PROGRAM ELEMENT 5
Assessment

Antibodies and Antibody-Based Sensors for Hexavalent Uranium and Chelators

Diane A. Blake,[1] Robert C. Blake II,[2] Haini Yu[1] and Andrey R. Pavlov[1]
[1]Tulane University School of Medicine, New Orleans, La.; [2]Xavier University of Louisiana, New Orleans, La.

The development of sensors that use antibodies as the recognition element appeals to a large number of potential end-users, since these devices may be used to monitor a very broad range of analytes. The variety of molecules that can be quantified by immunosensors is virtually unlimited, and depends primarily upon the binding affinities and specificities of the antibodies incorporated into the devices. The high sensitivity and selectivity of such sensors makes them attractive for situations where both speed and accuracy are required. Previous studies from our laboratories have demonstrated the feasibility of isolating monoclonal antibodies that recognize specific metal ions. The goals during the current grant period are to (1) isolate and characterize antibodies that recognize the most mobile form of uranium, UO_2^{2+}; (2) assemble, test and validate a new field-portable immunosensor based on these antibodies and a hand-held flow fluorimeter; and (3) prepare new monoclonal antibodies to the primary chelators (EDTA and DTPA) found in DOE wastes.

Three hybridoma cell lines have been generated that synthesize and secrete monoclonal antibodies that bind tightly and specifically to UO_2^{2+} complexed to 2,9-dicarboxyl-1,10-phenanthroline (DCP). Cloning and sequencing of the cDNAs that code for the light and heavy chain variable regions of these antibodies demonstrated that all three have distinct binding sites. Two antibodies (8A11 and 12F6) were selected for further studies, based upon their affinity for the UO_2^{2+} -DCP complex and their resistance to changes in pH and ionic strength.

A prototype competitive immunoassay for UO_2^{2+} was developed that accurately monitored UO_2^{2+} at concentrations from 10 to 120 nM in buffers amended with 12.5 μM DCP. These assays were conducted on the KinExA™, a semi-automated flow fluorimeter designed for sophisticated studies in the laboratory. As part of an effort to adapt this and related immunoassays for use in the field, an experimental version of a field-portable KinExA flow fluorimeter was assembled for testing purposes. Issues currently under investigation using this *alpha* unit include: (1) the effects of flow rates of liquid reagents on the accuracy and sensitivity of the immunoassay; (2) the necessity for pre-treatment of the sample prior to analysis; (3) practical limits on the volume of sample to be introduced into the field instrument; (4) the effects of lyophilization and reconstitution of disposable reagents on the performance characteristics of the assay. It is anticipated that these studies could generate a useful marketable product, a collection of portable field tests for uranium and related wastes that could be exploited both in government and private sectors.

Artificial Neural Networks as a tool for the Assessment of Microbial Communities

C.C. Brandt,[1] J.C. Schryver,[1] T.L. Marsh,[2] S.M. Pfiffner[3] and A.V. Palumbo[1]
[1]Oak Ridge National Laboratory, Oak Ridge, Tenn.; [2]Michigan State University, East Lansing, Mich.; [3]University of Tennessee, Knoxville, Tenn.

Microbial communities in soils can be characterized by the analysis of various biomarkers such as terminal restriction fragment length polymorphisms (T-RFLPs) and signature lipids. Biomarkers may be useful in assessing microbial communities during in-situ bioremediation since they have been shown to change in response to environmental conditions. However, the changes in biomarkers are often complex, nonlinear and not readily amendable to traditional statistical analyses.

One of the objectives of our research is to develop new data analysis techniques that can help in assessing microbial community structure from T-RFLPs and other biomarkers. We are evaluating artificial neural networks (ANNs) as a tool for relating changes in microbial T-RFLP patterns to the concentration of heavy metals. ANNs are nonlinear, nonparametric analysis methods that can learn from experience to improve their performance.

We tested ANNs on T-RFLP data obtained by T.L. Marsh and his colleagues from an abandoned tannery located in the upper peninsula of Michigan. Soil samples were subjected to chromium and T-RFLP analyses as described by Marsh et al. in this and previous proceedings. The abundance of the T-RFLP fragments were converted to a sample proportion, and those fragments with a maximum proportion of one percent or less were removed from the data set. The final data set of 51 samples was used to construct bootstrap training sets (41 samples) and a validation sets (10 samples) for subsequent data analysis.

