

A Report Summarizing Scientific Deliberations at the:
“Earth Life Interaction at the Microbe-Mineral Interface Workshop”
held at Pacific Northwest National Laboratory on November 4-6, 2003

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Workshop Coordinators

I. Biogeochemistry Grand Challenge Concept

The William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a U.S. Department of Energy (DOE) national scientific user facility, has initiated planning activities for a Grand Challenge research effort in biogeochemistry to align with research programs in the Environmental Remediation Sciences (ERSD) and Life Sciences Divisions (LSD) of the DOE’s Office of Biological and Environmental Research (BER). Specific programs within BER that are relevant to the Biogeochemistry Grand Challenge include the Natural and Accelerated Bioremediation Research (NABIR), Environmental Management Sciences (EMSP), and Genomes: Genomes to Life (GtL) Programs. The Biogeochemistry Grand Challenge is to be a coordinated, multi-investigator research effort focused on resolving a major scientific issue not accessible to the single investigator. The science themes, debated and identified by a group of experts at a workshop entitled “Earth-Life Interaction at the Microbe-Mineral Interface,” held at the Pacific Northwest National Laboratory (PNNL) from November 4-6, 2003 in Richland, WA., are cutting edge research topics with potentially broad impacts. Advanced experimental and computational capabilities in EMSL and other DOE user facilities will be leveraged to resolve complex science issues and questions associated with the grand challenge.

II. Science Concept for a Biogeochemistry Grand Challenge

Microbial life has been closely intertwined with the geosphere for nearly the entire history of the Earth. Microorganisms, because of their small size and incredibly diverse metabolism, have a tremendous influence on their environment through the transfer of energy and materials across a complex biologic-solvent-solid interface. Although the microbial “sculpting” of the



SIDEBAR 1. Cells of the dissimilatory metal-reducing bacterium *Shewanella putrefaciens* CN32 on the surface of a tabular hematite (Fe_2O_3) particle. The cells gain energy from the oxidation of lactate coupled to the reduction of mineral Fe(III) through an undefined mechanism, possibly via a direct electron transfer from cell-surface associated multi-heme cytochromes to Fe(III) associated with the oxide surface.

geosphere is often evident at large scales over the surface of the planet, the interplay between microbes and geological materials is dominated by processes at the molecular and microscopic scales. The microbe-mineral interface is a prime example of this interplay and represents a complex, and relatively unexplored region (see SIDEBAR 1).

This interfacial region is dynamic with chemistry and structure determined by interplay and response. The molecular workings and linkages across this complex region are poorly characterized and the science required for their resolution spans broad fields in biology and the physical sciences.

In a colloquium sponsored by the American Academy of Microbiology, “Geobiology: Exploring the Interface between the Biosphere and the Geosphere,” (see SIDEBAR 2) it was concluded that the “real action” in geobiology happens at the level of



SIDEBAR 2. **“Geobiology: Exploring the Interface between the Biosphere and the Geosphere”** (*American Academy of Microbiology Report, 2000*). “The interplay of Life & Earth has shaped our present environment. Life and Earth, through this “Geobiological Tango” continue to choreograph the co-evolution of the biosphere and geosphere.”

individual cells or groups of cells. In the ensuing report from the colloquium (Nealson and Ghiorse, 2000), it was emphasized that the details of such processes would only be revealed by observations and measurements made at small scales. New advances in microscopy, spectroscopy, and computational chemistry, as represented by capabilities in the EMSL and other DOE user facilities, have potential to provide unprecedented opportunities to probe, characterize, and resolve fundamental biologic and chemical phenomena that occur in this important and unique microscopic domain. DOE is uniquely positioned to take on a microbe-mineral interaction grand challenge because of the capability of its user facilities and the biogeochemical focus of its environmental and biologic research programs.

Biogeochemical phenomena driven by fundamental biologic and chemical interactions at the microbe-mineral interface are significant to major environmental and geoscience research areas including:

1. Rock weathering and soil formation
2. Contaminant fate and transport
3. Environmental mineralogy and surface chemistry
4. Biogeochemical cycling of C, Mn, Fe, and other elements
5. Bioremediation of organic and inorganic contaminants
6. Environmental sustainability
7. Enhanced oil recovery
8. Radioactive waste storage and disposal

Accordingly, the microbe-mineral interface theme supports DOE mission areas in the environment, but also contributes to broader science issues and questions.

III. The Biogeochemistry Grand Challenge Science Workshop

Planning

Planning for the Biogeochemistry Grand Challenge began in January 2003 with dialogue between the PNNL coordinators and BER/ERSD staff. A preliminary scientific theme of microbe-mineral interactions was advanced by the PNNL coordinators at that time. BER/ERSD staff agreed that this was a suitable example topic, pending informal review at the NABIR annual program meeting. It was also decided that a workshop focused on identifying and prioritizing scientific opportunities for the Biogeochemistry Grand Challenge and a workshop report that summarized the most exciting of these opportunities would represent the conclusion of the initial phase of planning. This document summarizes the results from the Biogeochemistry Grand Challenge workshop.

