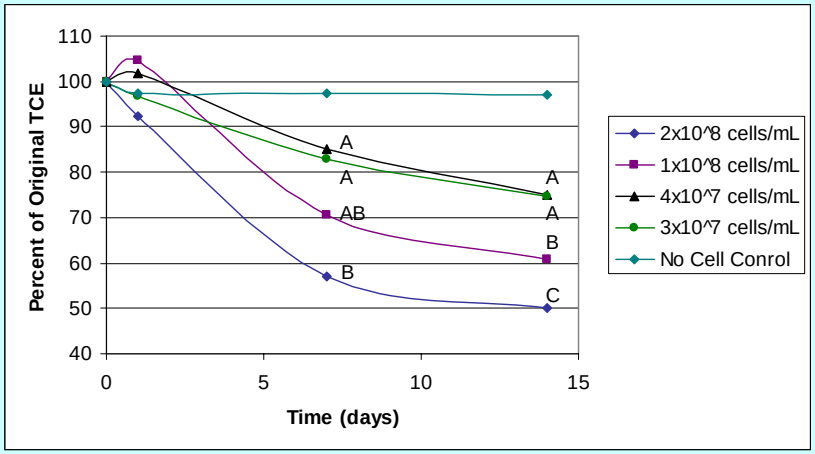
Lab Microcosm Studies





- Concentrate cells (1000 – 2000 x) used so that changes can be observed in a reasonable time ($T_{1/2}$ 10 years under ambient conditions; 7-14 days in lab)
- Determine Michaelis-Menton parameters that describe the effects of TCE, methane, and dissolved oxygen on TCE cometabolism
- Parameterize a competitive inhibition kinetic model
- INL – Corey Radtke

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Modeling Studies

- GW flow and transport modeling used to predict concentration at different locations over time
- Defensible predictions require having an appropriate description of kinetics (form and parameter values)
- First-order kinetics are typically used
- We will compare competitive inhibition model with first order kinetics; does the more complicated kinetic model provide a more credible prediction?
- North Wind – Bob Starr

Mathematical Model for Competitive Inhibition of Methane and TCE Oxidation with Inhibition at Low Dissolved Oxygen

$$r_g = \frac{-X\,k_g\,S_g}{K_{sg}\left(1+\frac{S_c}{K_{sc}}\right)+S_g}\,\frac{k_{DO}\,S_{DO}}{K_{DO}+S_{DO}}$$

Methane

$$r_c = \frac{-X\,k_c\,S_c}{K_{sc}\left(1+\frac{S_g}{K_{sg}}\right)+S_c}\,\frac{k_{DO}\,S_{DO}}{K_{DO}+S_{DO}}$$

TCE

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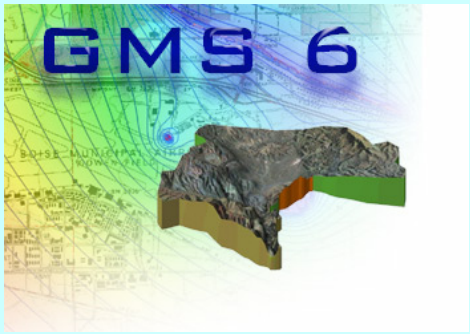
Modeling Approach



- GMS environment
- Use MODFLOW for groundwater flow simulation, RT3D for transport of kinetically-controlled, degrading solutes
- RT3D is being modified to simulate competitive inhibition reaction kinetics

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Status

- Lab techniques have been developed using lab cultures and biomass from the field
- Enzyme Probe studies have been successfully performed at TAN
- Anticipate performing enzyme probe studies at other aerobic TCE plumes in the near future
- Flow Through In Situ Reactors have operated successfully for ~5 months and will be deployed within a few weeks
- Mathematical model for TCE cometabolism has been developed and is being incorporated into flow and transport software
- Anticipate distributing biomass from the TAN Medial Zone for extensive lab studies in Spring/Summer 2007

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Acknowledgements

This research was supported by funding from the U.S. Department of Energy, Office of Science, Environmental Remediation Sciences Division.

The assistance of Evan Myler, Kevin Harris, Dana Dettmers, Lee Nelson, and Mark Delwiche for collecting and handling samples acquired from Test Area North is greatly appreciated.

Site access provided by the Idaho Completion Project is also greatly appreciated.

Overall project management by Rick Colwell of OSU

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