Several feed-forward architectures were tested by training bootstrap samples with 88 input variables (T-RFLP fragments) and a single output variable (chromium). Generalization performance of these networks was unsatisfactory, and we selected a subset of 25 fragment sizes for subsequent training. The most robust architecture with a limited number of inputs contained seven hidden nodes. One hundred realizations of this ANN architecture were tested. For each realization, we trained the ANN using a cross-validated early stopping procedure that terminated training prior to convergence when the error in the validation set no longer decreased. The best ANN explained more than 98% of the variance in the training data and more than 95% of the variance in the validation data set.

We are currently conducting sensitivity analyses to identify those T-RFLP fragments most strongly related to chromium concentrations. We are also exploring the use of autoassociative ANNs as a means of reducing the number of T-RFLP fragments prior to use in the predictive analysis.

Coupled Use of DNA Microarrays, Voltammetry and X-Ray Studies for Profiling Changes in Microbial Community Structure and Metal Speciation in Response to Metal Contamination

Darrell P. Chandler,[1] David A. Stahl[2] and Jean Francois Gaillard[2]
[1]Pacific Northwest National Laboratory, Richland, Wash.; [2]Northwestern University, Evanston, Ill.

The objectives of this project are to develop and apply DNA array technology to profile and monitor microbial communities through time in process-level microcosms and naturally contaminated sediments treated with increasing Cr, Pb and/or Zn concentrations, and correlate microbial community structure to changes in metal speciation and mobility. The central hypotheses relate to: (1) the sensitivity and specificity of tunable surface microarrays relative to standard hybridization chemistry; (2) the biochemical process associated with metal speciation in artificial mesocosms and natural environments; and (3) changes in microbial community composition in response to increasing metal contamination.

Natural sediments (ca. 10^9 cells g^{-1}) were obtained from three sites in Lake DePue, Ill., which contains substantial zinc, copper, cadmium, lead and arsenic. Three sites were characterized and used to establish microcosms, ranging from 50 ppm to 30% Zn. Methanogenic archaea and sulfate-reducing bacteria were quantified by MPN enrichments, and methanogens, iron-reducers and heterotrophic anaerobes isolated using acetate as an electron donor. Microbial biomass was significantly higher at the more contaminated site. Isolates are currently being evaluated for growth and Zn metabolism at different Zn concentrations, with the intent to compare biologically-governed Zn speciation in monoculture to Zn speciation observed in Lake DePue sediments.

Mesocosms designed to select for metal-reducing bacteria or methanogens were established and monitored for pH, acetate, metal concentrations (Zn and Mn) and headspace gasses (carbon dioxide, methane and hydrogen), with weekly samples taken for DAPI counts and DNA analysis. Differences in acetate consumption and methane generation were observed between sediment microcosms. X-ray absorption spectroscopy of bioreactor solids was used to investigate changes in Zn and Mn speciation due to Zn and Mn amendments. Total nucleic acids are currently being extracted from mesocosm sediments for analysis by T-RLFP to compare changes in microbial community structure with Zn addition. Predominant, novel or unexpected T-RFLP signatures will be cloned and sequenced to identify species-specific probes for a 'metal reducer' microarray.