An informal workshop was held in March of 2003 at the NABIR Principal Investigators Meeting to discuss the Biogeochemistry Grand Challenge concept and potential broad science themes in addition to the previously identified theme on microbe-mineral interactions. Approximately fifteen NABIR principal investigators participated in the meeting. These individuals had many questions on how a Grand Challenge activity would be initiated, managed, and supported (e.g., the conceptual model), but all participants were in agreement that the microbe-mineral interface theme was an excellent multidisciplinary topic with great potential for scientific impact. Competitive, alternative science themes that met the various programmatic and other constraints were not identified. After this affirmation of the science theme, and with DOE's guidance, a steering committee of prominent scientists was established (see Appendix 1) that covered a broad range of expertise related to the microbe-mineral theme. The represented disciplines on the steering committee included microbiology, biogeochemistry, geochemistry, mineral surface chemistry, and mineral physics. This steering committee assisted the PNNL coordinators in planning the program and identifying speakers for the workshop that is the subject of this report.

Workshop Objectives and General Organization

The primary scientific theme identified for the Biogeochemistry Grand Challenge, microbe-mineral interactions, was intentionally quite broad. The purpose of the scientific workshop was to utilize the breadth and expertise of the scientific community to narrow this theme to several workable, scientifically exciting topics. Specifically, a select group of sixteen experts from academia and the national laboratories (See Appendix 2) in the fields of microbiology, molecular biology, biochemistry, biogeochemistry, mineral surface chemistry and physics, and computational chemistry and biophysics were assembled to review the state of science in selected subdomains of microbe-mineral interactions and to help in identifying and prioritizing microscopic and molecular scale scientific subtopics that might align with ERSD research and EMSL capabilities.

The workshop was moderated by the PNNL coordinators with assistance from members of the steering committee and the workshop participants. The objectives of the workshop were to:

1. Identify and prioritize impactful research topics within the overall scientific theme to serve as subjects for the Biogeochemistry Grand Challenge
2. Determine key scientific issues and/or questions that underlie the two highest priority topics and identify example research that might be performed for their resolution
3. Evaluate computational and experimental capabilities that might be needed for the two highest priority topics, including those not currently available
4. Develop supporting information on why the selected subtopics are impactful, how they move the state of science forward, and how they contribute to DOE missions.

The workshop was divided into two major sections in order to accomplish the objectives (See agenda in Appendix 3). The first section (Day 1) was one of knowledge exchange where two sets of presentations were given by participants to define the state of science and research opportunities associated with several different subtopics of microbe-mineral interactions. In the first presentation set, two participants provided an overview of the microbiology and biogeochemistry of metal oxidation/reduction and biomineralization (“the environmental process as we know it”) stressing state of knowledge and research issues and challenges. The second presentation set involved five participants who discussed fundamental biologic/biochemical, microbiologic, and geochemical/mineral physical mechanisms involved in microbiologic metal oxidation/reduction and biomineralization. These presentations were intended to bring all participants up to a common knowledge state on biogeochemical electron transfer, one of the most important and ubiquitous microbe-mineral interactions in terrestrial and subsurface environments. The scope of these science discussions is summarized in the next subsection of the report.

The second section of the workshop (Day 2) was one of group deliberation to identify the two most promising grand challenge research topics. The day began with a presentation by the coordinators of an example science topic focused on electron transfer at the microbe-mineral interface. This example was derived from presentations and scientific discussions had on the first day of the workshop. The coordinator presentation identified why this was a suitable grand challenge topic from various perspectives. A series of potential research topics for the grand challenge were identified by the workshop participants through discussion of this example topic. These ideas were prioritized according to a series of criteria including multidisciplinary challenge and research opportunities, ERSD programmatic alignment, and potential scientific impact. These two research topics and supporting information on them were the primary products of the workshop.

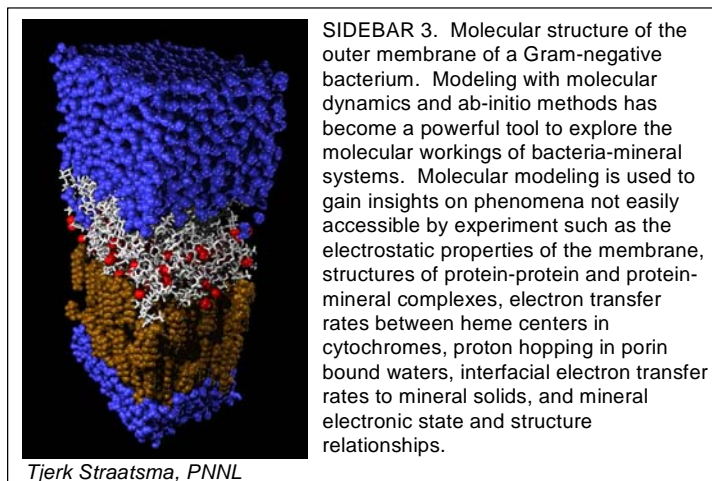
Scope of Science Discussions

By design and participant invitation, most of the scientific discussion focused on the microbe-mineral interface in terms of its structure and architecture, and the chemical and

energy exchange reactions that occur across that complex domain in the environment. Three prominent biogeochemical topics involving the microbe-mineral interface received the bulk of attention. These included: i.) how metal reducing bacteria sense and accomplish electron transfer, ii.) how metal oxidizing bacteria access reduced metal ion centers in mineral phases, and iii.) passive and active mechanisms of oxidative and reductive biomineralization on the outer membrane surfaces of bacteria. These scientific topics are prominent ones in the ERSD NABIR program, and underlie the environmental biogeochemistry of Fe, Mn, and trace metals and contaminants.

