PNNL SFA: Role of Microenvironments and Transition Zones in Subsurface Reactive Contaminant Transport

Microbial Ecology

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… with assistance of Microbiology staff

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Hanford IFRC: Deep Characterization Borehole

July, 2008: Samples retrieved in core liners from discrete strata

Hanford and Ringold formation
Microbiological characterization of sediments

- 40 ft of core over 175 ft borehole (Hanford and Ringold formations)
- 17 samples across geological formations and transition zones

**Cultivation-independent analyses**

- **Biomass**
  - Direct microscopic counts
  - Phospholipid fatty acids (?)
  - % Respiring cells
- **Phylogenetic / functional diversity and relative abundance**
  - Census of Bacterial/Archaeal 16S rRNA gene sequences
    - JGI approved CSP project for Sanger sequencing
    - Pyrosequencing by U Colorado collaborators
  - Real time PCR for specific phylogenetic and functional groups
- **Assessment of potential for TEA reduction**
  - Amend samples with electron donor
  - Add “natural” TEA: Mn⁴⁺, Fe³⁺, NO₃⁻, SO₄⁻²
  - Add U or Tc. Depend upon natural abundance of Fe(III) or exogenously added ferrihydrite as terminal electron acceptor (TEA)

**Cultivation-dependent analyses**

- Enrichment cultures with various TEA's
- High-efficiency cultivation strategies
- Analysis of metabolic versatility in cultivars

Provide Hanford-relevant microbes for molecular- to pore-scale projects

Multivariate statistical analysis of microbial census + geochemical / mineralogical data to generate hypotheses for field-scale studies
Biomass distribution in subsurface

**Graph 1:**
- **X-axis:** Acid-extractable Fe(II) (µmol/g)
- **Y-axis:** Depth (feet)
- **Legend:**
  - Hamford - Ringold contact
  - Oxic - reducing interface

**Graph 2:**
- **X-axis:** Redox sensor green positive cells (%)
- **Y-axis:** DAPI positive cells ($x10^8$ g$^{-1}$)
- **Legend:**
  - % active cells
  - Total cells

**Legend:**
- % cells w/ intact membrane
  - 36-60
  - 30-65
  - 80
  - 31-65
  - 23-30

**Notes:**
- Biomass distribution data is presented in two graphs, showing the distribution of acid-extractable Fe(II) and DAPI positive cells across different depths, with a focus on % cells with intact membrane.
Molecular ecology

DNA isolated from subsurface sediments

• 17 strata from Deep Characterization Borehole -- **Vertical heterogeneity**
• 68 samples (Hanford, Ringold oxidized, Ringold reduced) obtained from 27 of 35 wells in IFRC well field -- **Horizontal heterogeneity**

What we obtain:

• Yields: 120 ng DNA per g (Hanford formation) & 2-5 ng DNA per g (Ringold formation, 155 & 169 ft.)
• Fragment size: 10-23 kb
• DNA amplifiable with Taq polymerase and Phi-29 polymerase
Molecular ecology

Census of Bacterial / Archaeal populations

Based upon analysis of 16S rRNA gene sequences

- Joint Genome Institute – Community Sequencing Program
  - PNNL amplified 16S rRNA genes & constructed libraries for Bacteria (20 samples) and Archaea (12 samples)
  - JGI will provide 384 Bacterial and 192-384 Archaeal near-full length sequences per sample [Mar-Apr, 2009]
- U Colorado / Rob Knight -- pyrosequencing
  - Analyze ca. 100 different samples (vertical & horizontal heterogeneity)
  - Obtain several thousand sequences per sample
  - Sequence length = 240 bases (less phylogenetic resolution)
  - Ecological value: compare community similarities
16S rRNA gene sequences are molecular chronometers, useful for distinguishing microbes

>750K aligned sequences in RDP database

Figure by Jamie Cannone, courtesy of Robin Gutell; data from the Comparative RNA Web Site: www.rna.icmb.utexas.edu
Molecular ecology

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Molecular ecology: pyrosequencing 16S rRNA genes

UniFrac measures the phylogenetic distance between sets of taxa in a phylogenetic tree as the fraction of the branch length of the tree that leads to descendants from either one environment or the other, but not both.
Molecular ecology

Quantitative PCR --

- If PCR primers available to amplify a specific target gene, can quantify abundance of that gene in environmental DNA
- Bacteria vs. Archaea
  - Archaea comprise 0-8% of total 16S rRNA gene copies in DCB samples
- Functional groups (use of Terminal Electron Acceptors)
  - Nitrate (0.5 mM in GW) -- *nosZ* (nitrite reductase)
  - Sulfate (0.5 mM in GW) -- *dsrA* (sulfite reductase)
  - Fe / Mn -- no useful probes for functional genes
    - Phylogenetic (16S rRNA genes) for known groups
      - Geobactereaceae, *Anaeromyxobacter*, *Shewanella*
Molecular ecology – quantitative PCR

Relative abundance: gene copies normalized to 16S rRNA gene abundance
Neighbor-joining tree of 16S rRNA gene sequences amplified with *Shewanella*-specific primers 211f/1259r.

- Tree was constructed with MEGA4 program.
- Numbers in branches are bootstrap values (values less than 50 are not shown).
- Triangles represent Hanford clones with PCR products pooled from three different sampling depths.

**Preliminary analysis of sequenced clones**

Neighbor-joining tree of 16S rRNA gene sequences amplified with *Shewanella*-specific primers 211f/1259r.
Microbial activity in sediments: TEA consumption – a scoping experiment

Added Nitrate

Electron donors: Acetate + succinate + glucose

Added Hydrous Ferric Oxide or native Fe minerals

0.5 N HCl-ext. Fe(II) at day 32

- live - HFO
- sterile - HFO
- live - No acceptor
- sterile - No acceptor
What we are thinking about post census …

• Limiting factors for microbial activity
  – “Bioassay” expts w/ C, N, P
  – $^3$H-leucine incorporation

• Biomass distribution among particles in Hanford formation
  – Descriptive statistics: mean, variance, normal or log normal
  – ATP as sensitive, high-throughput biomass assay

• Groundwater DOC
  – Isolation
  – Characterization (NMR, FTICR MS)
  – Microbial degradation

• In-well experiments
  – Natural substrates or BioSep beads
  – Colonization rates
  – In situ activity
  – Stable isotope probing