Natural and Accelerated Bioremediation Research Program

Workshop Report

Research Opportunities in Biogeochemical Dynamics

Prepared by Raymond E. Wildung and Frank J. Wobber

U.S. Department of Energy
Office of Energy Research
Office of Biological and Environmental Research
Environmental Sciences Division

Contents

Introduction

This report summarizes the results of a workshop on Research Opportunities in Biogeochemical Dynamics, conducted August 4-5, 1998, in Vancouver, Washington, under the auspices of the U.S. Department of Energy Natural and Accelerated Bioremediation Research Program (NABIR). The report describes the background, objectives, approach, and organizational themes for the workshop and summarizes the results of deliberations to identify key research needs and collaborative opportunities in the biogeochemistry of metal and radionuclide behavior in heterogeneous subsurface environments at DOE sites.[a] The meeting agenda and the list of participants are given in Appendices A and B, respectively.

Background

Overall Program Goal

The primary goal of the NABIR Program is to develop a fundamental scientific basis for intrinsic and accelerated *in situ* bioremediation of metals and radionuclides at DOE sites. A key to the success of the program will be understanding the biologically mediated geochemical processes of importance in controlling contaminant behavior over the broad range of geohydrologic conditions at DOE sites. Major challenges include defining the principal biogeochemical processes operating in the subsurface at DOE sites, including the controls on the rate and direction of these processes, and upscaling this information to the field.

Biogeochemical Dynamics Workshops

Previous workshops were undertaken to survey bioremediation field technologies and to evaluate the manipulation of biogeochemical processes as a strategy for extending the range of subsurface conditions available for scale-up of laboratory research to the field. This workshop builds on the results of prior workshops and current NABIR research and begins the process of further defining and prioritizing research needed to understand the biogeochemical processes that influence the behavior of metals and radionuclides in heterogeneous subsurface environments.

Workshop Objectives

The objectives of the workshop were to (1) develop themes for focusing research on key biogeochemical processes underlying bioremediation of metals and radionuclides, and (2) define future research needed to understand how these processes are manifested at the field scale.

Research Themes

The workshop discussions were structured around proposed research themes or scientific topical areas of extraordinary benefit to field-scale bioremediation, in which there is (1) strong potential for scientific progress (major advances in the state-of-the-art are needed), and (2) collaborative, cross-disciplinary research that promises substantial scientific advancements compared to individual investigator studies.

(a) Workshop discussion was recorded by scribes identified in Appendix A.

Research themes included:

- Microbial transformation of subsurface mineral phases and effects on contaminant mobilization/immobilization (dissolution/desorption or promotion of strongly sorbed, occluded, or coprecipitated contaminants).

- Microbially mediated complexation processes (effects of complex formation/degradation on contaminant aqueous/solid phase interactions).

- Effects of natural subsurface heterogeneity on the distribution, rate, and direction of these processes (e.g., mass transfer controls on microbial distribution and nutrient and electron donor/acceptor dynamics) and scaling these processes to the field.

A series of questions was used to focus discussions on technical gaps, research needs, and approaches within these themes (see section on Technical Gaps, Research Needs, and Approaches, below) and to identify a possible technical focus for scaling biogeochemical processes in heterogeneous systems.

Workshop Approach

The workshop was organized in sessions designed to:

- Review the results of previous workshops that provided perspectives on DOE field environments, primary research issues, and possible use of core-scale approaches for extrapolating mechanistic studies to the field.

- Summarize ongoing NABIR research on the research themes and experimental resources/approaches for examining process coupling and upscaling.

- Based on the above, identify technical gaps and potential integrated experimental approaches for translating process-level understanding to natural systems.

The results of discussions addressing each of these topics are outlined in Appendix C and summarized in the following sections.