The molecular biochemical and mineral physicochemical interactions underlying these biogeochemical topics were explored by presentation and discussion. The molecular structure of the cell envelope and its implication to the dimensionality and chemistry of microbe-mineral interface, the structure and reactivity of outer membrane cytochromes and other enzymes involved in oxidation and reduction reactions, engagement mechanisms of cell surface molecules with mineral surface functional groups, and interfacial electron transfer into and within semi-conducting mineral material were discussed and judged to be

fundamental, underlying scientific issues that are not well understood. Newly evolving analytical molecular biogeochemistry capabilities at EMSL and other DOE user facilities and computational chemistry involving ab-initio, molecular dynamics, and larger scale simulations were seen, by presentation and EMSL tour, to offer great opportunities to unravel molecular scale

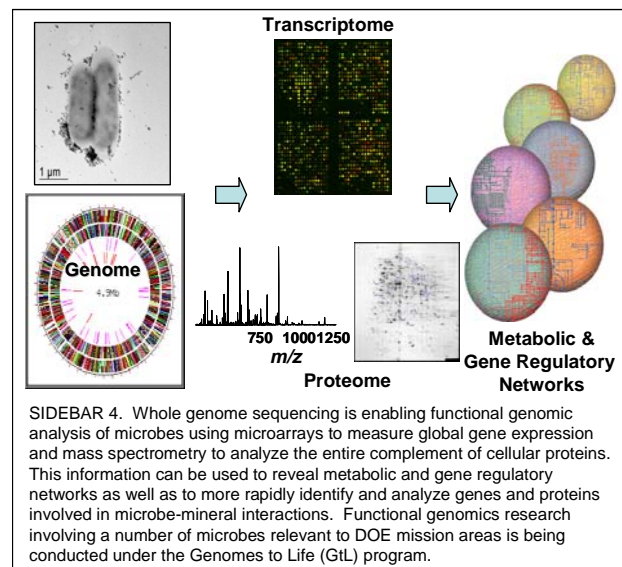


biochemical and mineral physicochemical phenomena that regulate the chemistry of the microbe-mineral interface (see SIDEBAR 3).

Scientific presentations about the electron transport chain in metal reducing bacteria, the induced formation of membrane bound redox proteins, and the electronic properties of oxide minerals illustrated the complex, subtle, and poorly-recognized interplay and chemical coupling that can occur between bacteria and the mineral surfaces upon which they reside. Many participants were not aware of this interplay and its fascinating implications to evolved biochemical functions of environmental organisms and the concept of a microbe-mineral association as a life unit. The recognition of the science involved in this interplay was a major workshop accomplishment. What are examples of this interplay? Metal reducing bacteria discriminate between isostructural Al(III)- and Fe(III)- oxides that have nearly identical surface properties. They preferentially populate the Fe(III) oxide surface that can be utilized as electron acceptor for respiration and that exhibits very different electronic properties from the Al(III) oxide surface. Bacterial reduction of Fe(III) in micron-sized layer silicates enhances layer charge, nutrient

retention, and crystallite structure all of which make the mineral-microbe association more hospitable.

Genomic information was found to offer great potential in the study of fundamental biogeochemical processes associated with the microbe-mineral interface. Its application was highlighted in several presentations where knowledge of the genome was used to delete or over express genes to test hypotheses about proteins involved in extracellular electron transfer. The study of fundamental biogeochemical processes has long been complicated by the fact that many bacteria employ multiple mechanisms to accomplish essential chemical tasks that are required for energy generation, such as electron transfer to an electron acceptor. While this mechanistic redundancy may be an effective defense against a variable and changing environment, it complicates the identification and characterization of specific controlling mechanisms.



Various examples of genetic manipulation to evaluate biochemical controls on reactivity at the cell surface were presented at the workshop, and these were an effective demonstration of the potential utility of the results and capabilities being developed by BER's Genomes: Genomes to Life Program (see SIDE BAR 4) to fundamental biogeochemical research.

The Two Top Science Topics Recommended by Workshop Participants

After considerable deliberation, two Biogeochemistry Grand Challenge topics (Table 1) were identified as holding the most promise for high impact research that aligned with the ERSD portfolio. Both of the topics involve fundamental molecular scale interactions that occur at the microbe-mineral interface and the complex interplay and response that occurs between the biotic and abiotic components. Multidisciplinary teams with a wide range of expertise and employing experimental and computational methods are required to address both of these topics.

