LBNL-49054

The NABIR Strategic Plan

2001

Natural and Accelerated Bioremediation Research Program

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The NABIR Strategic Plan

Berkeley Lab

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Forward

The Natural and Accelerated Bioremediation Research Program (NABIR) is a highly interdisciplinary, basic research program in the Office of Biological and Environmental Research (BER) in the Department of Energy's Office of Science. The program is beginning its fourth year of full funding, and it has grown and evolved since its original inception in the 1995 NABIR Program Plan. NABIR was conceived as a >$40M program that would cover all aspects of bioremediation including organic and inorganic contaminants in surface and subsurface soils and sediments. When the program was initiated at substantially lower funding, members of the scientific community recommended a focus on metals and radionuclides in subsurface environments. This focus would provide the greatest impact in terms of fundamental knowledge of DOE relevant contaminants. It is time to sharpen the focus of the program based on new scientific knowledge resulting from NABIR findings, additional fiscal constraints, and the increased emphasis on long-term stewardship. This need to sharpen the program focus led to the development of the NABIR Strategic Plan.

The NABIR Strategic Plan has greatly benefited from input from many individuals including the staff of BER and the DOE Office of Environmental Management (EM-50, EM-52, EM-53, and EM-54), the Biological and Environmental Research Advisory Committee (BERAC) subcommittee on NABIR, the NABIR Program Office at the Lawrence Berkeley National Laboratory, and, importantly, the NABIR scientists themselves.

A. THE PROBLEM: DOE LEGACY WASTES IN THE SUBSURFACE

For more than 50 years, the U.S. created a vast network of more than 113 facilities for research, development, and testing of nuclear materials. As a result of these activities, subsurface contamination has been identified at over 7,000 discrete sites across the U.S. Department of Energy (DOE) complex. With the end of the Cold War threat, the DOE has shifted its emphasis to remediation, decommissioning, and decontamination of the immense volumes of contaminated groundwater, sediments, and structures at its sites. DOE is currently responsible for remediating 1.7 trillion gallons of contaminated groundwater, an amount equal to approximately four times the daily U.S. water consumption, and 40 million cubic meters of contaminated soil, enough to fill approximately 17 professional sports stadiums.[*]

It is estimated that more than 60% of DOE facilities have groundwater contaminated with metals or radionuclides. The only contaminant that appears more often than metal and radionuclide contaminants in groundwater is chlorinated hydrocarbons. More than 50% of all soil and sediments at DOE facilities are contaminated with metal and radionuclides, the contaminants found with the highest frequency in soil at all DOE waste sites.[†] Indeed, while virtually all of the contaminants found at industrial sites nationwide can also be found at DOE sites, many of the metals and especially the radionuclides found on DOE sites are unique to those sites.

Current technology for treatment of groundwater contaminated with metals and/or radionuclides is "pump and treat," followed by disposal or reinjection of treated water. This process can be costly and inefficient due to the difficulty of completely removing the contaminated groundwater and sorption of contaminants on mineral surfaces. DOE's Office of Environmental Management (EM), which is responsible for the cleanup, has stated that advances in science and technology are critical for DOE to reduce costs and successfully address these long-term problems.[*] DOE's Environmental Quality R&D Portfolio includes environmental restoration and long-term stewardship as its highest priorities. A recent analysis of the portfolio (September 2000) suggested that R&D in these two areas is inadequate. The NABIR program aims 1) to provide the fundamental knowledge to support the development of new bioremediation technologies and 2) to advance the understanding of key processes that control the effectiveness of containment as a means of long term stewardship.

NABIR has the distinction of being the only federal program that funds fundamental research on metal and radionuclide contaminants in the environment. The program's greatest strength is in focusing talents and expertise from many disciplines to address challenging research questions. The products from NABIR will influence the development of effective bioremediation technologies as well as contribute new knowledge about the function of subsurface ecological systems at the microbiological and geochemical levels. These advances can lead to more effective stewardship of natural resources as well as to remediation of DOE sites.

B. BIOREMEDIATION OF METALS AND RADIONUCLIDES

The catalytic potential of microorganisms in nature is enormous, and yet still relatively untapped for use in environmental cleanup. Bioremediation is the use of microorganisms to decrease, eliminate, or contain hazardous and/or radioactive wastes to environmentally safe levels. While bioremediation of organic contaminants involves their transformation to benign products such as carbon dioxide, bioremediation of metals and radionuclides involves their removal from the aqueous phase to reduce

[*] *Status Report on Paths to Closure,* DOE/EM 0526, U.S. Department of Energy, Washington, D.C., March 2000.