Perspective

Previous workshops offered insights into the information and approaches that must be developed in order to take advantage of subsurface biogeochemical processes for remediation of metal and radionuclide contamination in heterogeneous field environments. Principal conclusions from previous workshops included the following:

- Biogeochemical processes are the essential foundation for intrinsic and accelerated bioremediation in natural subsurface environments.
- Prediction of the rate and direction of biogeochemical processes *in situ* is generally not possible based on current knowledge.
- Principal gaps in knowledge center on a lack of understanding of
  - biological mediation of coprecipitation, dissolution, and complexation reactions controlling contaminant mobilization/immobilization in mixed contaminant systems
  - secondary effects of biogeochemical processes, such as resorption of dissolved species and subsequent effects on reactive surface areas and contaminant adsorption
  - kinetics of nonlinear, slow-rate biogeochemical processes that often occur under subsurface conditions
  - spatial heterogeneity in microbial, geochemical, and physical properties and processes that govern scaling of biogeochemical phenomena from the molecular and microscopic scales, where biological mediation of geochemistry occurs, to the meter and larger scales where lithology and convective processes may dominate and remedial actions are instituted and measured
  - the integrated effects of manipulation of subsurface conditions for bioremediation
- New tools/approaches are needed to measure *in situ* properties (e.g., microbial biomass), processes (e.g., biologically induced chemical gradients at the pore scale), and process dynamics (e.g., effects of electron donor and nutrient fluxes resulting from advective processes) that serve as the foundation for bioremediation.
- Intact core analyses and manipulation offer potential for economically bridging the gap between the biogeochemical and the field scales, but major challenges exist in core retrieval, preservation, and experimental design.

Current Knowledge

The results of NABIR research on the principal biogeochemical processes operating in the subsurface are summarized in Appendix D, which outlines the central hypotheses, research approach, and key initial results of projects currently in the Biogeochemical Dynamics Program Element. Projects that include a significant scaling component also are identified.

Current research in the Biogeochemical Dynamics Program Element is highly pertinent to the workshop themes, encompassing microbially mediated iron, sulfur, and complexation processes influencing both contaminant immobilization and mobilization. The issue of co-contaminant effects (nitrate) is also addressed. Logical extensions of the research include other potentially important microbial processes (e.g., acetogenesis, methanogenesis, and denitrification) and the effects of other contaminants (e.g., metal mixtures and carbon sources), which may also influence contaminant behavior.

Advanced (e.g., synchrotron-based) chemical speciation methods, geochemical modeling, and scaling approaches provide a fundamental base for initial extrapolation of the results to other environments. However, issues of heterogeneity and scale-up are experimentally addressed in only two of the six projects, and new research emphasis in these areas is required to provide an adequate basis for design and assessment of bioremediation processes in the field.

Experimental capability and tools available in NABIR for extrapolation of mechanistic understanding of biogeochemical processes to the field are outlined in Appendix E. An impressive array of approaches is available, including use of the Subsurface Microbial Collection to expand mechanistic understanding to different organisms isolated from a range of environments; advanced geostatistical tools to describe and predict the distribution and activity of microorganisms; intermediate-scale flow systems to examine biogeochemical processes under controlled conditions of heterogeneity and flow; advanced tracer and imaging methods to examine the relationships between microbially mediated processes and pore scale physical and geochemical properties; and geophysical and manipulative hydrogeologic tools for examining the effects of heterogeneity on microbial processes at the lithologic and geologic scales.

Coupled with research underway in the Biogeochemical Dynamics Program Element these capabilities would offer unparalleled insights into the effects of heterogeneity on the distribution and function of biogeochemical processes in natural systems.

Technical Gaps, Research Needs, and Approaches

Research themes, approaches, and collaborative opportunities for extending biogeochemical process-level understanding to the field are described below. Conclusions derived from workshop presentations and discussions (summarized in Appendices C and D) are outlined according to key questions used to organize workshop discussion.

Do the research themes (and subsidiary questions related to heterogeneity) address the most critical biogeochemical processes for intrinsic and accelerated bioremediation?

The research themes, centered on microbial transformation of subsurface contaminants, complexing agents, and mineral phases involved in contaminant mobilization/immobilization, were considered to be scientifically appropriate. Clearly, mechanistic studies of the role of iron and sulfur-reducing and chelate-degrading bacteria in mobilizing/immobilizing metals in oxides or sulfides are important and proof of concept can be attained in batch and stirred reactor experiments. However, a critical question is: How well do these processes transfer to porous or fractured media in natural groundwater systems? Consideration should be given to the development of the concept of scaling itself as a research theme into which various biogeochemical processes important to contaminant remediation could be enfolded.