The first of these topics (Table 1.1) is related to cell envelope and mineral surface chemical factors that regulate electron transfer (e.g., oxidation and/or reduction) between microorganisms and mineral material, and the effect that attached mineral solids may have on cell metabolism and in inducing unique biochemical responses. Included in this topical area are how membrane-associated proteins and other biomolecules interact with mineral surfaces of differing composition and charge, and how the assemblage of different chemical components on the cell envelope interact collectively to accomplish specific chemical tasks at the mineral-water interface. These cell surface components create a unique architecture that assembles against the mineral surface; this nano-scale

domain is only now becoming accessible to characterization through advanced microscopy, spectroscopy, and computational methods. Key are the influences of biomacromolecular interfacial structure, conformation, and surface coordination environment on the thermodynamics and kinetics of electron transfer, proton transfer, and other types of microbiologically-mediated chemical reactions. Also germane are physiologic and metabolic responses of microorganisms to their localization at the electrically charged, structurally complex mineral interface. Anecdotal observations reveal that microorganisms display intriguing responses to mineral surfaces; however the involved biochemical and biologic mechanisms are not at all understood. Bacteria may utilize redox sensitive proteins or other biomolecules on their membrane surfaces to recognize favorable and unfavorable mineral surfaces for colonization. Once recognized, molecules promoting adhesion or electron transfer may be internally produced and exported to the cell surface to enhance specific reactions at the mineral interface.

Table 1. Top Biogeochemistry Grand Challenge Themes

<p>1.) Influence of bacterial cell envelope components on the regulation of electron flux between cells and solids, and bacterial sensing and response to microenvironments.</p> <ul style="list-style-type: none"> • Structure/conformation-reactivity relationships of bacterial biomolecules with mineral surfaces • Interfacial electron transfer mechanisms to the mineral phase and participating biomolecules • Sensing/recognition and response to extracellular minerals
<p>2.) Interplay between microorganisms and the surface, structural, and physicochemical properties of minerals</p> <ul style="list-style-type: none"> • Coupling between the electron/proton transport system of bacteria and mineral electronic states • Role of mineral defect sites and structures (surface/bulk, point/diffuse, chemical/physical) in microbiologic reactions • Local biogeochemical gradients, template effects, and reductive/oxidative biomineralization

The second topic (Table 1.2) pertains to the interplay between microorganisms and the surface, structural, and physicochemical properties of minerals, with emphasis on the colligative biogeochemical behavior of the microbe-mineral association. Poorly recognized and understood is the interplay between organisms and solids that occurs via the coupling of the electron and proton transport systems in microorganisms with the surface chemical and bulk electronic properties of the solid. Oxides of Fe and Mn are semiconductors with very different band gaps and electrokinetic properties, meaning that they respond differently as electrons and protons are added or withdrawn from their surfaces and structures. Changes in electron density induced by microorganisms may be localized to the surface or structurally dispersed depending on these properties and the size of the mineral particle. Accordingly, the associated mineral phase may modulate bacterial activity in complex fashion through these properties, or through structural rearrangements or surface chemical reactions that dissipate energy or alter electron or proton density. An important facet of this topic is the role of surface and structural

defects in the mineral phase that typically represent focal points for reactivity (e.g., Brown et al., 1999). These defects may be chemical or physical and often represent microscopic domains in the mineral phase that contain excess energy that may be more favorable for microbial exploitation. Alternatively, through their spatially directed microscopic activity, microorganisms may generate defects on or within the mineral phase that alter mineral stability and future reactivity with other system components. Included also in this topic is the important issue of biomineralization. The close spatial association of active bacteria with mineral surfaces creates chemical gradients that drive the formation of new biominerals through solid-state transformation and heterogeneous nucleation on mineral surfaces and cell surface components that serve as templates (Fortin et al., 1997). These oxidative and reductive biomineralization products typically exhibit small particle size and are the most reactive mineral phases found in the environment.

Example Research that Could be Conducted under the Two Topics

Specific examples are provided here of multidisciplinary research that could be conducted under each of the two topics. These two focused topics are still quite broad and a number of other good research examples could be cited for each of them.

1.) *Influence of Bacterial Cell Envelope Components on the Regulation of Electron Flux between Cells and Solids, and Bacterial Sensing and Response to Microenvironments*

Electron transfer between microbial cells and solids is a fundamental process that controls energy exchange throughout the geosphere yet the mechanisms by which this occurs remain obscure. A prime example is the problem faced by bacterial electron transport coupled to poorly soluble extracellular electron acceptors such as Fe(III) and Mn(III, IV) oxides (Figure 1). For the majority of respiratory processes involving O₂, nitrate, sulfate, or CO₂, the substrates freely move across the outer membrane into the periplasm, typically through porins or ion channels, where they engage with various electron transfer proteins. In the case of Fe and Mn oxides, direct engagement of the periplasmic proteins with Fe(III) or Mn(III, IV) is not possible because these exist as solids external to the cell surface and are unable to pass the outer membrane.

Certain bacteria that are proficient at metal oxide reduction appear to have solved this problem by localizing multi-heme cytochromes to the exterior of the outer membrane where the proteins can potentially engage directly with oxide surfaces and transfer electrons as depicted in Figure 1. Although this is an attractive model, many aspects remain unresolved including the fundamental problem of how electrons are moved from inside the cell to the cell exterior in an energy conserving manner, termed solid-state respiration (Nealson and Little, 1997). Some researchers have also suggested that soluble organic compounds with electron donating and accepting properties may be produced by metal-reducing bacteria that facilitate electron transfer across the outer membrane via a shuttle type mechanism. The biochemical mechanisms by which metal-reducing bacteria transfer electrons to poorly soluble metal oxides will have a profound impact on the electron flux from cells to solids.

