DOE/OBER Workshop Report
"Biogeochemical Manipulation for Scale-Up of Laboratory Research to the Field"

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The DOE/OBER NABIR program is evaluating approaches for extending the results of studies of metal/radionuclide biogeochemistry at the molecular, grain, and continuum scales to diverse hydrogeologic environments. A workshop was held April 28-29, 1998 at the University of Delaware Virden Conference Center to discuss and evaluate one such proposed approach for scaling laboratory based biogeochemical research to the field: the manipulation of subsurface cores containing natural heterogeneity.

The objectives of the workshop were to determine: 1.) the defensibility of using manipulative research on subsurface cores as a scientific strategy for scale-up to the field, 2.) the best chemical and microbiologic manipulation approaches available, and 3.) the scientific questions best resolvable with core-scale studies. The workshop agenda and participant list is included in the Appendix.

Workshop Motivation and Concept

The existing NABIR research portfolio has an emphasis on laboratory scale studies of microbiological and biogeochemical processes fundamental to the bioremediation of metals and radionuclides. Processes and reactions are being investigated by which microorganisms solubilize and immobilize metals and radionuclides by changes in valence, complexation, and aqueous and solid phase speciation. Because the microbiologic processes are not well understood, model systems are used to evaluate the rates and magnitude of these phenomena where the concentrations and flux of electron donors and acceptors, the of the microbiologic population, the mineralogy and morphology of solid phases, and the physical attributes of the system are controlled. A model system is both a simplification and abstraction of the natural environment typically created in the laboratory. Batch and column experiments with synthetic mineral material that have been inoculated with a single test organism are but one example of a model system. The microbiologic processes being studied by NABIR investigators occur slowly in the environment. To be effectively studied in the laboratory, however, these processes are accelerated by addition of electron donors, carbon sources, and nutrients at concentrations well above those found in the environment. The use of model systems to facilitate experimental interpretation and process acceleration to see impact, while necessary for laboratory experimentation, decouples the research from its field context.

The ultimate application of NABIR research is the bioremediation of metal and radionuclide contaminated lands within the DOE complex. It is therefore critical that approaches be put in place early in the NABIR program that both allow and facilitate the transfer of laboratory derived information on biogeochemical processes to the field. As a generalization, such "scaling" involves accounting for the effects of: 1.) heterogeneity in physical, chemical, and biologic properties; 2.) the intrinsic properties of the subsurface system including microbial ecology, diagenetic mineralogy and contaminant distributions, and pore structure and groundwater flow at the grain scale and above; 3.) the characteristically slow in-situ rates of biologic processes governed by nutrient, substrate, and electron donor limitations; and 4.) heterogeneous transport processes over different scales. The NABIR program has recognized the need for process scale-up to the field and is pursuing the establishment of a field research center (FRC) to address scaling issues and field application.

Because field studies are expensive and results are limited to the hydrogeologic conditions of the study site, the NABIR program is also considering other more modest approaches to evaluate metal and radionuclide biogeochemistry over a range of field relevant conditions. One approach that holds promise for scale-up and information transfer to the field is the use and manipulation of intact subsurface cores. Intact cores preserve important in-situ features of the subsurface environment, and thus their impact on biogeochemical processes may be investigated and understood. Furthermore, their microbiology and chemistry may be manipulated in the laboratory to test biogeochemical hypotheses or to extend mechanistic information to other hydrogeochemical environments pertinent to DOE lands. In some ways the use of intact cores to scale to the field may be viewed as a "poor man's" FRC.

Workshop Findings

1.) Role of Intact Core Studies in Scale-Up to Field

Participants felt that studies with intact cores were one of several essential approaches for scaling laboratory based research on biogeochemical processes to the field. Other essential approaches include intermediate scale flow cell experiments, field injection experiments, and evaluation of contaminant distribution and speciation in contaminated sites. All agreed, however, that intact core studies, and the other approaches as well, cannot be used individually as a stand-alone strategy for scale-up to the field. The overall recomendation was to identify biogeochemical scaling questions and issues first, and then select the most suitable approach for their resolution. If the question is best resolved using an intact core or its manipulation, then it warrants pursuit. Otherwise, a more suitable technique (e.g., an intermediate scale flow cell or push-pull injection experiment) should be selected. Workshop participants did not feel it appropriate to conduct experiments with intact cores for the "appearance of field relevance" or to attempt intact core experiments when the processes under investigation would be better understood or controlled in a less complex experimental system.