A theme for biogeochemical dynamics might be based on the question: What are the impacts of physical and chemical heterogeneities on microbially mediated complexation and mineralization processes? A theme driven by heterogeneity could lead to development of universal concepts and approaches for scaling biogeochemical processes and represent a major product of NABIR, providing a practical basis for addressing these issues in bioremediation.

Based on the current state-of-the-art in biogeochemistry, what are the pivotal issues that must be addressed in extending mechanistic studies at the microscopic, grain, and pore scale in the laboratory to larger scales in the field where heterogeneity may play a dominant role?

Iron reduction, sulfate reduction, and complexation processes likely serve as important biogeochemical controls on metal/radionuclide behavior at DOE sites nationwide and offer potential for manipulation to stabilize or mediate contamination in the subsurface. Depending upon the contamination and site conditions, other processes such as denitrification and methanogenesis may also serve as a basis for development of remediation processes. Mechanistic studies at the laboratory-scale offer insights into the microbial, aqueous, and solid phase controls on the rate and direction of these processes and the form of initial reaction products. However, in order to extend this information to the field, it is necessary to account for the intrinsic features of the subsurface system that control the relative distribution of microorganisms; the proximity of organisms to reactive mineral surfaces; the supply, speciation and flux of contaminants, nutrients, and electron donors; and, ultimately, the nature and stability of reaction products over long time frames. Mechanistic and field issues that must be resolved in order to understand how processes that occur at the mineral-microbe interface impact groundwater composition at the meter scale are illustrated in Table 1.

A pivotal (or central) issue in biogeochemical dynamics that emerges from a comparison of mechanistic and field requirements (Table 1) is the need for new approaches to understand the factors controlling the spatial relationships between contaminant transformation processes, which occur at the mineral-microbe and grain scales, and the diffusion and convective transport of key gases and solutes that control the

Table 1. *Examples of Mechanistic and and Field-Scale Biogeochemical Processes That Must be Understood in Order to Predict and Manipulate Metal/Radionuclide Behavior in the Subsurface. Issues identified may apply to more than one process.*

Biogeochemical Process	Mechanistic Issues	Core/Field-Scale Issues
1. Iron Reduction	• Identification of reactive iron oxides • Influence of iron reduction and aqueous geochemistry on biomineralization rate • Oxidation rates of ferrous biomineral (relationship of increased O_2 to iron solubilization rate, reactive surface area)	• *In situ* reactive surface area and diagenetic aspects • Accesibility; e.g., pore size and geometry • Location and reactivity of Fe(III) oxides • Fluxes of electron donors and nutrients controlling reaction rate • Location of iron reducers relative to electron acceptors • Oxidant transport • *In situ* reoxidation rates controlled by surface area and crystallinity • Other factors controlling reaction rate (e.g., electron shuffles) • Effects of time and substrate concentration differences • Transport/colonization (e.g., preferential flow paths)
2. Sulfate Reduction	• Competition with iron reducers and with iron reduction • Nature and stability of reduction products • Properties of sulfide phases - nature type - surface area and crystallinity - chemical association of contaminant with sulfide phase • Potential for remobilization of sulfide precipitates • Nature of oxidation products and contaminant scavenging • Relationship to S cycle and implications	• Factors controlling proximity of contaminant, sulfate, and sulfate-reducing organisms
3. Nitrate Reduction	• Factors favoring assimilative or dissimilative reduction pathways, dominant nitrogen reduction products • Competitive interactions between nitrate and iron reduction, effects on iron and metal chemistry	• Physical controls on - availability of natural and synthetic organic substrates - alternative electron acceptors
4. Complexation	• Complex dissociation (competition between metals and ligands, adsorption) • Reactive surface area, role of geochemically reactive Fe(III) • Oxidation/reduction rates of contaminants in complexes • Controls on complex bioavailability	• Pore accessibility (bacteria/solutes) • Sustenance of biodegrading community • Role of natural organic matter (ligand competition, sorption)

rate and direction of these processes at the pore scale in naturally heterogeneous systems.