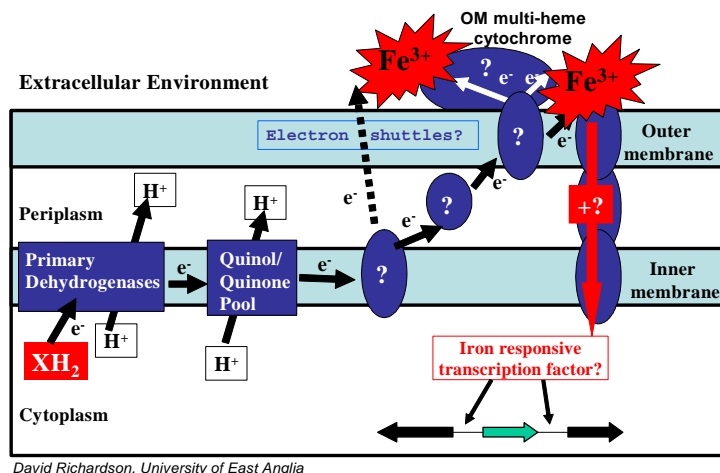


Figure 1. Model of electron transfer to extracellular Fe(III) oxides proposed for metal-reducing *Shewanella* species. Electrons are transferred from primary hydrogenases through an electron transport chain that extends from the cytoplasm and through the inner membrane to the periplasm. At this point the electrons may be transferred, by an unknown mechanism, through the outer membrane to extracellular Fe(III). The terminal electron transfer mechanism may include outer membrane redox proteins or electron shuttle compounds.

Another intriguing issue associated with the microbial respiration of insoluble electron acceptors is how the cells are able to sense the presence of extracellular substrates and switch on the expression of essential electron transport system genes. In *Shewanella*, the addition of Fe(III) to cells can cause large increases in the expression of a series of *c*-type cytochromes that are not observed when other electron acceptors, such as Mn(IV), are provided. This suggests that this bacterium has a specific system for sensing and responding to Fe(III). A potential mechanism for Fe(III)-specific gene regulation in *Shewanella* has recently been explored by D. Richardson (U. of East Anglia) and involves iron-induced flavocytochrome *c* (*ifc*) gene clusters that have been identified in several species of *Shewanella*. The *ifcA* protein, a periplasmic tetraheme cytochrome, is produced specifically in response to Fe(III). The *ifcR* gene, which encodes a regulatory protein, was shown to be involved in Fe regulation but was not subject to autoregulation. It is believed to be a component of a larger regulatory cascade and therefore is not directly responding to Fe itself but rather to another unidentified regulator within the cell. The specific iron response regulator remains to be determined but is suspected to be analogous the ferric uptake regulator or *fur* gene. *Fur* is a global regulator that functions as an iron-responsive repressor that utilizes Fe(II), as opposed to Fe(III), as a co-factor and binds to specific sequence elements in target promoters of iron regulated genes. In response to Fe limitation *fur* no longer binds the operator site and transcription from target promoters proceeds. In addition to identifying the Fe(III) global response regulator, a fundamental issue is the mechanism by which the regulator interacts with Fe(III) given its limited solubility, extracellular location, and surface structure and site characteristics.

2.) *Interplay between Microorganisms and the Surface, Structural, and Physicochemical Properties of Minerals*

Microorganisms adhere to and colonize mineral surfaces for various reasons. Some bacteria utilize Fe(III) or Mn(IV) containing solids as electron acceptors, others recover energy from structural Fe(II) or Mn(II) in mineral solids by enzymatic oxidation, while still others seek structural phosphorous or trace metals to satisfy nutrient requirements. Common to all of these microbiologic processes is the bacterial need to access and react with near surface and structural ions through microbe mediated phenomena at the mineral-water interface. Such access may be gained by slow dissolution processes mediated by biogenic organic acids or complexants that release the target ions, or by chemical transfer between cell surface components and ionically bound, inorganic structural constituents of the mineral medium.

A common tenet in mineral surface chemistry is that structural and chemical defects are focal points for both surface and bulk reaction. Defects may occur as structural vacancies (i.e., where an O or Fe is missing in an iron oxide structure), as foreign chemical substituents (i.e., where Al^{3+} substitutes for Fe^{3+} in an iron oxide), or as structural discontinuities (e.g., screw, step, or edge dislocations) (see Figure 2). Defects may occur at the surface or within the bulk and represent points or regions in the solid where energy is perturbed. Defects are ubiquitous in natural mineral matter but their role in microbe-mineral interactions is unknown. Important multidisciplinary research questions are whether organisms: i.) exploit the energetic perturbations present in defects to gain access to structural ions needed as energy or nutritional sources, and ii.) generate defects by their own action that regulate the overall biogeochemical behavior of the microbe-mineral association.

