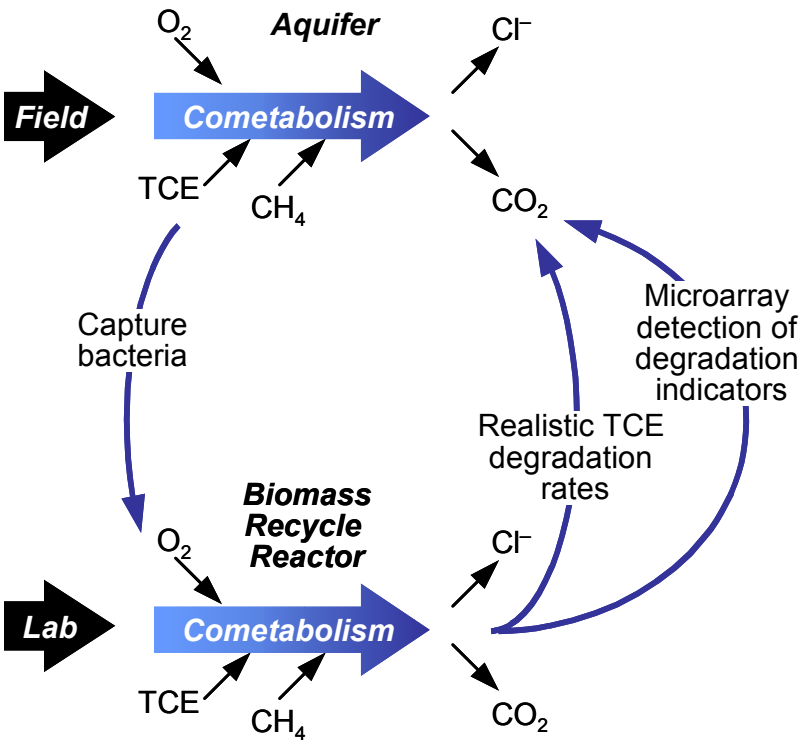
Coupling of Realistic Rate Estimates with Genomics for Assessing Contaminant Attenuation and Long-Term Plume Containment

