Summary of a 2005 NABIR PI Meeting Breakout Session

Tuesday, April 19, 2005

What changes in microbial community structure can be expected during and after biostimulation?

Organizers: Terry Hazen – *Lawrence Berkeley National Laboratory*

Lee Krumholz – *University of Oklahoma*

Invited Presenters: Joel Kostka – *Florida State University*

Anne Spain – *University of Oklahoma*

Aaron Peacock – *University of Tennessee*

Frank Loeffler – *Georgia Tech*

Cheryl Kuske – *Los Alamos National Laboratory*

Matthew Fields – *Miami University*

Lee Kerkhof – *Rutgers University*

Abstract

In this breakout session, we will discuss recent results studying changes in microbial communities associated with biostimulation of aquifer bacteria. Research field sites to be reported on include the Oak Ridge National Lab FRC, the UMTRA site at Rifle, CO and a contaminated site at Hanford, WA. These sites represent a broad range of geochemical and microbiological conditions faced by DOE. The participants will identify critical and unresolved issues specific to their experiences and research needs specific to NABIR goals.

The goals of the breakout session are as follows:

1. Present results from NABIR investigators on biostimulation experiments. These will include results from studies addressing biomass estimates, various techniques for assign microbial diversity using environmental 16S rRNA gene sequences, diversity of functional groups by analysis of functional genes, and stable isotope analysis. We will hear results from: Joel Kostka –FRC, Lee Krumholz – FRC, Aaron Peacock – Rifle, CO, Terry Hazen – Hanford, WA, Jack Istok – FRC model systems, Frank Loeffler – FRC and others, Cheryl Kuske – Recent molecular tools and Pu simulations, and Matt Fields – FRC.

2. Define critical questions developed from these experiments that can be addressed at the existing and future NABIR field sites. These will include but will not be limited to questions such as:
   a. What other approaches are available which are useful for understanding changes in microbial communities?
   b. What are the best strategies for sampling in order to obtain representative communities and incorporate data on heterogeneity?
   c. Can we link structure of communities to their function?
   d. How can we incorporate results into a model to develop an understanding of common changes in microbial communities?
   e. How can we use this information to develop more efficient and long-term protocols for bioremediation?

3. Discuss where the NABIR program is heading in this area.

What changes in microbial community structure can be expected during and after biostimulation?

Introduction and Workshop Format

In this breakout session, we discussed recent results studying changes in microbial communities associated with biostimulation of aquifer bacteria. Research field sites included the Oak Ridge National Lab FRC, the UMTRA site at Rifle, CO and a contaminated site at Hanford, WA. These sites represent a broad range of geochemical and microbiological conditions faced by the DOE NABIR Program. (Note: Many of the participants felt that this session overlapped significantly with the April 18, 2005 session on Monday afternoon: " How distinct are microbial communities at different field sites? Tony Palumbo and Fred Brockman" and many of the same points were further elaborated from this session.)

The goals of the breakout session were as follows:

1. Present reports of recent results from NABIR investigators on biostimulation experiments. These included results from studies addressing biomass estimates, various techniques for assigning microbial diversity using environmental 16S rRNA gene sequences, diversity of functional groups by analysis of functional genes, and stable isotope analysis. Participants included: Joel Kostka-Florida State University, Anne Spain-University of Oklahoma, Aaron Peacock-University of Tennessee, Terry Hazen-LBNL, Frank Loeffler-Georgia Tech, Cheryl Kuske-LANL, Matt Fields-Miami University, and Lee Kerkhof-Rutgers University.

2. Conveners discussed relevant talks from morning presentations by Derek Lovley, David White, and Darryl Chandler. These studies were compared based on sampling approaches, analysis techniques and results obtained. In each case, sampling, analysis and results were different, indicating that either sampling approach or analytical methods can dramatically influence the results obtained.

3. Presenters were then asked to first define critical questions developed from these experiments that could be addressed at the existing and future NABIR field sites. They were also asked to present research needs and new ideas for future research. Audience participation was encouraged.

Invited Presentation Summary

Matthew Fields
Despite geochemical and phylogenetic differences, low and high contamination sites were functionally similar (based upon sequence data). The bacterial numbers were only 1-log lower between low and high contamination sites. These data support the idea that despite geochemical effects that population shifts maintained underlying structure. Groundwater bacterial communities might respond in a predictable fashion in the

contexts of a dynamic equilibrium model. A dynamic equilibrium model would help predict changes in diversity patterns within functional groups that differ in growth rate and competitive displacement (Huston, 1994). Results of community analysis of enrichment cultures were discussed and Matt showed that many of the enrichments for Fe(III) and nitrate reducing bacteria contained Gram positive clones as well as Anaeromyxobacter.

