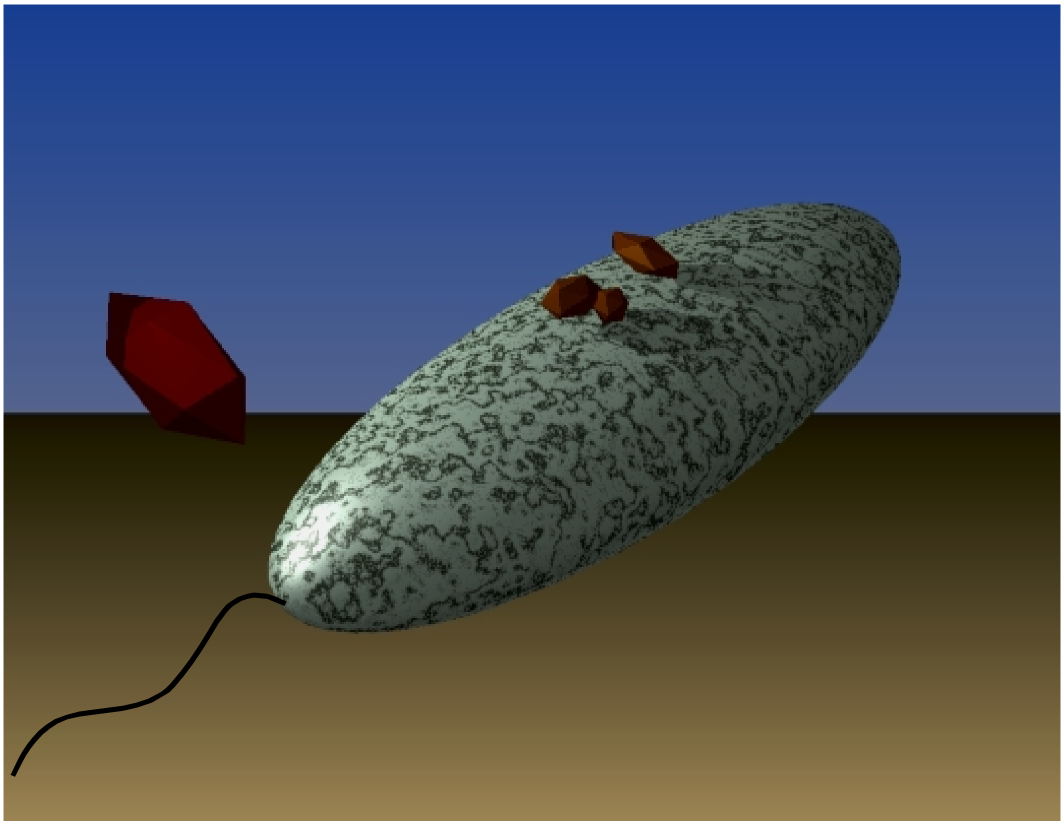
METAL/MICROBE INTERACTIONS WORKSHOP

October 11-13, 2000

Warrenton, Virginia



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Hosted by:

Yuri A. Gorby
Battelle PNNL
PO Box 999, P7-50
Richland WA 99352
Yuri.gorby@pnl.gov

Gill Geesey, Ph.D.
Montana State University, Bozeman
PO Box 173520
Bozeman MT 59717-3520
gill_g@erc.montana.edu

The Department of Energy's Office of Biological and Environmental Research sponsored a two-day workshop (October 12 – 13, 2000) that targeted the microbial and geochemical processes governing the interactions of bacteria with heavy metals, radionuclides, and mineral phases relevant to contaminated subsurface sediments. The objective of the workshop was to (1) provide a current state of knowledge of metal/microbe interactions, (2) to identify the knowledge gaps associated with these interactions, and (3) suggest scientific and technological approaches to address these gaps. Fifty scientists and educators from within and outside the Natural and Accelerated Bioremediation Program (NABIR) attended the workshop. Scientist with noted expertise in one of five topical areas lead general sessions (see agenda) and coordinated a series of short presentations that covered the current state of knowledge and identified gaps in our understanding of issues related to metal/microbe interactions. Breakout sessions were organized the following day to foster more detailed discussions of issues presented in the general sessions, and to provide some recommendations for research needs and approaches for meeting such needs. Implicit in these recommendations are benefits toward meeting the goals of the NABIR program while advancing our understanding of natural and accelerated bioremediation in general.