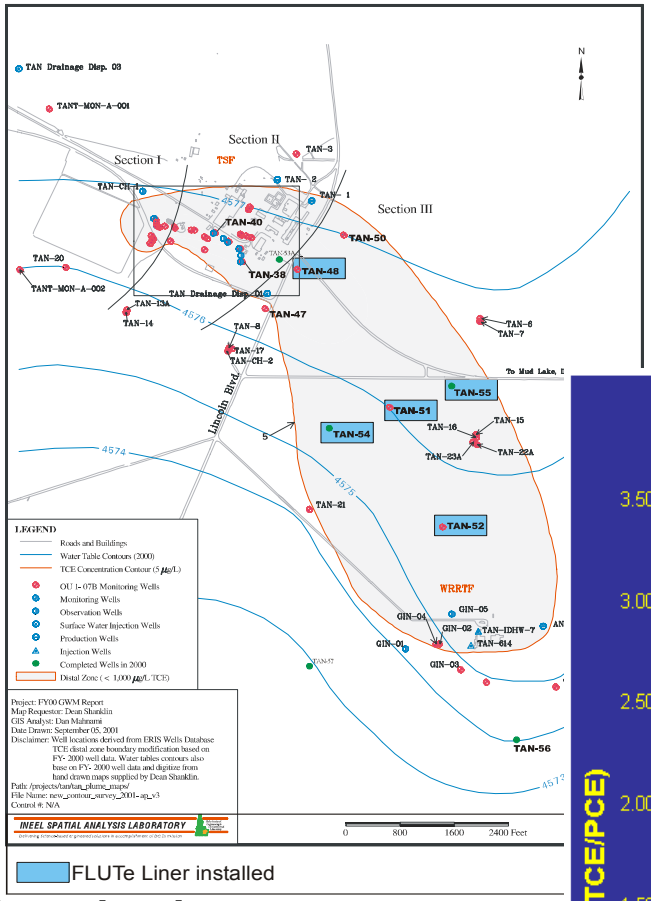


Frederick Colwell, Mark Delwiche, and Cindi Brinkman, INEEL

Ronald Crawford and Dan Erwin, University of Idaho

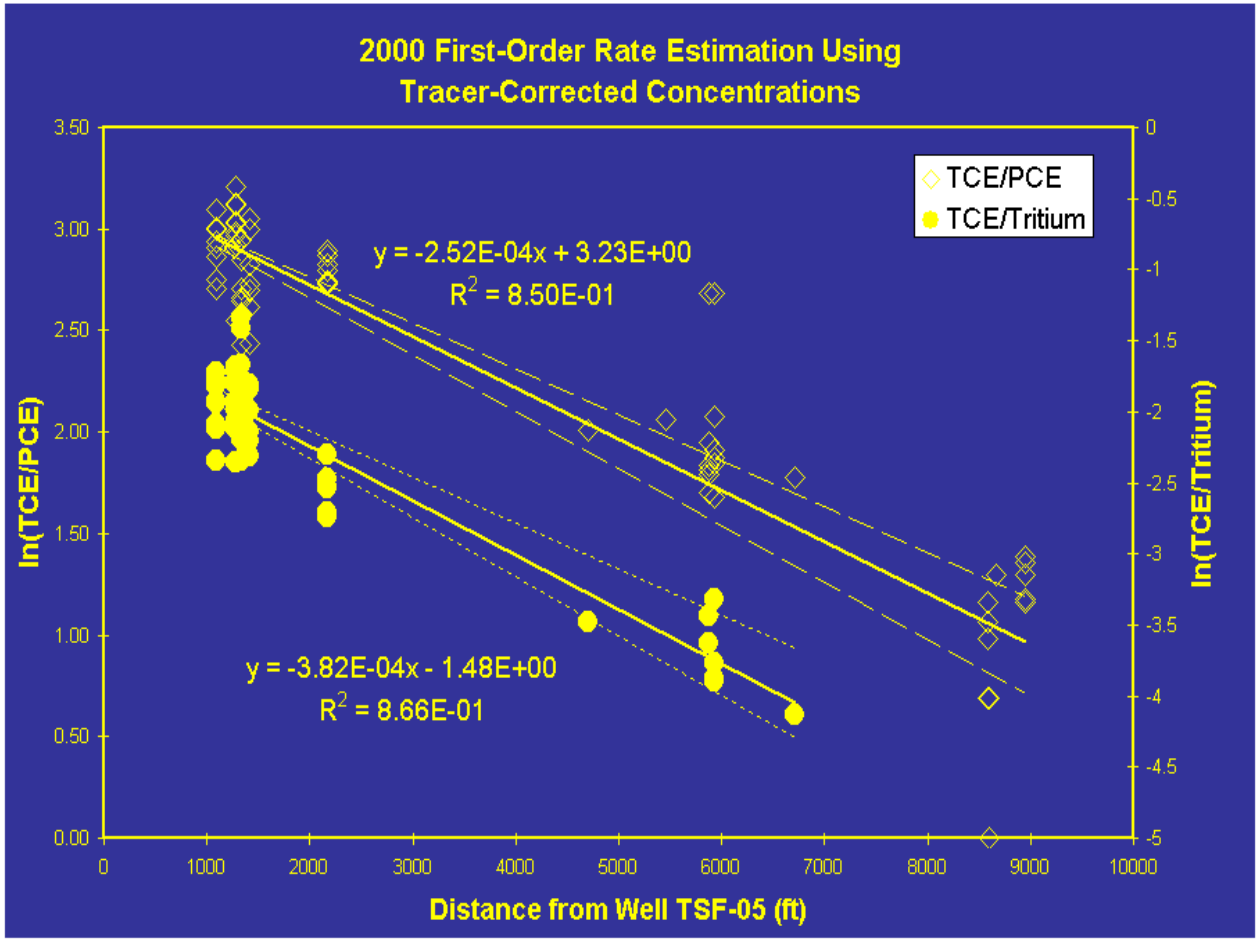
Kent Sorenson, North Wind Environmental

TAN TCE Plume Map