[†] Riley, R.G., and J.M. Zachara, *Chemical Contaminants on DOE Lands and Selection of Contaminant Mixtures for Subsurface Science Research,* DOE/ER-0547T, U.S. Department of Energy, Washington, D.C., April 1992.

risk to humans and the environment. Microorganisms can directly transform metals and radionuclides by changing their oxidation state to a reduced form that leads to in situ immobilization. Or, microorganisms can indirectly immobilize metals and radionuclides through the reduction of inorganic ions which can, in turn, chemically reduce contaminants to less mobile forms. The long term stability of these reduced contaminants is as yet unknown. Other mechanisms whereby microorganisms can influence mobility include alteration of pH, Eh, oxidation and complexation.

Currently, the fundamental knowledge that would allow the cost-effective deployment of in situ subsurface bioremediation of metals and radionuclides is lacking. Research on in situ bioremediation of metals and radionuclides has received less attention than research on solvents, fuels, and other organic contaminants; however, successful in situ applications of bioremediation of petroleum products and chlorinated solvents provide experience from which scientists can draw. Scientists in DOE's NABIR program are exploring the potential of bioremediation to solve DOE problems of radionuclide and metal contaminants in subsurface environments.

The focus of the NABIR program is on radionuclides and metals that 1) are of great concern at DOE sites, and 2) are tractable by means of bioremediation. Thus, research is focused on the metals chromium and mercury, and the radionuclides uranium, technetium and plutonium. Radioactive contaminants such as tritium and cobalt are not a focus because of their relatively short half lives, and strontium and cesium are not addressed because they are not readily amenable to biotransformation. Research is focused on the subsurface below the zone of root influence and includes both the vadose (unsaturated) zone and the saturated zone (groundwater and sediments). NABIR research is oriented toward application in areas that have low levels of widespread contamination because it is too costly to clean up those situations with existing technologies. Chromium, uranium and technetium can be especially mobile in the subsurface under certain conditions; they are risk-driving contaminants at some DOE sites. The effects of co-contaminants such as nitrate, complexing agents (such as EDTA) and chlorinated solvents (such as trichloroethylene and carbon tetrachloride) on the behavior of metals and radionuclides in the subsurface is also of interest to the NABIR program.

A. NABIR PROGRAM GOAL

The goal of the NABIR program is to provide the fundamental science that will serve as the basis for development of cost-effective bioremediation and long-term stewardship of radionuclides and metals in the subsurface at DOE sites. The focus of the program is on strategies leading to long-term immobilization of contaminants in place to reduce the risk to humans and the environment. The NABIR program encompasses both intrinsic bioremediation by naturally occurring microbial communities, as well as accelerated bioremediation through the use of biostimulation (addition of inorganic or organic nutrients) or, if necessary, through the use of bioaugmentation (addition of microorganisms).

NABIR will provide an improved, multidisciplinary understanding of the biogeochemical functioning of the subsurface environment. Sophisticated new findings related to molecular/microscopic workings of mineral-microbe association and their regulation by hydrologic, physical and other field scale processes and features will have broad applicability. The science base will enable remediation programs within the Office of Environmental Management while providing fundamental new insights into the biogeochemical cycling of nutrients, trace elements and trace gases in subsurface environments.

Natural attenuation is the removal of a contaminant from the aqueous phase or transformation of that contaminant to a less toxic form by natural biological, chemical, and physical processes, without human intervention. The biological component of natural attenuation is sometimes referred to as intrinsic bioremediation. By contrast, accelerated bioremediation involves manipulating the contaminated area to enhance the rates of microbial transformation of hazardous wastes. There are two types of accelerated bioremediation: biostimulation and bioaugmentation. Biostimulation is the addition of nutrients to stimulate natural microbial communities. Bioaugmentation involves the addition of microorganisms with special capabilities, and it is used if the requisite microorganisms for bioremediation are lacking at a site. Bioaugmentation is usually used in conjunction with biostimulation to provide nutrients to enhance the growth of the introduced microorganisms. Bioremediation strategies may in some cases be coupled with existing physicochemical approaches to clean up a contaminated site.