Notes Rendered by Workshop Participants

Microbe/mineral interfaces.

Most bacteria (>90%) in subsurface or sedimentary environments are attached to particulate phases. Hence, investigating the nature of the microbe/mineral interface is vital for understanding the role of bacteria in influencing or controlling the fate and transport of heavy metals and radionuclides in contaminated environments. Scanning and transmission electron microscopy have provided the most complete and detailed images of bacteria on minerals. Advances in imaging of hydrated samples or of samples that were flash frozen and analyzed at liquid nitrogen temperature has revealed that surfaces of cells rarely contact mineral surfaces directly. Rather, they are often separated by a layer of extracellular organic matrix or exopolysacharride. Recent work suggests that even metal reducing bacteria, which use oxide minerals as terminal electron acceptors, are shrouded with exopolysacharride that bridges distances of a few microns between the cells and minerals. How electrons are transferred from the bacterial surface to the oxide mineral during respiration is not known.

Ion-specific microelectrodes and fluorescence imaging tools are becoming available to investigate biogeochemical conditions with spatial resolutions needed for investigating microbe/mineral interfaces. Recent results have shown that steep gradients of oxygen, pH, and nutrients exist within the complex networks of microbial biofilms. It appears that the structure of biofilms respond to environmental conditions, such as hydrodynamic flow, in such a way as to optimize mass transfer of substrates, nutrients, and electron donors to members of the attached microbial community.

Most research examining microbe/mineral interactions is conducted using 'laboratory strains' operating under a defined set of conditions. Yet more fundamental questions remain regarding natural populations of attached bacteria on subsurface sediments.

1) What is the structure of natural microbial communities on subsurface materials? Are cells sparsely distributed, arranged in microcolonies, or present as members of a complex biofilm?
2) What are the critical differences between attached and suspended cells in the subsurface environment and how do these differences influence microbe/mineral interactions?
3) Do bacteria and minerals develop long-term relationships and, if so, how do these relationships impact long-term stability of reduced minerals?
4) What is the effect of mixed bacterial populations on porosity and transport within attached bacterial communities?
5) Does chemical sensing through specific intercellular molecular signaling contribute to bacterial migration, communication among bacteria, influence attachment of cells to mineral substrates with accompanying colonization, account for resistance to toxic minerals, and contribute to cellular transport of soluble toxic mineral?

By improving our understanding of the structure and members of natural microbial communities in the subsurface, investigators can define relevant laboratory systems to evaluate fundamental issues of microbe/mineral interactions. One caveat to this approach is that research should not be restricted to a specific organism or mineral as the "model", but should continue to generate information on a variety of organisms and minerals. Selection of organisms for which complete genomic sequences are known has certain benefits. Within a few years the number of microbes whose genomes have been sequenced will be much higher as the sequence technology improves. Greater emphasis should be given to approach, whether as a reductive or as a holistic focus, to provide new information of high impact.

Controlled laboratory experimentation is still needed to more fully understand the specific interactions between bacteria and minerals. An interdisciplinary approach is vital for addressing the complex interfacial interactions between bacteria and mineral surfaces. No obvious gaps of instrumentation were noted, but existing technologies need to be improved for 'real time' imaging of chemical states. Of particular importance is the development of high-energy probes with various spatial scales to study the bacterial micro-environment and characterize the mineral surface in the vicinity of attached bacteria. Microchips or other high-throughput technologies should be investigated as approaches to evaluate the genes expressed by bacteria at mineral surfaces over the range of conditions found in contaminated subsurface environments. These data will advance our understanding of the potential for bacteria to immobilize metals and radionuclides in subsurface environments and will address the follow set of fundamental scientific questions.

1) Where on the cell or on the solid mineral does the interaction occur and what degree of specificity is involved?

2) What are the temporal (i.e. kinetic) issues that characterize interfacial processes?

3) Do bacteria express phenotypic changes as the cells are exposed to the different minerals? What are the chemical specificities at the surface of the cells, and are these fixed or are they subject to change with different minerals?