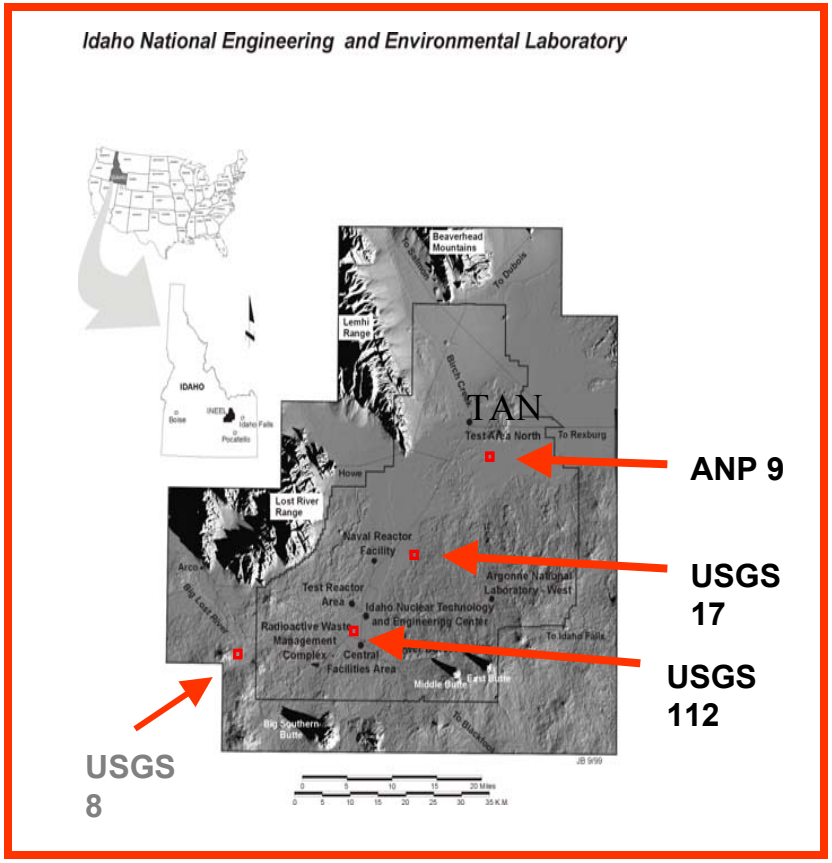
2-mile long TCE plume at INEEL's Test Area North is stable by natural attenuation

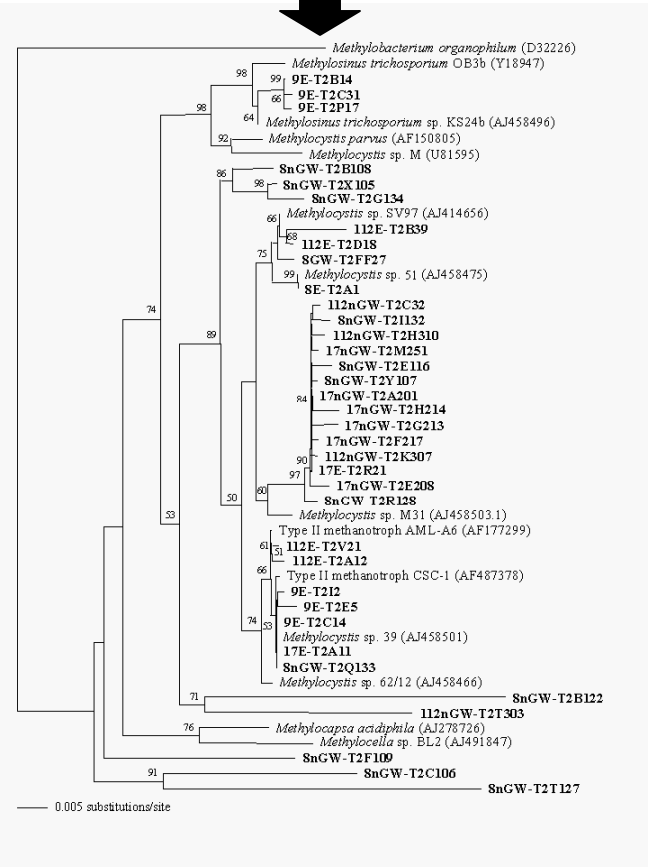
Natural attenuation verified by TCE/PCE and TCE/tritium ratios; first-order degradation rate apparent for TCE