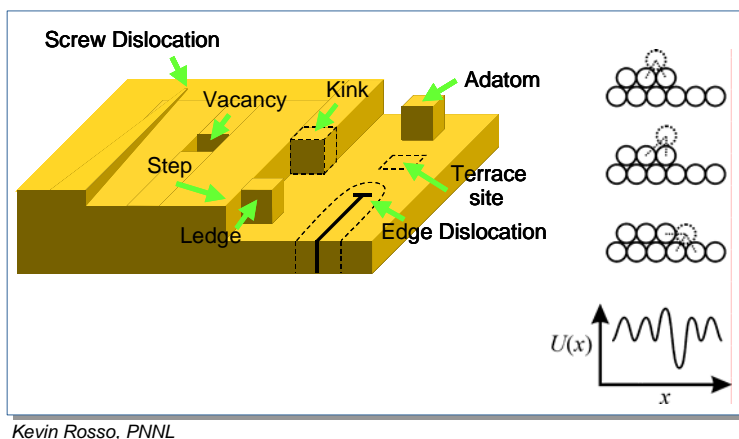


Figure 2. There are many forms of surface defects that range in size from subnanometer (vacancies) to micron (structural dislocations in size). These are sites that exhibit different energetic properties from the bulk. Theoretical and anecdotal lines of evidence confirm their reactivity.

Published research has shown striking patterns of bioreductive dissolution on mineral surfaces that contrast sharply with the elliptical-shaped organism footprint. In Figure 3a, the dissolution features display crystallographic control and alignment with structural defects associated with screw and step dislocations. Implied is the preferential dissolution of high energy structural regions associated with the defects promoted by a nonlocal electron transfer process, or electron migration to defect sites from the point of biotic discharge. Either way, the mechanism remains entirely speculative. Resolving this mechanism would provide insights on how microorganisms extract energy from complex natural Fe(III) oxide minerals.

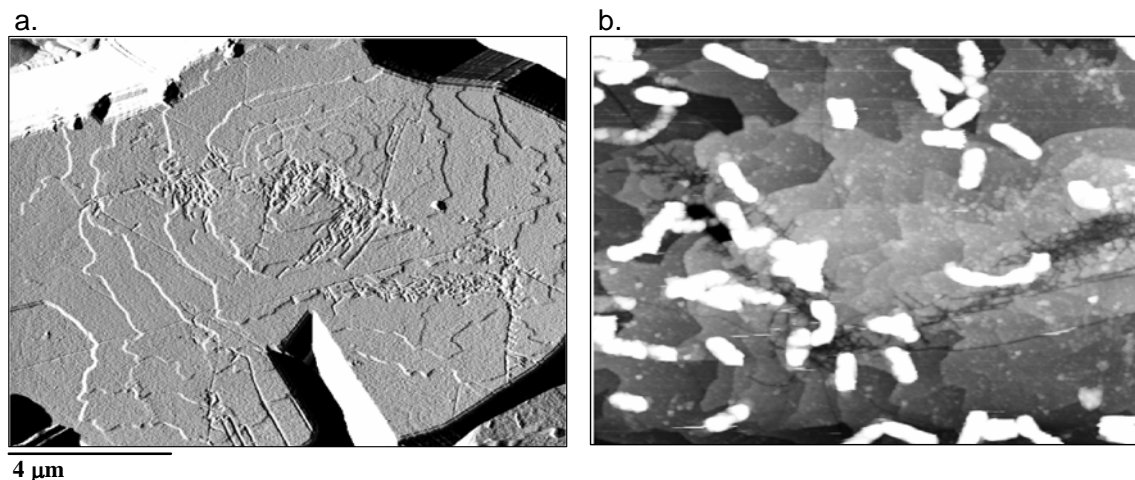


Figure 3. Dissolution features on the surface of hematite (a) resulting from the activity of attached iron reducing bacteria correlate with defect locations and crystallographic structure, rather than the organism's footprint (white rods in b). The images were obtained by scanning probe microscopy.

Fruitful areas of research could probe issues of electron and proton mobility in relevant mineral solids and mixtures with different defect densities and types as driven by surface associated bacteria. Fundamental studies would seek to ascertain the influence of the individual defect structures and their integrated features on the electronic and other physicochemical properties of the solids, and the attendant effects of these on the spatial locations and overall extent of microbiologic reactivity. Physical-chemical techniques (microscopy, scanning probe microscopy, spectroscopy, and computation) merged with modern molecular biology and microbiological techniques could yield fascinating results. Mechanistic issues associated with biotically driven oxidation/reduction front propagation in Mn and Fe oxides and Mn and Fe-containing silicates (layer silicates, basaltic glass, olivines, etc.) could be evaluated to provide molecular scale insights on reaction types common to weathering and the bioremediation of metals and radionuclides.

Another variation on this area of research would be to investigate the reactivity of chemical or structural defects caused by microbiologic activity. Microorganisms often induce chemical change to local spatial regions of mineral particles proximate to the areas that they colonize (e.g., Figure 3b). The nature of defects that they cause and their

density and structure are different from those created by abiotic processes, and these differences may affect subsequent particle reactivity. Examples of this could include the behavior of bacterially generated Fe(II) sites on the surfaces of Fe(III) oxides or within the structure of layer silicates. These sites serve as reductants for many contaminants, and their biologically driven spatial distribution may impart unique electronic effects that cause different thermodynamic/kinetic properties from analogous abiotic systems.

IV. Potential Science Contributions

Since early field and experimental observations of microbes dissolving and forming minerals, scientists have puzzled and speculated at the underlying mechanisms. With the increasingly sophisticated techniques for probing, at high resolution and sensitivity, the chemical and physical attributes of solids and solutions in combination with the emerging biological details of microbes being afforded by genomics, the mechanistic details of microbe-mineral interactions can potentially be revealed. These opportunities were highlighted in a recent review of geomicrobiology (Banfield and Nealson, 1997).