4) Can changes in phenotype be verified and quantified through the use of molecular biology approaches (m-RNA, proteomics, etc.)?

5) How does stress response (starvation, stationary phase, pH, oxygen, etc) influence global gene regulation and contribute to surface changes of the cells?

Interfacial conceptual and mathematical models that incorporate biotic, as well as abiotic, processes at mineral surfaces need to be developed and tested. Non-linear modeling would be useful to evaluate the spatial and temporal scales over which bacteria and minerals interact. Establishment of such models has been hindered by a lack of understanding of what parameters should be included. Another challenge is how to normalize data collected at the micro-scale for construction of models that will be used to predict and interpret processes of interest at the field scale.

Characterization of natural attached communities.

A general lack of understanding of natural microbial communities hinders our understanding of biogeochemical processes of subsurface and sedimentary environments. Preliminary characterization of particle-associated microbial communities could exploit available imaging techniques. Scanning Electron Microscopy and Environmental Scanning Electron Microscopy remain powerful, but under-utilized, tools for directly imaging samples. Post-imaging geostatistical tools could be applied to quantify the spatial distribution of bacteria, protozoa, and fungi on particles obtained from subsurface sediments. Environmental Scanning Electron Microscopy can be used to image hydrated samples, minimizing alterations to attached communities that can occur during dehydration. Images of surface-associated bacterial populations may also be generated using cryopreservation and imaging techniques currently available for transmission electron microscopy.

As more phylogenetically-based oligonucleotide probes become available for subsurface microbial populations, it will be possible to define microbial community structure on mineral surfaces by coupling Fluorescent In Situ Hybridation (FISH) with confocal laser microscopy. Polysaccharide-specific fluorescent probes also offer the opportunity to evaluate and image the extracellular matrix between cells and populations. Collectively, these approaches provide information on the physical structure of attached microbial communities. Morphology and community structure of subsurface biofilms should then be correlated with predominant metabolisms or terminal electron-accepting processes.

Access to environmentally-relevant samples stands as an identifiable need for advancing our understanding of subsurface microbial communities. In-situ (or down-hole), extractable cylinders that can be packed with synthetic or natural metal oxide-bearing materials were suggested to facilitate retrieval of microbial/mineral samples from subsurface environments. By coupling packer technology with imaging techniques listed above, such samples could be used to monitor the temporal, in situ development of microbial communities on particle surfaces under environmentally relevant conditions.

Biogeochemical characterization of subsurface microbial populations is necessary for understanding the factors that control the fate and transport of heavy metals and radionuclides. Such characterization requires development and application of analytical techniques for evaluating environmental parameters, such as pH, Eh, and solute concentrations, at the microbe/mineral interface or within the influences of mineral surface-associated microbial populations. A better understanding of the surface properties of microorganisms and their spatial/temporal variation, and minerals in the subsurface environment, is needed to advance our knowledge of the physical, chemical, and enzymatic interactions of minerals and microbes.

Surrogate experimental systems are needed for contaminated, remote, subsurface sites. Accessible natural ore deposits offer promise as analogues for sites contaminated with heavy metals and radionuclides. Natural ore deposits could provide samples needed for characterizing the geochemical, mineralogical, and microbial nature of a naturally stabilized, reduced site. Samples could reveal geological records and mineralogical signatures of depositional processes. DNA and microfossil records should reveal the dominant microbial communities that directed the formation of the deposit, and possible clues on how to maintain system stability. Because many of the uranium ore-bearing formations are located outside the U.S., it was agreed that we should pursue collaborations with scientists and engineers in countries where these formations exist.

Biosorption/bioaccumulation of metals by bacteria attached to mineral surfaces.

Bacteria can sequester heavy metals and radionuclides through passive sorption mechanisms involving charged constituents on the cells surface (biosorption) or by an energy consuming process involving the transport to the interior of the cell (bioaccumulation). The current understanding of sorption and accumulation of heavy metals and radionuclides by bacteria attached to mineral surfaces compromises our ability to predict microbiological immobilization of contaminants in subsurface and sedimentary environments. The use of microorganisms and mineral phases that more closely mimic conditions in the field would aid in narrowing the knowledge gaps in this area.