Microarray research during year one focused on specific technical challenges associated with the *direct* detection (i.e., no PCR amplification) of full-length rRNA targets relative to PCR-amplified, functional gene targets. Surface chemistry and probe attachment methods, target labeling and detection strategies, and novel solution conditions were evaluated to achieve specific and reproducible hybridization of *Geobacter chapelleii* 16S rRNA to universal and species-specific 16S rRNA probes. The specific detection and allelic discrimination of PCR-amplified, functional gene targets from an E. coli model system was very successful, regardless of probe attachment chemistry, labeling or detection strategy. However, the specific, reproducible hybridization of full-length 16S rRNA or rDNA targets to a 2-dimensional (versus 3-dimensional membrane or gel-pad) microarray remains a significant technical challenge. Sandwich hybridization and chaperone systems have been developed and compared to direct chemical labeling reporter systems. Short rRNA fragments (ca. 300 bp) hybridized with greater signal intensity than full-length (1500 bp) products, although both size fragments generate anemic signals relative to similar functional gene target assays. We postulate that the high degree of secondary structure, large target fragments, steric constraints at a two-dimensional surface and limited range of ionic strengths amenable to DNA:DNA hybridizations may limit the efficacy of DNA probes in a 16S rRNA microarray. To address these possible limitations of DNA probes for community-level 16S rRNA analysis, we are evaluating peptide nucleic acid probes under low-salt, high temperature buffer conditions to overcome steric constraints associated with large, 16S rRNA targets.

Rapid Gene Probe for Microorganism Monitoring by Novel MS Approaches

C.H. Winston Chen,[1] Jizhong Zhou,[2] H. Huang,[3] N.R. Isola,[3] R. Hurt[3] and N.I. Taranenko[3]
[1]Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.; [2]Environmental Science Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.; [3]Oak Ridge Associated Universities Postdoctoral Fellow

The goal of this work is to develop new assessment technologies by using laser desorption mass spectrometry (LDMS) for microbial community analysis. The approach involves technology developments and the application of innovative mass spectrometry technologies for microbial DNA analysis. For technology development, we have achieved the following major tasks: (1) initiation, design, installation and test of the idea of laser induced acoustic desorption; (2) demonstration of hybridization probes detected by LDMS; and (3) demonstration of direct DNA sequencing by LDMS with selective fragmentation. For microbial DNA analysis, we have demonstrated: (1) evaluation of mass spectrometry based detection methods for analyzing the RFLP patterns of 16S rRNA genes amplified from microbial communities; (2) development of quantitative PCR methods and coupling with LDMS for measuring copper nitrite genes in environmental samples; and (3) development of methods for simultaneous extraction of RNA and DNA from soil samples.

The principle of laser induced acoustic desorption is based on the shaking force for biomolecule desorption. Since no direct absorption of laser photons to raise the temperature of substrate or matrix molecules occurs, soft desorption can be achieved. We recently obtained mass spectra with better mass resolution by laser induced acoustic desorption compared to matrix-assisted laser desorption/ionization. At present, most hybridization to probe DNA sequence is pursued as one hybridization reaction per site. The hybridization probed needs to be tagged with either radioactive material or fluorescent dye. With mass spectrometric for DNA probe detection, more than one reaction per site can be pursued. We have demonstrated the detection of multi probes on a single hybridization site by mass spectrometry. Since thousands of probes can be used for hybridization on a single chip, it is important to have a quick and reliable method to sequence short DNA probes. For short ss-DNA probes, it is extremely difficult to use Sanger's enzymatic method to prepare DNA ladders for sequencing. We have developed the selective fragmentation for sequencing DNA probes. Future work will be concentrated on the mass resolution improvement for laser-induced acoustic desorption, increase of multiplexing on hybridization detection and direct sequencing of probes on chips.

The size of the intergenic regions between 16S and 23S ribosome genes varies among different bacterial species. We measured the replicated DNA products in this region with the size of ~1600 bp, which is the largest DNA fragments detected by ultraviolet laser desorption. We further measured RFLP to demonstrate that mass spectrometry technology can be used for microbial population determination by the patterns of RFLP. Future effort will be placed on the demonstration of various probes designed to characterize microbial populations in soil and sediment samples.

To examine the potential for mass spectrometric detection in quantitative PCR assays, we developed primers directed to the eubacterial glutamine synthetase (EGS) gene. PCR primers were designed to recognize positions that exhibit the least divergence (highest similarity) among individual EGS DNA sequences in a multiple sequence alignment. This approach allowed us to design PCR primers that generate a 153 or 156 bp product from representatives of a maximum range of evolutionary divergence. Tests have demonstrated that a 174 bp internal standard molecule yields a quantitative result based on assays using known copy numbers of *Escherichia coli* DNA. We are currently in the process of comparing quantification results based on gel electrophoresis with results obtained using mass spectrometry.