There was general consensus that the basic criteria for pursuing an intact core study was 1.) need based upon the scientific question, and 2.) experimental focus on processes or phenomena where the in-situ structure of the geochemical media, the intrinsic physical/spatial relationships of the microbial community, or cm-dm scale heterogeneities in physical, chemical, and biologic properties or processes may play an important role. The scientific questions most effectively resolved using intact cores involve the following:

- Evaluating the effects of intrinsic, in-situ biologic and geochemical features of subsurface materials that have resulted from long-term water-rock interactions and microbiologic colonization. Factors such as mineral micro-environments, microbial populations and consortia, pore space distribution and diagenetic features fall in this realm.

- Exploring the physical distribution and chemical speciation of contaminants in cores from DOE sites slated for remediation to identify the spatial relationships between metal species,
in-situ flow paths, field mineralogy, and microbial distributions and functions that have evolved over long term contact and in-situ transport and reaction.

- Determining unique biogeochemical effects resulting from the coupling of physical, biologic, and chemical processes during water and chemical mass transport through heterogeneous, structured porous media. Issues of non-equilibrium transport and preferential flow paths, and their biogeochemical implications, as controlled by intact geologic structure and fabric are important.

- Investigating slow biogeochemical processes where rates are controlled by the intrinsic microbiologic population and the in-situ dynamics (solubilization) and transport of nutrients, electron donors/acceptors, and carbon sources.

Workshop participants felt that these were extremely important issues or questions related to field scale behavior, and that their resolution through the application of intact core studies should be strongly encouraged within the NABIR Program.

2.) Experimental Challenges in the Use of Intact Cores

While intact cores offer the possibility to explore the impacts of in-situ subsurface features and properties on biogeochemical dynamics, there are some important limitations and demands imposed by the technique that need be recognized. These include 1.) physical and microbiologic disturbance during/after core collection, 2.) replication and controls, and 3.) characterization and measurement challenges. Workshop participants felt that these limitations could be overcome or worked around in most cases, but they are important and require explicit consideration in experimental design, performance, and interpretation.

- Disturbance - Minimizing physical and chemical disruption in core sampling is essential, as a primary motivation for intact core experiments is to investigate impacts of in-situ properties. If the in-situ features are damaged then the experimental utility of the intact core may be compromised. Physical disruption may include compaction, fracturing or cracking, or water content or temperature changes. Excavation has been used effectively by DOE researchers for years to obtain vadose zone cores with a high degree of physical integrity. Chemical disruption (change) must also be avoided as it may have large impacts on the microbiology and reactivity of the core. Oxygen or carbon dioxide exchange are foremost among concerns. Even a small amount of uncontrolled oxidation of an anoxic or anaerobic core may render it useless for further experimentation.

A major concern discussed at the workshop was microbiologic change after sampling. Research was presented by participants that showed that microbiologic populations increased while diversity decreased after sampling and mixing vadose zone materials. Factors such as physical disruption; water content, temperature, and gaseous atmosphere change; and alteration of nutrient flux vectors were collectively believed to induce evolution of the natural community. Even unavoidable vibrations associated with mechanical coring are speculated to influence the endogenous microbiologic community in a retrieved core. Microbiologists in attendance at the workshop were themselves unsure as to whether the in-situ microbial ecology with its associated biogeochemistry could realistically be sustained after core collection.

- Replication and Controls - Because subsurface materials are intrinsically heterogeneous, obtaining suitably comparative cores from the same location for experiment replication or validation of findings may be difficult, or even impossible in some cases. Thus, an intact core experiment may have to be viewed as a single point observation that exhibits temporal characteristics. Much can be learned from this observation point, but it cannot provide statistical information on the population from which it was sampled. Clearly the chances for replication can be improved if the subsurface geology of the site has been characterized sufficiently to allow informed sampling, and if the site is not overly physically or chemically heterogenous at the core scale.

  Another problem is that of control. If the core itself cannot be easily replicated how then does one establish a control? Is a valid control simply a replicate core that does not receive the target treatment (e.g., nutrient or electron donor amendment)? These issues were discussed, but not necessarily resolved at the workshop. One recommendation that gained some acceptance was that a given intact core could first be studied under conditions representing “baseline” or “background” conditions. Once the baseline core biogeochemistry was characterized, the system conditions could be manipulated, perturbed, or adjusted in some way to induce change in a desired direction for hypothesis testing. The baseline conditions would then represent an effective control for the manipulation experiment.

- Characterization and Measurement Challenges - A limitation of an intact core study is that the in-situ properties are not easily determined either before or after the experiment, and a rather complex set of destructive analyses, in-situ measurements, and modeling is required for successful interpretation.