Techniques are needed to obtain nonintrusive, *in situ* measurements of metal speciation, mobile, soluble, low molecular weight and colloidal organic phases that bind metals and radionuclides. Nonintrusive technologies should be developed for these determinations. Research is needed that integrates an experimental approach for data collection with mathematical modeling and other computational approaches to achieve better predictive capabilities of biosorption and bioaccumulation processes in the field. Information is needed from experiments designed and conducted by interdisciplinary teams of scientists and engineers. New experimental approaches need to be developed that incorporate processes of bioaccumulation and biosorption in studies of iron and sulfate reduction and biooxidations.

Bioreduction.

Bioreduction is generally recognized as an important metabolic process controlling the fate and transport of heavy metals and radionuclides, as well as the over all geochemistry of subsurface and sedimentary environments. Bioreduction of multivalent metals can (1) increase the concentration of dissolved Fe(II) and Mn(II) in groundwater, (2) transform oxide minerals into reduced mineral phases, such as siderite and magnetite, (3) and convert dissolved, oxidized forms of multivalent heavy metals and radionuclides, such as U(VI) and Cr(VI), to reduced forms that readily precipitate from solution. Much of the work conducted in the area of bioreduction has focused on controlled laboratory experiments, typically using axenic cultures and synthetic metal oxides as electron acceptors. Results from laboratory and field research indicate that most (>90%) metal-reducing organisms associate with mineral surfaces. However, little is know concerning the nature of this association and the specific mechanisms by which organisms transfer electrons to solid phase oxides during respiration. Investigations to describe the physical, chemical, and biological interactions between mineral surfaces and attached biota were unanimously recommended.

“Biofilm” was proposed as an operational term to refer to organisms and organic material that accumulate at mineral surfaces or other solid phases. However, biofilms that form on solid phases at contaminated subsurface sites may differ significantly in form and function from those found in other environments described to date. Knowledge of the arrangement of surface-associated microorganisms is necessary in order to more fully understand the factors (e.g., electron donor and acceptor availability, pH, enzyme kinetics, diffusional limitations) that control the biogeochemistry of redox-sensitive minerals. Hence, it is important to investigate the composition, structure, and activities of microbial communities associated with solid phases in iron-reducing environments.

The process by which metal-reducing bacteria transfer electrons to a metal oxide surface is poorly understood. Laboratory studies reveal that strategies for metal reduction may be species specific. Some organisms, such as *Geobacter* species, appear to require direct contact with oxide minerals. Others, such as *Shewanella*, may produce soluble electron shuttles that mediate electron transfer. Though *Geobacter* species appear to dominate iron-reducing communities in some subsurface environments, the dominant mechanism of iron reduction in natural iron-reducing environments is not fully known. Moreover, deployment of species other than *Geobacter* that produce soluble electron shuttles may be, in some cases, advantageous. Studies are warranted to address gaps in our understanding of the influence of various electron transport strategies on controlling (1) the rate and extent of metal and metal oxide reduction, (2) formation of various biogenic mineral phases, and (3) fate and transport of radionuclides and heavy metals.

Investigation of mineral surface-associated, metal oxide-reducing bacterial populations will be facilitated by developing techniques for investigating *in situ* iron reduction.

Specific technology development needs include non-invasive spectroscopic techniques for detecting and quantifying redox-active molecules that may be involved in electron shuttling. Towards this goal, some researchers (G. Luther and D. McKnight) are currently developing a quinone-specific microelectrode that may be able to detect quinones at the microbe/mineral interface.

Workshop participants recommended building collaborations between microbiologists, chemists, and physicists to identify and design probes that are needed to more fully describe the biogeochemistry of microbial communities on mineral surfaces.

Biooxidation.

Biooxidation is the use of reduced metals as the source of electrons for energy transduction. Biooxidation reactions may play an important role in the transformation of metals and minerals in the environment, including iron and multivalent contaminants such as uranium. Gaps exist in our knowledge of microbiologically-induced oxidation of subsurface environments. The extent to which reduced minerals are susceptible to biooxidation under the conditions that exist in subsurface environments is uncertain. The relative importance of biologically-mediated and abiotic oxidation reactions in metal-contaminated subsurface environments needs to be assessed. Molecular approaches that probe for genes encoding key oxidative enzymes in bacteria from these areas may be useful in identifying microorganisms that have the ability to oxidize mobile or immobile forms of reduced metals at contaminated field sites.