Development and Evaluation of Stable Isotope and Fluorescent Labeling and Detection Methodologies for Tracking Injected Bacteria During In-Situ Bioremediation

Mark E. Fuller,[1] Tullis C. Onstott,[2] David C. White,[3] William P. Johnson[4] and William E. Holben[5]
[1]Envirogen, Inc., Princeton Research Center, Lawrenceville, N.J.; [2]Princeton University, Princeton, N.J.; [3]Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tenn.; [4]University of Utah, Salt Lake City, Utah; [5]University of Montana, Missoula, Mont.

The goal of this research is to develop new methods for tracking bacteria in the subsurface. Methods to label bacteria with a variety of fluorescent dyes will be tested. The effects of each dye on cell culturability and adhesion to sediment, as well as the stability and longevity of the fluorescently-labeled bacteria in microcosms, will be assessed. The level of detection for fluorescently-labeled cells using microplate spectrofluorimetry will be determined. The fluorescent tracking method will be evaluated during both transport experiments using intact sediment cores and in the field, and compared with several other detection/enumeration methods.

Additionally, tracking methods involving the analysis of stable carbon isotopes incorporated into cells will be examined. Bacteria will be grown on ^{13}C-only substrates to achieve very highly ^{13}C-enriched cells. Standardization between the number of cells and the amount of ^{13}C in membrane fatty acids, determined by either gas chromatography/chemical reaction interface mass spectrometry (GC-CRIMS) or high performance liquid chromatography/electrospray ionization/mass spectrometry (HPLC/ESI/MS), will be performed. The stable isotope tracking method will be evaluated during transport experiments using intact sediment cores and in the field, and compared with several other detection/enumeration methods.

Once developed, these methods will allow the movement of bacteria injected during in situ bioremediation to be accurately and easily assessed, thus improving the overall effectiveness of bioaugmentation efforts.

During the past year, the fluorescent tracking method has undergone extensive evaluation. A green fluorescent compound, 5-(and-6-)-carboxyfluorescein diacetate, succinimidyl ester (CFDA/SE), was shown to stain bacteria without significant effects on cell viability or adhesion to sediment. The stained cells remained fluorescent for at least 21 days in both groundwater and sediment microcosms. CFDA-stained cells were quantifiable by epifluorescent microscopy, flow cytometry and microplate spectrofluorimetry. Optimization of the microplate enumeration method resulted in a lower detection limit of approximately 10^5 CFDA-stained cells per well. Cell concentrations in the effluent of intact cores during bacterial transport experiments, as determined by viable plate counts, scintillation counting, direct microscopic counts of CFDA-stained cells, and microplate enumeration, were all similar. Evaluation of the fluorescent tracking method during a field-scale bacterial transport experiment was also performed, with near real-time measurement of the cell concentrations in individual samples using the microplate reader.

Work on the CRIMS stable isotope tracking method has been limited by difficulties with the prototype instrument. However, an alternative stable isotope method using HPLC/ESI/MS is being pursued in collaboration with D.C. White (Univ. of Tennessee). Intact core and field-scale bacterial transport experiments using ^{13}C-labeled cells have been performed, and samples are currently being analyzed.

An In-Situ Tracer Method for Establishing the Presence and Predicting the Activity of Heavy Metal Reducing Microbes in the Subsurface

Kirk Hatfield
University of Florida, Gainesville, Fla.

The objective of this study is to establish nondistructive in-situ tracer methods for detecting the presence, distribution and activity of subsurface heavy-metal-reducing microorganisms.

Research efforts have focused on the critical areas of: (1) the identification and characterization of potential biotracers; (2) the development of mathematical models; and (3) the development of methods to extend biotracer technologies to the field.