What approaches or combination of approaches offer the highest probability for success in addressing pivotal issues?

A comprehensive NABIR research effort to address the impacts of geochemical and physical heterogeneity on biologically mediated processes in the subsurface must involve parallel investigations at multiple scales. A general scheme for use of multiscale approaches for scaling biogeochemical processes to the field is given in Table 2. Advanced capabilities available in NABIR for application to heterogeneity and scaling issues are summarized in Appendix E. A well-planned, hypothesis-driven approach is critical to ensure that information obtained at each scale is used to focus and refine experiments at other scales. A general description of how multiscale approaches can be used in the context of the entire NABIR program is given below:

Core Scale

Intact cores (mm-dm scale) offer a compelling alternative to field studies for (1) obtaining specific, detailed information on mineralogical/microbiological heterogeneity and pore/fracture-scale physical heterogeneity (e.g., pore size and structure; fracture spacing, aperture, length, and geometry); (2) establishing the role of these features in determining the endproducts of biomineralization (using archeological investigations of cores); and (3) assessing contaminant mobilization/immobilization resulting from manipulation. However, successful use of intact cores will require careful development of hypotheses that can be legitimately tested within the limitations of core-scale experimentation. Challenges that must be overcome include difficulties in obtaining unperturbed samples, establishing comparable replicates and controls, characterizing heterogeneity, and separating localized effects from effects that may have resulted from processes occurring at a larger scale when the core was in place. Many of the approaches and tools discussed in the workshop (Appendices D, E) may have special relevance for core experimentation (e.g., x-ray computed tomography for non-destructive measurement of pore structure and connectiveness and x-ray absorption spectroscopy for aqueous and solid phase chemical speciation).

Biogeochemical processes at the core scale must be placed in context of larger (m)

Table 2. *Examples of the Use of Multi-Scale Approaches for Scaling Biogeochemical Processes to the Field. Approaches are not mutually exclusive, and their use separately or in combination depends on the research question being addressed.*

Information Needs	Approaches		
Contaminant transformation/sequestration, process interactions			
Spatial relationships for physical/chemical and microbiological processes	ore-scale observation/ experimentation		
Non-linear interactions (e.g., preferential flow paths)			
Concepts/strategies for process simplification/ field-scale modeling (to reduce complexity, focus on first order features)		Intermediate-scale experimentation	ld-scale observation and experimentation
Concept/model validation			
Quantification of uncertainty			

scales. Ongoing NABIR research at the intermediate and field scales, if properly integrated with core-scale research through joint hypothesis testing, can provide investigative mechanisms to address scaling issues.

Intermediate Scale

Intermediate-scale flow cells (dm-meter scale) offer the opportunity to predesign physical/chemical heterogeneity and control fluid flow for testing specific hypotheses relative to the effects of non-uniform convection on biogeochemical reaction rates and microbial transport. Intermediate-scale experimentation thus provides a capability for upscaling (diffusion-dominated) micron-scale batch studies and (dispersion-dominated) centimeter-scale homogenous column studies. When coupled with the application of new conceptual approaches, such as streamtube modeling for upscaling nonlinear reactions involved in microbial and reactive chemical transport, intermediate-scale experimentation can provide a vital predictive and support tool to augment and extend the results of core scale and field studies.

Field Scale

Field-scale observations (multi-meter scale) are ultimately the most relevant, but often only represent one set of observations in time and space. Field studies must therefore be (1) focused to address hypotheses that cannot be evaluated at other scales, and (2) properly designed to ensure integration with research at other scales and to optimize interpretation and extrapolation of results. It is necessary to establish (1) a conceptual and geologic framework that includes the physical, chemical, and biological properties of the system and their spatial arrangement as well as the processes occurring in the system that are either directly involved in, or influence, the biogeochemical reactions under study, and (2) a framework for dynamic processes (e.g., iron reduction) that can be superimposed on this static framework. While establishing the static framework involves heavy reliance on the most advanced geophysical methods, the dynamic framework relies heavily on mechanistic studies and flow and transport models that incorporate pertinent biogeochemical reactions linked to hydrodynamics. Core and flow cell experiments and field manipulation methods (Appendix E) would aid in the development of the fundamental relationships between heterogeneity structure, reaction mechanisms, and dynamics.