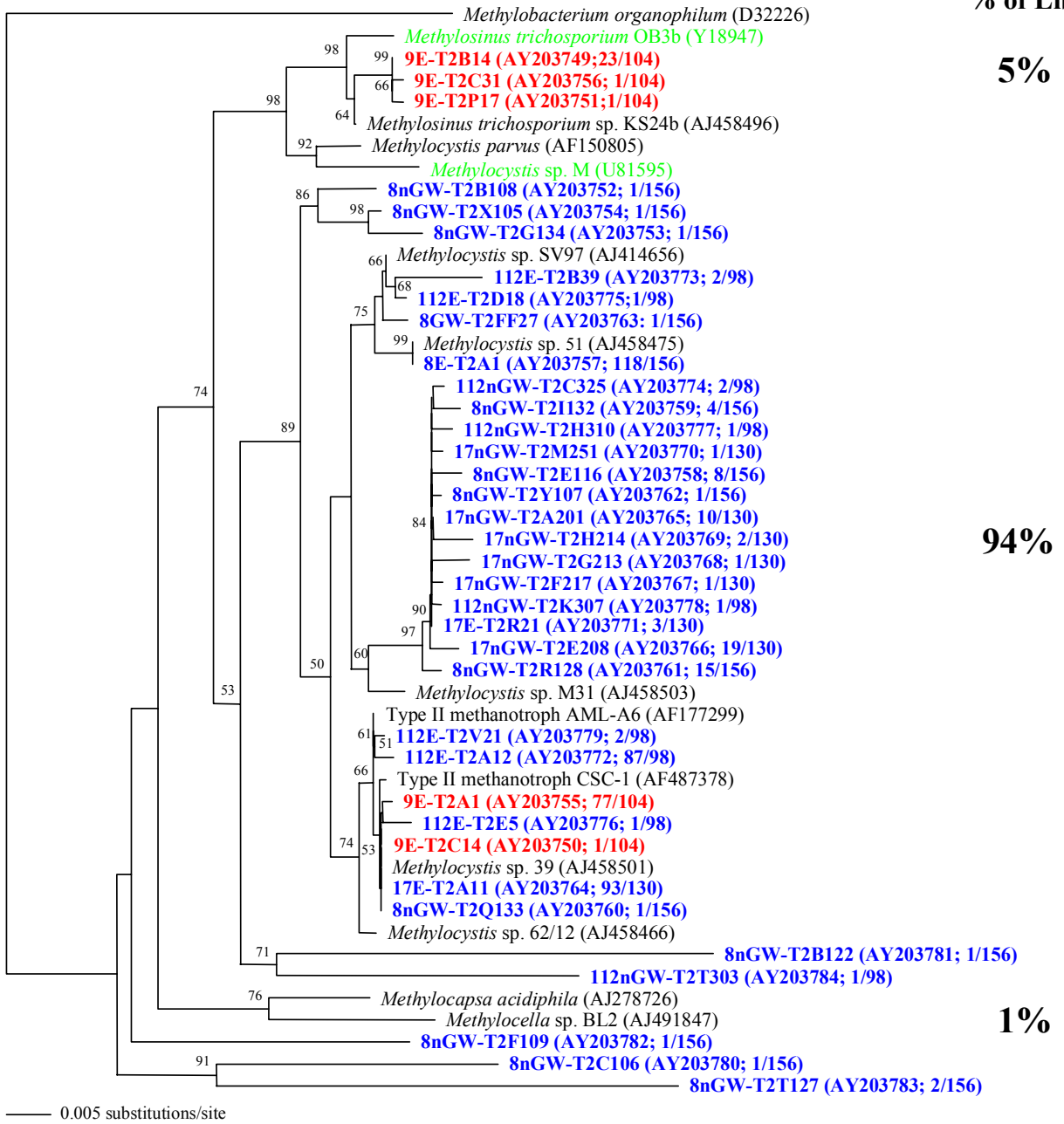


But what is responsible for the natural attenuation? Need cause-and-effect relationship between contaminant loss and mechanism (NRC, 2000)

- *Amplify methanotroph DNA from groundwater, enrichments, isolates (16S rDNA, mmoX, pmoA)*
- *Clone products, screen clones, sequence unique products*
- *Compare to RDP/GenBank*
- *Phylogenetic analysis*