With regard to the first effort, of identifying and characterizing potential biotracers, a series of bacterial enrichment cultures, batch experiments and flow-through column studies were initiated to identify electron donor-acceptor systems that would likely lead to efficient reduction of chromium and iron under anaerobic conditions in a test soil. The ultimate goal of these studies was to identify electron donors and/or electron accepting systems that would serve as indicators of Cr(VI)-reducing activity in this soil. It was thought that electron donors leading to Fe(III) and Cr(VI) reduction might be candidates for reactive biotracers indicative of Cr(VI)-reducing activity, and that electron accepting systems might prove useful as conservative tracers of these processes. Recent research efforts have examined anthraquinone disulfonate (AQDS) as a conservative electron acceptor and possibly an electron shuttle. Preliminary experiments have shown the reduced form of AQDS, anthrohydroquinone disulfonate (AHQDS), reduces Cr(VI) and Fe(III). Thus, AQDS/AHQDS shuttle has been identified as a potential system of biotracers for iron- and chromium-reducing activity.

Data from the first effort are immediately used in the second to development models that will serve as tools to characterize subsurface microbial distribution and activity through the fate and transport of biotracers. Modeling efforts have focused on the developing analytical and numerical multi-component batch reactor models and a 3-dimensional multi-component finite element transport model. Numerical methods originally developed for large-scale atmospheric transport models have been applied here to solve systems of nonlinear reactive transport equations. Data generated from batch and column experiments have been used to formulate these models with appropriate stoichiometric and kinetic relationships.

The third and final area of focus is a recently initiated effort to address issues pertinent to extending biotracer technologies to potential field applications. Research is under way to develop in-situ methods for measuring water, tracer and chromium fluxes in the subsurface. At a minimum, the technology will quantify changes in tracer mass in 3-dimensional transient flow field. Preliminary laboratory tests show evidence of success; however, a field test has not been performed.

SIMS for Direct Interrogation of Microbe/Mineral Interfaces

Jani C. Ingram, R. Michael Lehman, F.S. (Rick) Colwell and Gary S. Groenewold
Idaho National Engineering and Environmental Laboratory, Idaho Falls, Idaho

The goal of this work is to evaluate static secondary ion mass spectrometry (SIMS) as a tool for direct assessment of microbial populations at mineral surfaces. Because SIMS is a sensitive, surface analysis technique, it has the potential to directly interrogate interfacial interactions between microorganism and the mineral substrate. A number of controlled microbial samples have been characterized; this benchmark research is leading toward the microbial surface characterization of sediments found at the Uranium Mill Tailings Remedial Action (UMTRA) site near Shiprock, N.M.

The basis for our approach is to use static SIMS to probe phospholipid fatty acids and other biomolecules associated with the cell membrane of intact microorganisms. We hypothesize that since static SIMS probes only the top layers of the sample surface, it could be used to collect unique mass spectral signatures of the cell membrane chemistry of a microorganism by analyzing intact cells (no sample preparation). In order to test this hypothesis, SIMS spectra of >50 microorganisms were collected and the results were compared to a standard method for probing the cell membrane chemistry (Microbial Identification System, MIS). The mass spectral results from the SIMS analyses showed marked differences in spectral features. Comparing the SIMS results to the fatty acid profiles generated by MIS, many of the fatty acids were identified on the basis of specific anions observed in the SIMS data. By applying principal component analysis to the SIMS data, microbes having similar phospholipid compositions could be statistically grouped.

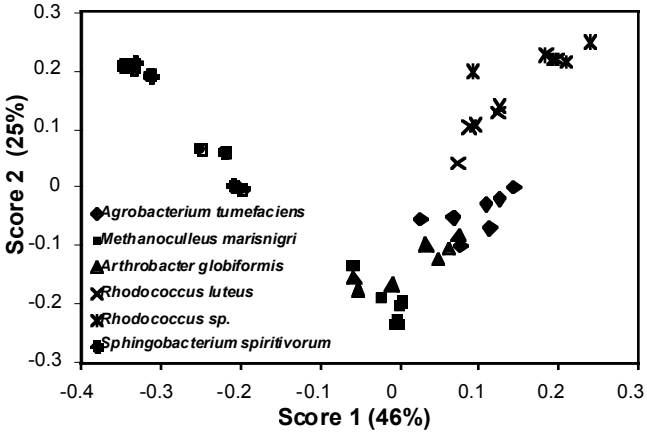

Figure 1: Principal component analysis of SIMS spectra from six microorganisms

A second approach for microbe identification is to utilize mass spectrometry/mass spectrometry (MS/MS) to detect specific biomarker molecules which are contained in the cell membrane. Results from the early stages of this research will be reported as part of this presentation.