Design and Evaluation of Bioremediation Processes

Currently, the design and evaluation of bioremediation processes is based largely on simplified geophysical tools, batch equilibration studies, and hydrodynamic models. The new concepts and approaches based on fundamental principles and evaluated in an iterative manner as described above need to be incorporated into user-friendly bioremediation design tools. However, this will require advances in establishing quantitative linkages between physical/geochemical properties and biological processes at the appropriate scales and in representing these linkages at the field scale over a range of hydrogeologic regimes.

What additional capabilities/skills are needed to address scientific gaps and implement research?

> There is a critical need to be able to characterize and understand the impacts of heterogeneity at different spatial scales on contaminant behavior. Emphasis in this program element should be on interdisciplinary linkages to relate microbial distribution and activity to physical/geochemical features in subsurface materials and the impacts of these associations on the rate and direction of biomineralization processes under field-relevant conditions. New collaborations within NABIR and new capabilities will be needed to implement an effective research program addressing these issues.

New emphasis and capabilities are required in addressing reaction mechanisms at the molecular, organism, and grain scales; visualization and study of microbe-mineral associations, the structure and reactivity of mineral-water interfaces, and pore-scale gradients and mixing; and in the manifestation and modeling of these processes in heterogeneous environments at the core and field scales.

The Path Forward

A proposed research theme for future research in biogeochemistry and dynamics that evolved from workshop discussions is as follows: **Impact of physical and geochemical heterogeneity (micron to decimeter scale) on microbial mediation of contaminant complexation and mineralization processes**. This theme would encompass the pivotal issue identified by this workshop, would be achievable within resource limitations, and represent a niche for unique NABIR contributions.

A logical focus would be on intact core systems isolated as part of NABIR field research. Initially, cores would be available from DOE field sampling of sediments at western (UMTRA) sites and sediments from sites located on the Eastern Coastal Plain (e.g., Oyster II). Ultimately, it is expected that the NABIR Field Research Center would be the primary source of cores for hypothesis testing. Core-scale studies would need to be carefully designed to accommodate the limitations of these studies (see previous workshop report) and closely integrated with NABIR research at the intermediate and field scales to ensure that information at the cores scale was effectively used to address bioremediation in the field.

Core-scale investigations are particularly well-suited to address spatial relationships between contaminants, *in situ* flow paths, mineralogy, and microbial distributions and function that have evolved through *in situ* reaction and transport and long-term rock-water interactions. Manipulation at the core scale would offer fundamental observations of non-equilibrium biogeochemical and transport phenomena as influenced by geologic features (e.g., the effects of pore connectivity and preferential flow paths).

Integrated core-scale experiments, using a combination of archaeological and manipulative approaches, should be structured to address three primary issues:

- Intrinsic, *in situ* microbiologic and geochemical features resulting from long-term sediment-water interactions (and implications for contaminant immobilization)
- Spatial relationships between microbiological, mineralogical, and pore/fracture-scale physical heterogeneities (insights into the factors controlling the distribution and scaling of biogeochemical processes)
- Effects of nutrients, electron donors/acceptors carbon sources and flow rates on microbial distribution/activity and nonequilibrium contaminant transport (basis for examining the effects of heterogeneity, e.g., preferential flow paths and accelerated remediation measures)

Information derived from well-planned, hypothesis-driven experiments using intact cores will assist in providing fundamental understanding of the factors controlling the mobilization/immobilization and transport of contaminants at the grain and pore scales. This information, when used in conjunction with related investigations in other program elements (reaction mechanisms, effects of controlled heterogeneities in flow regimes at the meter scale, and field-scale observations of biogeochemical dynamics at the lithologic and geologic scales, should provide a sound basis for assessing the effects of biological processes on contaminant behavior over a broad range of site conditions and for extrapolating information across sites.