% of Library

5%

94%

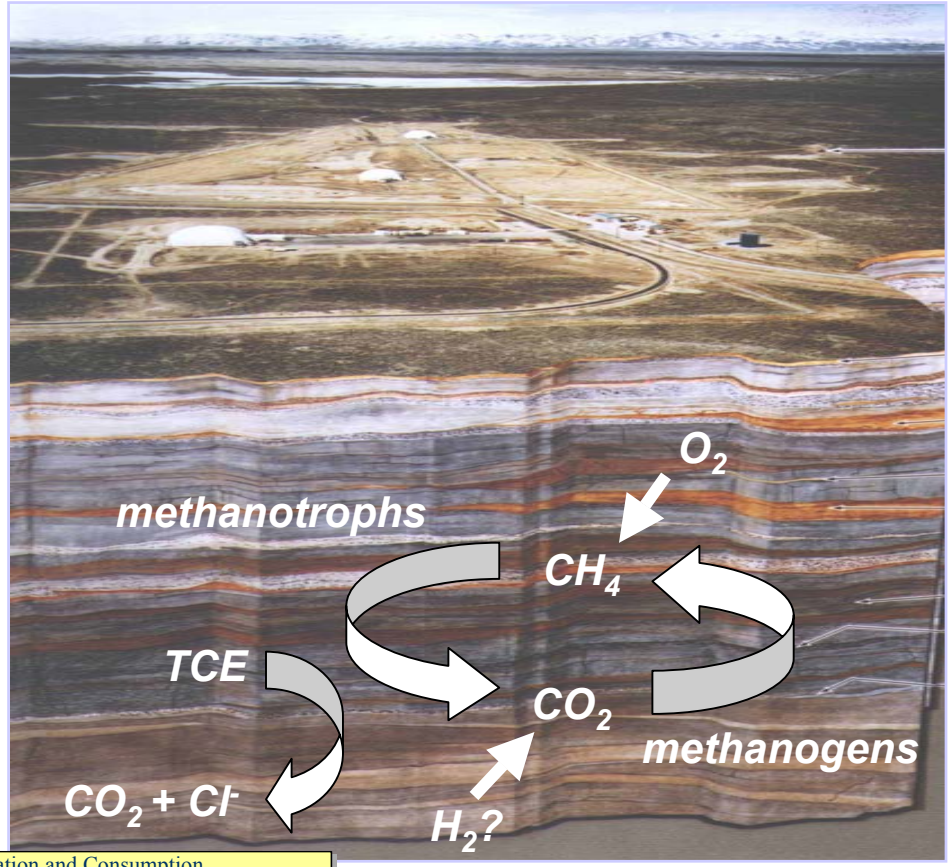
1%

Type II methanotroph tree

- **5% of clones related to *Methylosinus trichosporium.* All from ANP 9 (near TAN)**
- **94% of clones related to *Methylocystis* spp. TCE co-metabolism not evident in this clade**
- **1% of clones are unique, undescribed methanotrophs**

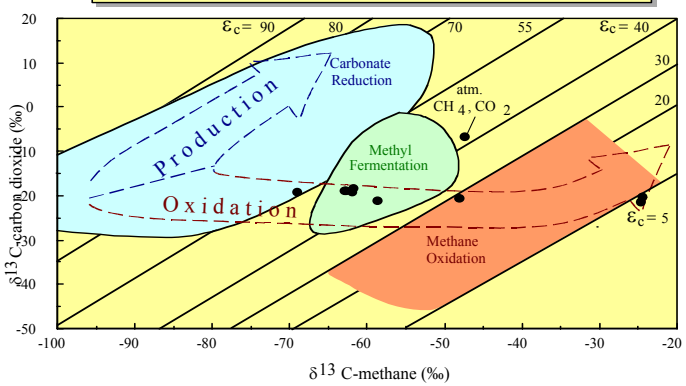
Clones from ANP 9; clones from other SRPA wells; TCE co-metabolizing methanotrophs

C-1 Cycling in the Snake River Plain Aquifer



- *Dissolved methane is biogenic by stable isotope analysis; appears to be used by methanotrophs*
- *Aquifer contains both methanogens and methanotrophs*
- *Some of the methanotrophs appear to be related to TCE-cometabolizing strains*
- *Activities are LOW; need to study cells at appropriate rates*

Bacterial Methane Formation and Consumption



after Whiticar, M.J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, Vol. 161, No. 1-3, p. 291-314.

$$\varepsilon C - 10^3 \ln \alpha_C - 10^3 (\alpha_C - 1) - \delta^{13} C_{CO2} - \delta^{13} C_{CH4}$$

Goals

- *Determine realistic (in situ) rates of methane turnover and TCE cometabolism by indigenous methanotrophs at subsistence activities.*
- *Determine if these rates account for stabilization of the TAN plume given the estimated methanotrophic biomass in the plume.*

- *Identify the genes that are expressed by the subsisting cells during cell starvation and TCE cometabolism.*
- *Determine whether specific gene expression can be used as evidence of TCE cometabolism.*
- *Evaluate whether aquifer communities express genes consistent with low activity existence and TCE cometabolism.*