Currently, we are investigating detection limits and how specific microorganisms can be typed (groups, species, subspecies) by SIMS. We are also investigating isolates collected from the Shiprock UMTRA site, and plan to discuss those results as part of this presentation.

In-Situ Determination of Microbial Metabolic Activity

Jonathan "Jack" Istok and Jennifer Field
Oregon State University, Corvallis, Ore.

The goal of this project is to develop and apply the single-well, "push-pull" test method for measuring in-situ rates of microbial metabolic activity in the subsurface. Activities are measured as the rate of transformation of an injected substrate to a specific product. Push-pull test assays are being developed and field tested to: (1) estimate the size of the metabolically active microbial biomass, and (2) quantify rates of SO_4^{2-} and Fe(III)- reduction, at metals-contaminated groundwater aquifers within the Department of Energy complex.

To estimate microbial biomass, assays were developed to measure activity expressed by a broad spectrum of subsurface microorganisms, including those that express aerobic respiration, glucosidase and phosphatase activity, and hydrogen utilization. Laboratory and field experiments were conducted to examine the correlation between activity measured with the push-pull test and independent measures of biomass (e.g., lipid-bound phosphate) obtained for groundwater and sediment. As part of this work, push-pull tests were designed to obtain in-situ estimates for Michaelis-Menton kinetic parameters that describe the transformation of substrate to product. Experiments were conducted at non-DOE sites as well as UMTRA sites, including Shiprock, N.M., and Gunnison, Colo. Field push-pull assays will be used to monitor changes in biomass that occur following growth-substrate addition. In collaboration with other NABIR investigators, an extensive series of field tests was conducted to quantify rates of sulfate reduction in the presence and absence of exogenous electron donors.

Microbiological and Biogeochemical Characteristics of Subsurface Sediments at Uranium Mill Tailings Sites at Gunnison, Col., and Shiprock, N.M.

Philip E. Long,[1] James P. Mckinley,[1] David Balkwill,[2] Dwayne Elias,[3] Jennifer Field,[4] Kevin Finneran,[5] James K. Fredrickson,[1] Craig Goodknight,[6] Jonathan Istok,[4] Mark Kautzky,[6] Lutz Kinderman,[6] Lee R. Krumholz,[3] Derek R. Lovley,[5] Steve Nold,[7] Sarah Macnaughton,[8] Stan Morrison,[6] John Stephen,[8] Joseph M. Suflita,[3] James Tiedje[7] and David C. White[8]
[1]Pacific Northwest National Laboratory, Richland, Wash.; [2]Florida State University, Tallahassee, Fla.; [3]University of Oklahoma, Norman, Okla.; [4]Oregon State University, Corvallis, Ore.; [5]University of Massachusetts, Amherst, Mass.; [6]MACTEC ERS, Grand Junction, Colo.; [7]Michigan State University; [8]University of Tennessee, Knoxville, Tenn.

Uranium Mill Tailings Remedial Action (UMTRA) sites provide an opportunity to study the microbiology and biogeochemistry of field sites contaminated with metals and radionuclides. Subsurface sediments at UMTRA sites have been in contact with contaminants for 30 to 50 years, a time period comparable to that for contaminants at DOE weapons complex sites. Contaminants include a wide range of metals dominated by uranium. Other anthropogenic solutes include nitrate, ammonium and sulfate. Given these characteristics, DOE's NABIR Program is collaborating with the UMTRA Program to study selected UMTRA sites.

The objectives of this research are to: (1) determine the dominant electron accepting processes at sites with long-term metal contamination, and (2) define the biogeochemical transformations that may be important to either natural or accelerated bioremediation. Sampling of sediments and groundwater has been completed at Shiprock, N.M., and Gunnison, Colo. Preliminary results for Gunnison indicate: (1) low to moderate microbial activity (based on p-nitrophenol production rates), and (2) enrichment of viable sulfate-reducing, nitrate-reducing and Fe(III)–reducing bacteria. A peak in sulfide, acetate and p-nitrophenol activity in a single sample suggests that microbial activity is relatively heterogeneous at this site.