Lee Krumholz
Used two lines of evidence to show that *Alcaligenes* species are enriched for in Area 1 by ethanol addition: clone libraries, culturing. Detected novel groups of organisms using clone libraries that would have been overlooked using other approaches (of total 16S clones sequenced, 7.9% belonged to unclassified bacteria).

Lee Kerkhoff
Discussed technical issues related to techniques that he has used including mRNA analysis, Bromodeoxyuridine incorporation, ribosome fingerprinting and stable isotope fractionation.

Joel Kostka
Discussed their work on community analysis of biostimulated sediments as well as microcosms undergoing bioremediation.

Results from in situ biostimulation experiments:

a) Community composition of metal reducers and controlling environmental parameters elucidated for conceptual models
b) With proper integration of approaches and communication, relevant organisms may be detected, quantified, related to chemical parameters

Results from microcosms:

a) Rates of electron-accepting processes and carbon utilization can be used in reactive transport models
b) Provide more accurate rates than pure cultures for modeling
c) Effective for screening for organisms and substrates under realistic subsurface conditions

Frank Loeffler
Discussed the assessment and monitoring of groundwater bacteria specifically within the group Dehalococcoides and how techniques developed for that system could be used for monitoring metal bioremediation. Techniques include monitoring of gene products (mRNA), 16s rRNA genes and metabolites.

Cheryl Kuske
Discussed some technical questions and presented her ideas on critical needs in the area. Raised the question of how isolates can be used to understand the function of the community.

Discussion Summary

1. Identification of major players (microbial taxonomic groups) in metal bioremediation.

Related Questions

- How do we relate community structure to function?
- Establish cause and effect relationships among specific members of community and radionuclide precipitation.
- Spatial and temporal factors affecting sampling, modeling and interpretation of community structure.
- What are the mechanisms by which disturbances in subsurface conditions (pH change, donor addition, etc.) change the composition of the microbial community?
- We must think of succession in microbial communities and how it affects structure.
- Can minor populations play a vital role in the microbial community?

Recommended methodologies

- FISH probes for metal reducers
- Quantify active members of the population.
- Compare t-RFLP, clone libraries and microarrays and protein analyses to understand environmental microbial processes, i.e. standard protocols that would allow comparisons and rigorous side-by-side validation and verification of technologies to determine underlying uncertainties.
    - Biases associated with each.
    - How many clones should we sequence?
    - How quantitative are these techniques? Are there more quantitative techniques?
    - How much DNA to use for clone libraries? Template concentration of 10-15 ngs?
    - Specificity of probes?
- Can we eliminate the use of PCR?
- Stable isotope probing and stable isotopes combined with fatty acid analysis and DNA analyses, i.e. link signature analyses for species or functional groups to electron donors and/or electron acceptors to see who is being stimulated? Is bromodeoxyuridine incorporation a possible alternative to this approach?

2. Assessment of the effectiveness of bioremediation.

Related questions

- Determine whether lab sediment column and microcosm results can be extrapolated to the field, i.e. are there issues of scale and source acclimation that make microbial community changes resulting from biostimulation difficult or impossible to extrapolate to the field?
- What is the role of attached versus planktonic bacteria, i.e. do groundwater analyses really indicate changes in sediment communities that may be relevant to bioremediation?
- Sample diverse sites to determine common trends, i.e. do heterogeneities between sites still follow fundamental principles of necessary community functional components based on the background biogeochemistry?
- Identify functional genes for use in microarrays.
- Targets for deciding on promising treatments, long term monitoring.
- Specific probes for U(VI) and Tc(VII) reduction.

Recommended methodologies

- RNA techniques to identify important metal reducers:
  - Find ways to deal with the fact that RNA is very short lived.
  - Develop more sensitive RNA extractions techniques.
- Compare cores to samplers and groundwater.
- Develop effective sampling strategies for extreme heterogeneity in sediment characteristics (mineralogy, pore geometry.)
- Use PI coordination to increase replicability of approaches within the same field experiment (to combat sample heterogeneity.) Faster methods are needed to make data available for EM.