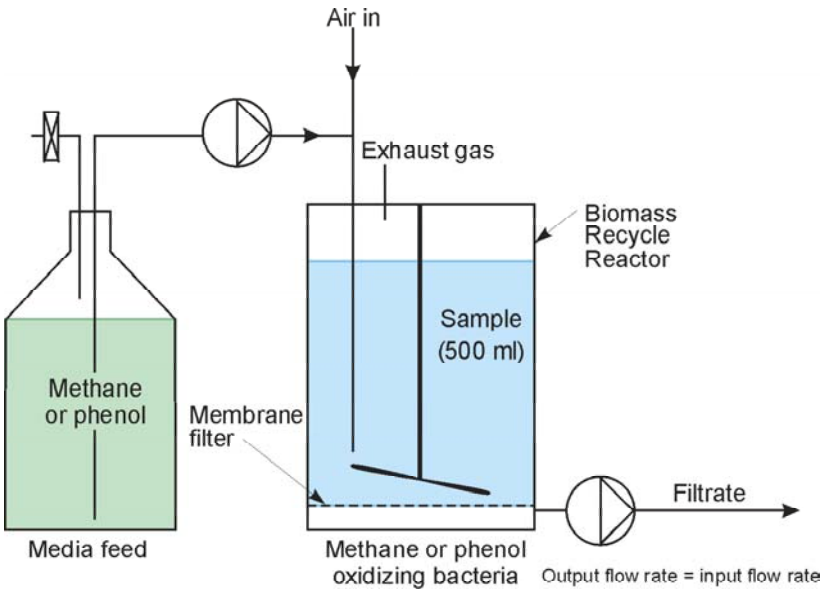
Biomass recycle reactor

- *Use type II methanotroph from SRPA*
- *Biomass retention*
- *Substrate in at same rate as filtrate removed*
- *Trapped cells with just enough substrate for maintenance energy requirements*
- *Post-exponential phase, chronic starvation*
- *Derive catabolic rate est*

Maintenance energy demand =

substrate provision rate ÷ biomass

- *Introduce TCE (25 ppb) and achieve new steady-state, new catabolic and TCE cometabolism rates estimated*



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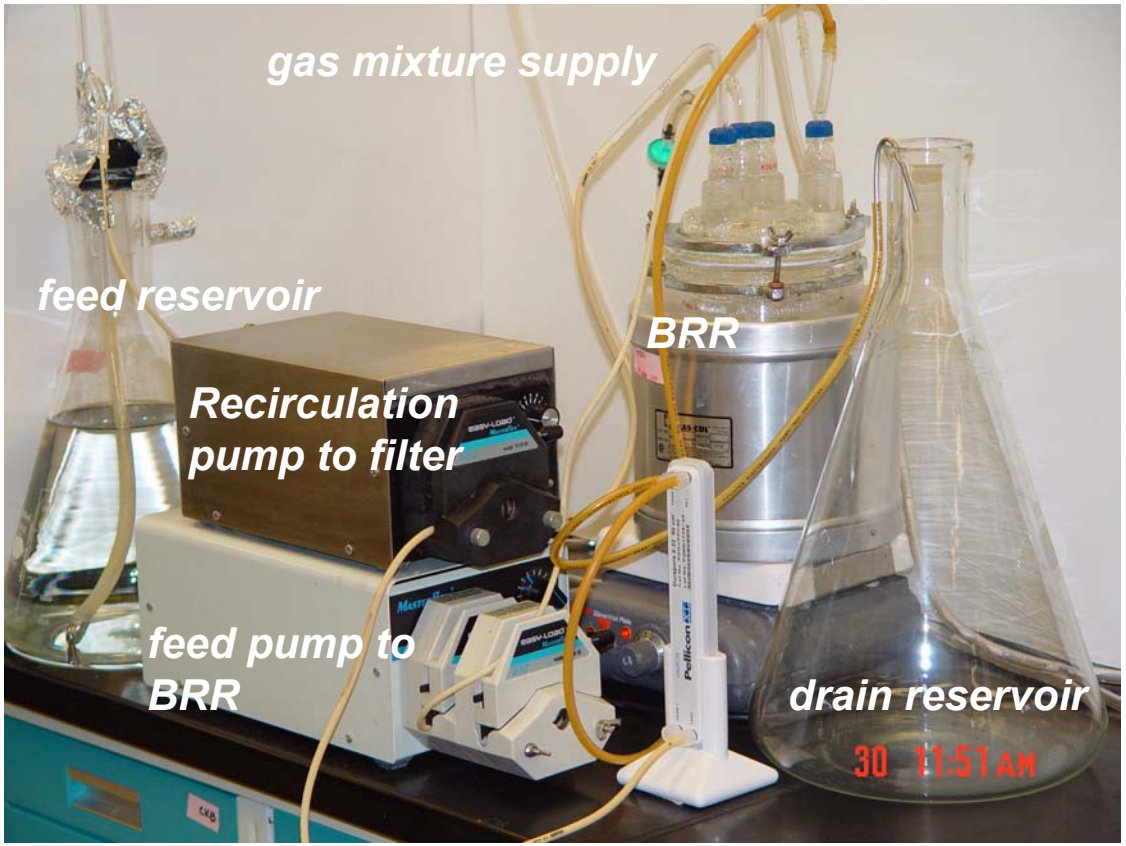
- ***BRR currently contains <u>Methylosinus</u> <u>trichosporium</u> OB3b***
- ***Enrichment, isolation underway to obtain methanotrophs from filtered water (ANP-9) or basalt chips from (ANP-10)***