Preliminary results for the Shiprock site include: (1) phospholipid fatty acids ranging from 50 to 200 picog/g sediment, indicating the presence of a diverse, active microbial community, including SRBs and actinomycetes; (2) viable anaerobic bacteria, including NRB, SRB and methanogens in flood plain sediments; (3) low levels of Fe-oxidizing bacteria that use nitrate as an electron acceptor under anaerobic conditions; (4) flood plain sediments also exhibit U-reducing bacteria but none in shale samples; (5) DGGE results from groundwater filtrate demonstrate a diverse microbial community with representatives from most functional groups occurring in samples from a single well. These results suggest the importance of biogeochemical processes in the natural attenuation of uranium in alluvial sediments, particularly at the Shiprock UMTRA site. They also suggest that indigenous microorganisms present at both sites could contribute to in-situ stabilization of U via reduction to insoluble U(IV) species.

Core-Scale Interrogation of Permeability and Geochemical Heterogeneity for Assessment of Bioremediation Effectiveness

Philip E. Long,[1] Timothy D. Scheibe[1] and John L. Wilson[2]
[1]Pacific Northwest National Laboratory, Richland, Wash.; [2]New Mexico Institute of Mining and Technology, Socorro, N.M.

Quantitative, field-scale understanding of reactions between microbes and natural porous media is critical to solving many contemporary subsurface environmental problems. Because these reactions occur at water-mineral-cell interfaces and are strongly controlled by local biogeochemical conditions, knowledge of small-scale variations (heterogeneity) in natural porous media properties and their net effect on field-scale transport is needed. However, small-scale heterogeneity of physical properties such as permeability and porosity combines with that of biogeochemical properties to give rise to complex behaviors that are difficult to quantify at relevant field scales. Detailed descriptions of small-scale heterogeneity and observations of their relationship to bacterial attachment are needed to form a defensible foundation for quantitative modeling and theoretical developments.

Progress has been made on integration of a number of innovative, core-scale imaging technologies which will significantly enhance detailed assessment of physical and biogeochemical heterogeneity at sub-core scales. The technologies used have been applied, in varying degrees, to geological characterization problems, but have not been integrated and applied to quantify joint physical and biogeochemical core- and outcrop-scale heterogeneity. Basic issues to be addressed by this research include: (1) the interpretability of mineral abundance in natural porous media from spectral response of sediments; (2) relationships among observations of physical properties (especially permeability) at several scales; (3) the significance of preferential flow paths in microbial transport and attachment; and (4) determination of optimal moisture contents for estimation of permeability using air mini-permeameters and infrared imaging methods.

Significant progress has been made in developing a detailed dataset describing millimeter- to centimeter-scale joint physical and biogeochemical heterogeneity. Specifically, we have collected ultrasensitive IR images (256x256 pixels), high-resolution color scanner images and air permeability data of both halves of a core from Oyster, Va., used for a bacterial transport experiment. The microorganism used in the transport experiment was Comamonas sp. DA001 (Fuller et al., 1999; DeFlaun et al., 1990). We are currently in the process of obtaining 20-um resolution x-ray microtomography (XMT) data on core segments and 3-um resolution synchrotron light source XMT on subcores. When the physical parameter measurements are integrated with microbial distribution and transport data for the same core, we expect to identify specific controls on microbial attachment in heterogeneous porous media. These results will fill a key gap in the knowledge required for assessment of in-situ field-scale bioremediation.

Spatial Heterogeneity of Microbial Iron Reduction Potential at the South Oyster Focus Area

Chris Murray,[1] Eric Roden,[2] Ken Overstreet[2] and Susan Hubbard[3]
[1]Pacific Northwest National Laboratory, Richland, Wash.; [2]University of Alabama, Tuscaloosa, Ala.; [3]Lawrence Berkeley National Laboratory, Berkeley, Calif.