If biooxidation of reduced metals is an important metabolism in subsurface environments, further study would certainly be warranted. However, if biooxidation is shown as a minor contributor to natural cycling of multivalent metals, one should not dismiss the potential of this metabolism to affect the fate and long-term stability of contaminants as part of an engineered system. Controlled oxidation of reduced heavy metal and radionuclide precipitates through *in situ*, biologically-mediated reactions offers the possibility to mobilize the contaminant as part of a planned recovery operation. Controlled biooxidation may also have a role in the co-precipitation of reduced metal species, thereby buffering the immobilized metals from unpredictable natural oxidation reactions. Additional research is needed in the following areas: (1) mechanisms and products of biological oxidation, (2) the diversity, distribution, and physiology of metal oxidizers in natural ore deposits, (3) the specificity of oxidative enzymes, cytochromes, and other redox-active biomolecules, and (4) influence of biooxidation on metal toxicity.

Contaminant-mineral interactions.

Control over the biological processes at heavy metal- and radionuclide-contaminated sites is not likely to be achieved without an understanding of the abiotic processes taking place at the mineral surface. Information is lacking on the capacity of minerals to bind and interact with mobile metal and radionuclide contaminants. The long-term stability of many metal-mineral interactions is poorly understood, as is the kinetics of metal binding to mineral surfaces under environmental conditions. Information is also needed on the nature of films of organic molecules adsorbed to mineral surfaces and their role in metal interactions with mineral surfaces.

Summary of Recommendations

1. Investigate the nature of microbes associated with mineral surfaces in a variety of subsurface and sedimentary environments and under a range of terminal electron accepting metabolisms (aerobic, nitrate reducing, sulfate reducing, iron reducing).
2. Develop technologies (e.g., microprobes, non-intrusive spectroscopic methods) for investigating physical, chemical, and biological interactions at mineral surfaces.
3. Evaluate the effect of mineral surfaces on microbial physiological activities and responses.
4. Integrate experimental and computational approaches to better predict and interpret biogeochemical processes (e.g., biosorption, bioaccumulation, bio-oxidation, bioreduction). Define relevant parameters needed for kinetic and thermodynamic computational models focused on microbe/mineral interfacial processes.
5. Avoid using a "model" organism or environment. Support holistic approaches for investigating subsurface and sedimentary biogeochemistry.
6. Exploit microchip technology for investigating genes expressed by bacteria at mineral surfaces over the range of conditions found in contaminated subsurface environments.
7. Evaluate the role and relevance of biooxidation for influencing metal cycling and contaminant mobility in contaminated subsurface environments.
8. Continue to investigate mechanisms of iron/metal reduction and the various strategies used by bacteria to transfer electrons between bacterial and oxide mineral surfaces.
9. Determine best approaches for stimulating microbially-mediated mobilization or immobilization of metal contaminants in the subsurface.
10. Invest in meso-scale research to link bench-scale observations to field-scale processes. Develop laboratory bioreactor systems that more accurately reproduce relevant processes in the field, and are used by Field Research Center investigators.

Workshop Agenda

October 12

8:00 AM	Introduction and Objectives: Gill Geesey
8:30 AM	Bacterial/Mineral Interfaces: Larry Barton
10:00 AM	Break
10:15 AM	Biosorption/Bioaccumulation: Anne Summers
11:45 AM	Lunch
1:00 PM	Biooxidation: John Coates
2:30 PM	Break
2:45 PM	Bioreduction: Derek Lovley
4:15 PM	Controls on Iron Oxide Reduction: Jim Fredrickson
6:00 PM	Dinner

October 13

8:00 AM	Convene and Assemble Breakout Sessions
12:00 PM	Lunch
1:00 PM	Reconvene for Breakout Session Summaries and Open Forum Discussion
3:00 PM	Summary: Yuri Gorby
5:00 PM	Depart