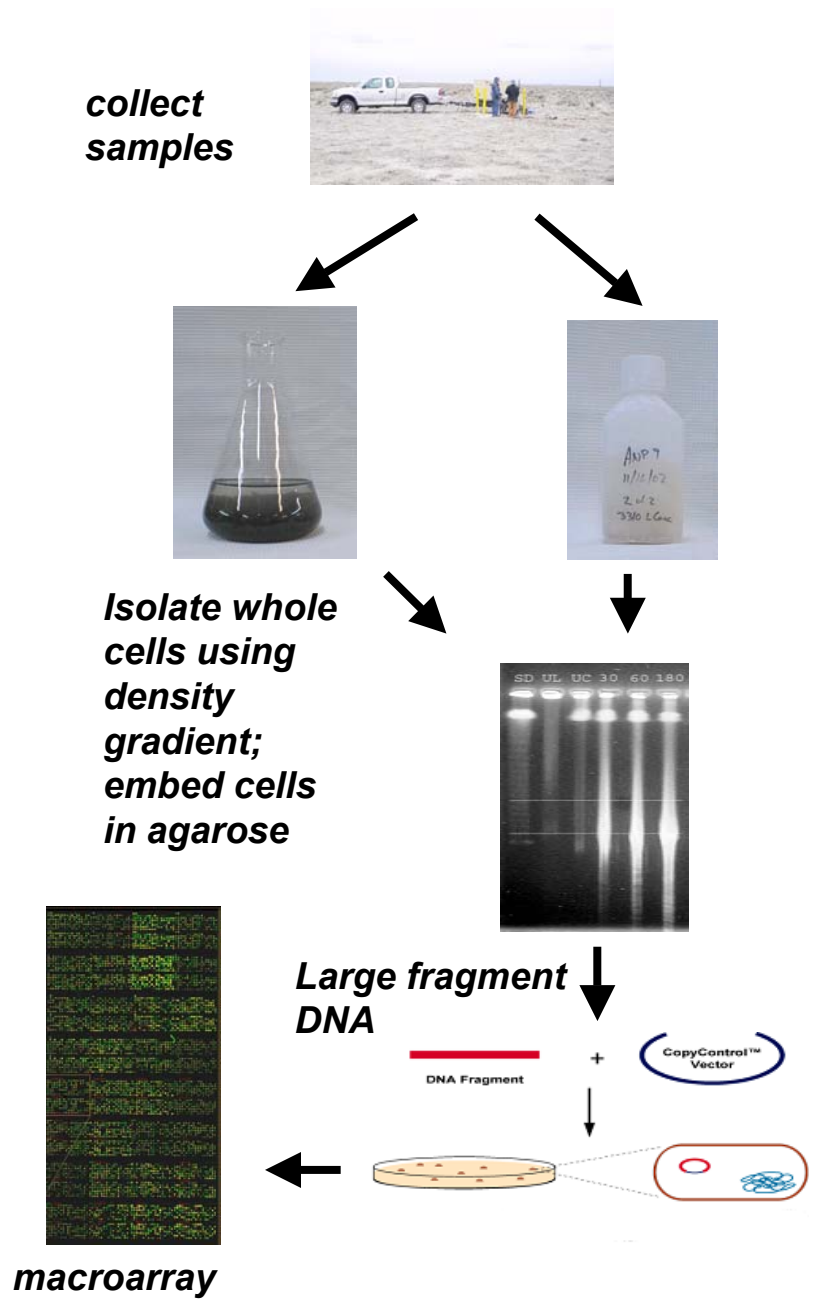
Aquifer biomass, methane and TCE determinations