We are addressing the spatial distribution of subsurface microbial iron reducers at the field-scale, which will provide significant information required to understand and predict the effect of heterogeneity on the reduction and immobilization of radionuclides and metals by iron-reducing bacteria. In our initial research we have intensively sampled three boreholes at the South Oyster Focus Area, a DOE/NABIR analog site near Oyster, Va. The samples are being analyzed for their microbial iron reduction potential (MIRP) using a low-cost batch measurement method. We have also measured the extractable iron oxyhydroxide content, the hydraulic conductivity, the bulk density, the concentration of organic matter, and the grain size of the samples. We will also determine the detailed mineralogy of the solid mineral phases present for a subset of the samples which are representative of the different biogeochemical sediment types detected in the boreholes. High-resolution crosshole seismic data was recorded between each pair of boreholes.

Preliminary results indicate that the heterogeneity of the site is more pronounced than that of other DOE-sampled locations in the Oyster area, all of which were dominated by sandy sediments. The sediments at the site include fine-grained lagoonal and back-bay sediments as well as sand-rich sediment layers. The fine-grained sediments include both black organic-rich peat layers and light to medium gray clay beds that contain relatively high concentrations of extractable Fe(II) and organic matter. Three distinct types of sand layers appear to be present at the field site, based on differences in the presence of MIRP, the hydraulic conductivity and the concentration of extractable Fe(III).

The variations in physical properties of the sediment layers appear to be traceable on geophysical data recorded at the site, which should make it possible to predict the sediment types between the boreholes. Geostatistical analysis will be used to predict the levels of MIRP between the existing boreholes using stochastic simulation techniques that integrate the MIRP data with geological, geochemical and geophysical data. Further drilling and sampling will then test the predictions. The research will provide a model for the distribution of microbial iron reduction potential on the Atlantic Coastal Plain, and a methodology that can be applied to develop similar models for other locations.

In Situ Assessment of Effective Reactive Surface Area of Chemically Heterogeneous Porous Media

Robert W. Smith
Biotechnologies Department, Idaho National Engineering and Environmental Laboratory, Idaho Falls, Idaho

The relationship between effective reactive surface area (i.e., the surface area that reacts with locally advected solutes) of heterogeneous porous media and advective groundwater will be evaluated using reactive tracer experiments on cores collected at Oyster and Abbott's Pit. Inverse reactive transport modeling techniques relating tracer breakthrough to effective reactive surface area will be developed. Research results will provide a validated physicochemical scaling approach to assess the role of variable reactive surface area for field-scale contaminant and bacterial transport.

The characteristics and amount of reactive surface areas for subsurface materials have long been recognized as key to controlling the adsorption of contaminants (e.g., metals, radionuclides, organic ligands) and dissolved constituents (e.g., electron donors/acceptors, nutrients) required by subsurface microorganisms. In addition, the activity of (e.g., FIRB) and the retention of (bacterial transport) bacteria in subsurface are also influenced by the reactive surface area of subsurface materials. In water-saturated homogeneous systems devoid of advective fluxes (e.g., batch experiments), the surface area available for reaction is similar to the surface area (as measured by conventional means) of the subsurface media. However, in physically and geochemically heterogeneous systems with advective fluxes, the effective reactive surface area is smaller than the laboratory measured surface area and is a complex function of advective velocity, which is in turn a complex function of the correlation structures of the physical and chemical heterogeneities.

We propose to investigate the coupled relationships of small-scale (e.g., microfractures or sedimentary laminae) geochemical heterogeneity (spatial distribution of reactive minerals) in the presence of nonuniform groundwater flow and the effective reactive surface area of intact weakly consolidated sedimentary media. The experimental focus of the proposed research will be the use of cationic and anionic reactive tracers to estimate effective reactive surface areas in repacked columns with controlled heterogeneity of hydrous metal oxide coated sands, and intact core of from the South Oyster Site (Oyster, Va.) and Abbott's Pit, (Mappsville, Va). The selection of tracers and approaches will be done so that intermediate-scale (IS) reactive tracer experiments can be conducted using Oyster site materials in the latter part of the second year of the project (FY 2000). This research builds upon our extensive investigations of geochemically heterogeneity at the Oyster sites and will expand our previous studies of correlation structure to assess the effects of this structure on reactive transport of contaminants and bacteria.

.