  Intact core experiments generally involve the water-borne perfusion of nutrients, microorganisms, or chemicals through the core, followed by measurement of solute or microorganism concentrations in downgradient effluent solutions. In-situ instrumentation such as micro-electrodes can be installed along the core axis to provide real time analyses to augment the breakthrough data. Linking the measured concentrations in the effluent solutions with the spatial location of and the rates, magnitude, and identity of biogeochemical phenomena occurring in the column, however, is a major challenge. Such linkage is typically accomplished retrospectively by core dissection with destructive analyses of microbiologic, chemical, and physical properties, and through reactive transport biogeochemical modeling.

Non-destructive methods, however, are required to determine the initial physical characteristics of the intact media and to ascertain changes that may have occurred during the experiment. Two forms of in-situ interrogation tools, tracers and spectroscopy/geophysical methods, were discussed in the workshop. Water-borne tracers such as Br, He, organic macromolecules, and charged microparticles can be used to characterize the transport process in intact, structured porous media; including the differentiation of colloid and solute transport, macropore/micropore transport, and matrix diffusion. These techniques, possibly combined with biosensors (molecular tags) to trace organism populations, provide information critical to understanding solute, substrate, and nutrient flux and biogeochemistry in intact core materials. CT imaging can yield 3-dimensional density maps of the core providing insights on macroscopic pore structure, fracture distribution, and distribution of mineral phases with markedly different density, and a physical basis for interpretation of tracer measurements. Techniques such as nuclear magnetic resonance (NMR), gamma-ray tomography (GRT), and other geophysical methods can yield complementary information on 3-dimensional porosity and microorganism distribution ($>10^6$ microorganisms/g) by H_2O imaging but are largely untried.

3.) Experiments Recommended for Intact Cores

It was uniformly agreed that intact cores are best suited for use in transport studies that are 1.) exploring the activity and function of the endogenous microbiologic community, 2.) investigating the impact of unique in-situ features such as mineral-microenvironment associated with diagenetic fabric or porous media structure, or 3.) evaluating the effects of core scale heterogeneity on biogeochemical processes. As such, the hypotheses for these experiments should all have focus on the impacts of in-situ features, structures, or populations. Intact cores are not suited to investigate basic biogeochemical or microbiological reactions which are better studied in more controlled batch systems with single organisms/substrates or reconstructed flow through systems (homogeneous columns) of reactive porous media.

Two schools of thought were present on how well a given microbial metabolism or biogeochemical phenomena need be understood before progressing to a heterogeneous, intact column. Some felt that the basic biogeochemistry and geomicrobiology was insufficiently understood at molecular, microbe, grain, and pore scale to devise a rigorous and defensible intact core study. With respect to this perspective it was stated: “It should be a goal of NABIR to support the needed science base in the early part of the program to enable design of good experiments in intact cores.” Others, however, felt that the microscopic process need not be fully understood before moving to a heterogeneous core. An intact core experiment may assist in qualitatively identifying key factors controlling a biogeochemical process under field relevant conditions that would never be seen in microscopic studies of reaction mechanism. One participant stated: “I do not agree that all relevant biogeochemical processes must be fully understood at the microscopic scale before experiments involving core-scale manipulations can be meaningful. The crux lies in focusing on core-scale phenomena, many of which will not be best understood by reducing them to microscopic (or molecular) scale processes. Research on these core-scale processes should be hypothesis driven...”

Table 1. Current and Proposed Uses of Intact Core.
Effect of in-situ pore structure and particle size gradation on bacterial transport
Biogeochemical function of in-situ microbiologic communities including competitive metabolisms
Role of disturbance on in-situ microbiologic function
Feasibility of bioaugmentation or biostimulation
Evaluating the effect of porous media structure and geology on bigeochemical process.
Determining transport controls on in-situ microbiologic processes regulated by in-situ physical features
Contributions of surface reactions on diagenetic mineral phases with in-situ morphologic features and surface area
Providing realistic biogeochemical data sites for testing linked, multi-process models
Experiments with realistic DOE contaminants

The workshop participants in group session identified a list of activities thought to represent the best uses of intact cores (Table 1). Intact cores are not well suited to investigate fundamental microbiologic or biogeochemical mechanisms or their kinetics; transport independent processes; or studies of large scale heterogeneity, rapid processes, or those requiring a large number of treatments or replications.