In selected wells:

- ***Determine biomass of free-living and attached SRPA methanotrophs using culture- and nonculture-based methods (MPNs, real time PCR, and FISH?)***
- ***Determine methane and TCE concentrations***

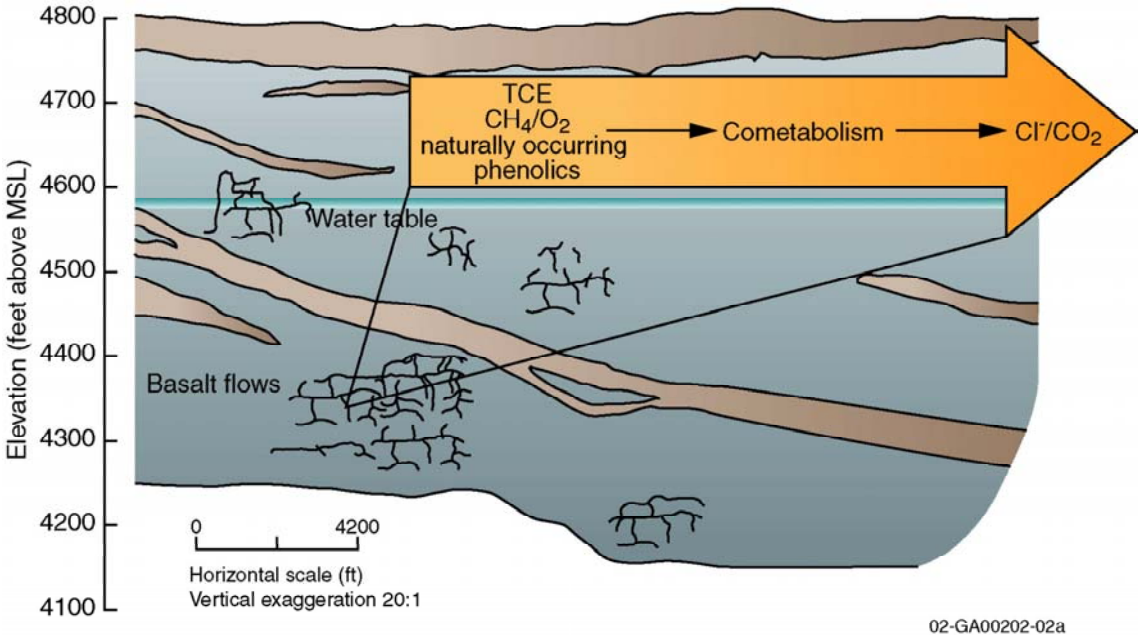
Genomics of TCE cometabolism

- Create large-fragment genomic DNA BAC library to identify TCE cometabolizing microbes and their genes
  - Obtain attached and free-living SRPA cells
  - 100-300 (50?) Kb fragments, 10,000-50,000 clones arrayed
  - Probe BAC library with synthesized monooxygenase sequences (e.g., mmoX, pmoA)
  - Sequence probe-positive clones; detect associated 16S DNA? design FISH probes?
- Create cDNA library from BRR to detect differential expression in response to TCE exposure
  - cDNA signals suggesting TCE-associated injury (in BRR and aquifer)
  - Genetic evidence of injury



Modeling and estimates of volumetric productivity

- ***Based on prior modeling of natural attenuation at TAN for CERCLA cleanup***
- ***GMS platform, MODFLOW to generate flow fields, and MT3D-MS for transport and attenuation***
- ***Lab attenuation rates will be approximated as first-order processes with a reactive transport code (RT3D) as an alternative***
- ***TAN will be modeled first but a second DOE site will be modeled***



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Hypotheses

- ***Catabolic Rate Hypothesis: Catabolic rate estimates from starved methanotrophs, when matched to in situ biomass of these populations, will yield realistic values for in situ rates of methane oxidation as well as TCE cometabolism. These values will agree favorably with prior determinations of the rate of TCE natural attenuation in the aquifer that have been ascribed to biological communities.***
- ***Genetic Marker Hypothesis: Distinctive patterns of functional gene expression will be evident in starved methanotrophs carrying out TCE cometabolism, and such patterns can be used to assess TCE cometabolic potential when these cells are taken directly from the aquifer.***