Microbial reduction of metal oxides, a topic examined in detail during the BGC workshop, provides an excellent example. An understanding of how microbes regulate electron flux to metal oxides or metal-containing silicate minerals can provide important underpinnings for remediation science. For example, biogenic ferrous iron can function as a reductant of organic and inorganic contaminants, promoting their *in situ* transformation and/or immobilization as a means of remediation. The same fundamental scientific contributions could also lend important insights into the microbial weathering of oceanic and basaltic glass, thought to be a potentially important global mechanism of weathering. The potential energy and nutrients within basalts may also support chemolithotrophic microbial communities in aphotic environments. These same scientific insights can also lead to an understanding of how microbes contribute to the dissolution and precipitation of economically important minerals.

The physical association between microbes and mineral surfaces is often a requisite for reactivity yet the mechanisms and biomolecules involved in microbial attachment to surfaces are poorly understood. Microbes produce a myriad of compounds and biomolecules that are secreted to the outside of the cell or are associated with the cell envelope that are involved in binding and transporting metals or in promoting the physical attachment of cells to surfaces. For example, many Gram-negative bacteria produce adhesions and pilins or other fiber-like structures such as curli that have been implicated in cell-cell and cell-surface attachment. The genomes of microbes known to interact with metals and minerals contain genes for the compound, and structures yet their molecular role in microbe-mineral interactions is unknown. The understanding of the regulation and function of these genes is critical for establishing how microbial cells physically adapt to, interact with, and modify the reactivity of mineral surfaces. In addition, the results from microbe-mineral research are certain to more broadly impact other science areas including biocorrosion and microbe-host interactions where the surfaces of interest are not inorganic but rather biologic.

The compelling microbe-mineral interaction topics identified for the Biogeochemistry Grand Challenge have potential for drawing together a broad range of disciplines in ways that have not been previously achieved. In fact, it is likely that achieving true integration of disciplines will be essential for the success of the Grand Challenge. In this way it can serve as a model for other emerging, interdisciplinary collaborations in other fields. The science that will be accomplished is anticipated to lead to groundbreaking scientific results in geomicrobiology and related fields.

References

Banfield, J. F., and Nealson, K. H. (1997) Geomicrobiology: Interactions Between Microbes and Minerals. *Reviews in Mineralogy Volume 35*. Mineralogical Society of America.

Brown, G. E., Jr., Henrich, V., Casey, W. H., Clark, D. L., Eggleston, C., Felmy, A. R., Goodman, D. W., Gratzel, M., Maciel, G., McCarthy, M. I., Nealson, K. H., Sverjensky, D., Toney, M. F., and Zachara, J. M. (1999) Metal Oxide Surfaces and Their Interactions with Aqueous Solutions and Microbial Organisms. *Chemical Reviews* 99:77-174.

Fortin, D., Ferris, F. G., and Beveridge T. J. (1997) Surface-Mediated Mineral Formation by Bacteria. *In: Geomicrobiology: Interactions Between Microbes and Minerals. Reviews in Mineralogy Volume 35:161-180*. Mineralogical Society of America.

Nealson, K., and Ghiorse, W. (2000) Geobiology: Exploring the Interface between the Biosphere and the Geosphere. *American Academy of Microbiology Report*.

Nealson, K. H., and Little, B. (1997) Breathing Manganese and Iron: Solid State Respiration. *Advances in Applied Microbiology* 45:213-239.

Appendix 1

Biogeochemistry Grand Challenge Steering Committee

Terry Beveridge, *Univ. of Guelph*, ultrastructure of bacterial cells

Jim Fredrickson, *PNNL*, microbial ecology and biogeochemistry

Gill Geesey, *Montana State Univ.*, microbial biofilms and biogeochemistry

Mike Hochella, *Virginia Poly. Inst.*, molecular geochemistry

Ken Nealson, *Univ. of So. California*, microbial geochemistry

Jim Tiedje, *Michigan State Univ.*, molecular analysis of microbial communities

John Zachara, *PNNL*, biogeochemistry and mineral surface chemistry

Appendix 2

Earth-Life Interaction at the Microbe-Mineral Interface Workshop Participants

DOE/OBER Representatives

Teresa Fryberger, Paul Bayer, and Jeff Day

PNNL/EMSL Representatives

Bill Rogers, Allison Campbell, Doug Ray, and Harvey Bolton, Jr., Andy Felmy, Michel Dupuis, Yuri Gorby, Margaret Romine, Alex Beliaev, Mike Bowman, and Mike Kennedy

Other

Bob Marianelli

Invited Participants

John Bargar, *SSRL*, X-ray spectroscopy and biogeochemistry

Daniel Bond, *Univ. Massachusetts, Amherst*, microbiology of metal reduction

Tom DiChristina, *Georgia Tech*, molecular biology/biogeochemistry of anaerobic bacteria

Carrick Eggleston, *Univ. of Wyoming*, mineral surface structure and electron transfer