4.) Core Manipulation for Hypothesis Testing

The concept of core manipulation was posed to the workshop participants to evaluate the merits of its application in the NABIR Program. Within the context of the workshop, manipulation was defined as changing the microbiologic (ecology, functional group distribution) or chemical properties (pH, Eh) of a core and its contained biogeochemical processes via addition of chemical contaminants; substrate, nutrients, e-donors/acceptors; or exogenous/endogenous microorganisms in presence of water flow (e.g., transport). Manipulation may be used to accelerate or enhance a given microbiologic process, change conditions so that a minor process becomes dominant, or to alter biogeochemical conditions to influence chemical flux or speciation. The motivation for such action is to further understand a given biogeochemical process, to enhance beneficial function for remediation, and to develop a broader information base for extension to DOE sites of diverse hydrogeochemistry.

The general consensus was that some, but not all, types of manipulation make sense and could be pursued if scientific questions and hypotheses so dictate. The addition of substrates, e-donors, C-sources, and other nutrients to selectively or globally stimulate the microbial population were manipulations most thought were acceptable. Major chemical manipulation such as pH (H^+/OH^- addition) modification was argued against because of the large intrinsic buffering capacity of most subsurface sediments. Futhermore, the pH dynamics are controlled by a complex reaction network that would need to be understood to some degree. Controlled biotic reduction and abiotic oxidation, however, were two system changes that most thought would be fruitful to investigate and pursue if driven by valid core-scale hypotheses.

Participants had mixed views on the merits of bioaugmentation as a manipulative strategy. For some types of study, such as microbial transport, bioaugmentation is a necessity. For studies of biogeochemical processes, however, many thought that bioaugmentation was complicated by issues of transport, survivability, colonization, and competition; and possibly should be avoided because of experimental challenges and difficulties associated with their resolution. Others, however felt that the ability to inoculate a core with an organism of known metabolism and function was of great experimental and scientific advantage and should be pursued in spite of the above noted concerns. While retention of function after emplacement was noted as an important question, most thought selective pressure could be applied through substrate monitoring to provide competitive advantage for long term survival. Obtaining a suitable, undisturbed core to function as a control for the bioaugmentation study was noted as essential.

A general finding was that core manipulation could provide a means to extend research results to sites or conditions that differed from those under which the core was collected. However, participants urged caution in this regard because more simple batch and homogeneous column experimental systems are better suited to investigate ranges of physical and chemical variables, and these opportunities should be initially exploited. The manipulation of intact cores should be limited to the study of the impact of in-situ features, properties, or microbiologic populations on the targeted process. Extension of intact core results to different environments requires well designed experiments and supporting information, including a.) background scientific information on the manipulated biogeochemical process, b.) in-situ characterization of physical features and flow properties of the core, and c.) relatively comprehensive bio-hydrochemical models that include relevant chemical reactions, microbiologic population dynamics, and physical transport processes.

5.) Alternative Approaches to Intact Cores

Workshop participants felt that intact core studies have significant merit in scaling knowledge on biogeochemical processes to the field. Few however, felt that the approach had unique advantages over other ways of studying intermediate scale processes that are summarized in Table 2. The overall recommendation was to select the experimental approach that was economically viable and best suited to resolving the scientific questions at hand.

Table 2. Alternative Approaches to Undisturbed Cores
Archeological investigation of cores from contaminated sites (contaminant distribution and speciation)
Down-hole, in-situ microcosms placed in the vadose or saturated zone
Investigating groundwater biogeochemistry by well access along a known flow path (upgradient-down gradient evolution)
Intermediate-scale flow cell experiments with controlled heterogeneities and microbiota
Field injection experiments- Forced and natural gradient
Permanent observatory - In-ground well array with multi-level samplers
Down hole manipulation of biogeochemical processes by substrate or electron donar injection

Overall Recommendation

Studies of intact subsurface cores hold great promise for evaluating the effects of field-scale physical, geochemical, and microbiologic features on biogeochemical processes involved in or fundamental to bioremediation. For that reason, they merit consideration as one of several experimental tools useful in scaling biogeochemical research to the field, and inclusion in a research program emphasizing field scale behavior. Other complimentary (intermediate scale) techniques include meter-scale flow cell experiments with controlled heterogeneities, in-situ microcosms, and small push-pull injection experiments in the field. Intact core studies were found to have few unique experimental advantages over the other techniques. All the techniques have significant limitations, and each has several strengths. The main strengths of intact core studies is the ability to 1.) experimentally evaluate the effects of natural heterogeneity (as opposed to that which is engineered or controlled) and unique diagentic structural, mineralogic, and biologic features at the cm to dm scale and 2.) manipulate in-core conditions for hypothesis testing. The choice of one intermediate scale technique over the other should be determined by experimental question and hypothesis, and critical evaluation of the strengths and weaknesses of the different approaches.