Scott Fendorf, *Stanford Univ.*, biogeochemistry and surface chemistry

Volkhard Helms, *Univ. of Saarland*, computational biology

Sam Kaplan, *Univ. of Texas, Houston*, gene regulation

Terry Meyer, *Univ. of Arizona*, structure and reaction of cytochromes

David Richardson, *Univ. of East Anglia*, bacterial electron transfer

Eric Roden, *Univ. of Alabama*, bacterial metal reduction

Kevin Rosso, *PNNL*, molecular modeling of electron transfer at mineral interfaces

Alan Stone, *Johns Hopkins Univ.*, surface complexation at mineral surfaces

Tjerk Straatsma, *PNNL*, molecular modeling of membranes and cytochromes

Brad Tebo, *Univ. of CA San Diego*, bacterial metal oxidation

Lynmarie Thompson, *Univ. Massachusetts*, NMR spectroscopy of membrane bound proteins

Sam Traina, *Univ. of California, Merced*, contaminant biogeochemistry

Appendix 3

Earth-Life Interaction at the Microbe-Mineral Interface **Workshop Agenda**

Tuesday, November 4

- 8:00 am Registration – EMSL Lobby
- 8:30 - 8:45 Introduction
- 8:45 - 9:00 DOE perspective and workshop motivation (Teresa Fryberger, OBER/DOE-HQ)
- 9:00 - 9:30 The Grand Challenge concept and theme, and workshop objectives (PNNL coordinators)
- 9:30 - 9:45 *Question & Answer Session*

The Environmental Process As We Know It

Presentations to define the state of science and key research issues and challenges in the microbiology and biogeochemistry of metal oxidation/reduction

- 9:45 - 10:30 Microbiology and biogeochemistry of metal oxidation (B.M. Tebo, UCSD)
- 10:30 - 11:15 Microbiology and biogeochemistry of metal reduction (S. Fendorf, Stanford Univ.)
- 11:30 - 11:45 Discussion
- 11:45 - 12:30 *WORKING LUNCH [The morning presentations will be reviewed and discussed, and an introduction will be provided to the EMSL Facility in advance of the tour]*
- 12:30 - 1:45 EMSL tour [selected facilities relevant to grand challenge theme; two groups]

Mechanistic Aspects

Presentations to highlight fundamental mechanisms and experimental/computational capabilities, and identify major research opportunities and issues. Presenters are to allow 10-15 minutes for discussion.

- 1:45 - 2:25 Electron transfer in microbial respiration and iron reduction (D. Richardson, Univ. of East Anglia)

- 2:25 - 3:05 Structure of the organism/mineral interface – ultra structure, adhesion, protein alignment (T. Beveridge, Univ. Guelph)
- 3:05 - 3:20 *Question & Answer Session*
- 3:20 - 4:00 Bacterial strategies of electron exchange with metals and solids (T. DiChristina, Georgia Tech)
- 4:00 - 4:40 Environmental surface chemistry of metal reduction and oxidation (A. Stone, Johns Hopkins Univ.)
- 4:40 - 5:20 Molecular mechanisms of electron transfer at mineral interfaces (K. Rosso, PNNL)
- 5:20 - 5:30 Wrap-up
- 6:30 - 9:30 Group Dinner

Wednesday, November 5 – Identification of Biogeochemistry Grand Challenge Research Topics

- 8:00 - 8:45 Example BGC research topics (Coordinators/Advisors)
- ❖ Example topics that align with OBER programs and science areas
 - ❖ Discussion of their merits and limitations and potential broader impacts
- 8:45 - 10:15 Solicitation and discussion of additional topics from the group (session to include opportunity for each participant to make a recommendation)
- 10:15 - 10:30 *Question & Answer Session*
- 10:30 - 12:00 Prioritization of all topics
- ❖ Scoring matrix and criteria (all to score)
 - ❖ Discussion and calibration of results
- 12:00 - 1:00 *WORKING LUNCH [Discussion of the initiation, organization, and integration of grand challenge activities]*
- 1:00 - 3:15 Discussion of two “best” topics
- ❖ Potential science questions
 - ❖ Short, medium and long term research opportunities
 - ❖ Model systems
 - ❖ Available and needed capabilities (experimental and computational)

- ❖ Anticipated accomplishments
- ❖ Estimated level of effort

- 3:15 - 3:30 *Question & Answer Session*
- 3:30 - 4:15 Requirements and components of a successful grand challenge
- ❖ Scope
 - ❖ Assembly
 - ❖ Linkages and integration
- 4:15 - 4:45 Potential spin-offs of BGC research to other science areas and topics
- 4:45 - 5:15 Concluding remarks and final discussion (Coordinators and DOE)
- ❖ Summary of deliberations and recommendations
 - ❖ Next steps to be taken including the workshop report
- 5:15 Adjourn

Thursday, November 6

Attendees: John Zachara, Jim Fredrickson, Teresa Fryberger, Paul Bayer, Jeff Day, Bob Marianelli, Terry Beveridge, Gill Geesey, Mike Hochella, Ken Nealson, Allison Campbell, Doug Ray, and Harvey Bolton, Jr.

- 8:00 am CLOSED SESSION
- ❖ Evaluate workshop accomplishments
 - ❖ Discuss priority science ideas
 - ❖ Determine content and organization of workshop report
 - ❖ Identify next steps in grand challenge process
- 12:00 pm Adjourn