Naturally occurring subsurface microbes may be involved in intrinsic bioremediation of metal and radionuclides by reducing and immobilizing, either directly or indirectly, metal and radionuclide contaminants. However, these natural processes typically occur at fairly slow rates, and there may be a need to use biostimulation. The primary focus of the NABIR program is on biostimulation strategies, due to the ubiquity of metal-reducers in nature. If biostimulation studies show that enhancing the activity of naturally occurring microorganisms is insufficient to immobilize radionuclides and metals in situ, bioaugmentation approaches will be considered as a possible option for further research.

In situ immobilization of contaminants is one approach to long term stewardship, which is the post-closure responsibility of DOE at its contaminated sites. Long term stewardship involves long-term monitoring and other maintenance activities to ensure that residual in-ground contaminants do not spread further. Immobilization is focused on contaminant capture from both vadose zone and groundwater plumes. As such, it may be a strategy applied to prevent the discharge of deep or widely distributed contaminants from the vadose zone to groundwater, or from groundwater to a receiving water body (e.g., the Columbia River at Hanford). Immobilized metals and radionuclides are not removed from the subsurface as may occur with excavation, pump and treat, or biodegradation of organic contaminants. Therefore, an important aspect to the NABIR program is to assess factors controlling the long-term stability of the immobilized contaminants and to devise approaches (biological/chemical) to maintain their immobilization through the stewardship phase. In the future, engineering approaches may be designed to extract the immobilized contaminants that are captured in highly localized, biostimulated zones.

B. NABIR DELIVERABLES

The primary customer for research products from NABIR within the DOE is the Office of Environmental Management/Office of Science and Technology. Within this office, the responsibility for developing new technologies to address contamination in the subsurface at DOE sites falls under the Subsurface Contaminant Focus Area. Other customers may include agencies such as the Department of Defense (DOD) and industries dealing with metal contamination. Deliverables include:

- ***An integration of the disciplines of microbiology, geosciences and environmental engineering in a manner not previously accomplished.*** This fundamental understanding of the dynamics of microbial communities will provide the scientific underpinnings required to address a myriad of existing subsurface science problems as well as provide new insights on the biogeochemical cycling of elements on Earth.

- ***Bioremediation strategies for immobilization of metals and radionuclides in subsurface environments.*** These immobilization strategies may include intrinsic bioremediation, as part of the natural attenuation of metals and radionuclides, or biostimulation through the addition of key nutrients to accelerate the rate of immobilization of metals and radionuclides in situ.

- ***Defining the state-of-the-art in bioremediation.*** Fundamental knowledge will help delineate the limits of technology—what can and cannot be achieved relative to other technologies within the context of disposal site conditions; aid in determining the system attributes and features critical for process optimization and success; and provide the basis for criteria to assess bioremediation effectiveness and regulatory compliance.

- ***Science-based understanding of biogeochemistry of metals and radionuclides in the subsurface.*** NABIR is conducting fundamental research on biogeochemistry that is contributing to our overarching understanding of the fate and transport of metals and radionuclides in the subsurface. These findings may be immediately useful in determining the risk to the humans and the environment, developing remediation strategies, and determining cost factors. The knowledge that these studies will supply will also support DOE's effective long-term stewardship of these sites.

- ***Innovative tools for determining the bioremediation potential of microbial communities at DOE sites.*** Novel tools developed by NABIR researchers will ascertain if naturally occurring microorganisms in the subsurface at a contaminated site are genetically and physiologically capable of immobilizing metals and/or radionuclides. These tools may include nucleic acid microarrays for determining microbial community structure and antibody-based biosensors for radionuclides. Innovative approaches such as "push-pull" tests will allow site managers to determine the in situ activity of microbial populations in the subsurface.

C. NABIR PROGRAM STRUCTURE: An Overview

The NABIR Program consists of four closely interrelated Science Elements, two Cross-Cutting Elements, and the Field Research Center. The Science Elements are Biogeochemical Dynamics, Biotransformation, Community Dynamics and Microbial Ecology, and Biomolecular Science and Engineering. A detailed description of each of these Science Elements can be found in Section III. The two Cross-Cutting Elements—Bioremediation and Its Societal Implications and Concerns (BASIC) and Assessment (innovative method development)— are described in Section IV. The NABIR program has established a Field Research Center (FRC) on the U.S. Department of Energy Oak Ridge Reservation in Oak Ridge, Tennessee, at Bear Creek Valley (BCV) within the Y-12 National Security Complex. The FRC is a focal point for integration of process level understanding and field research in the NABIR Program. The FRC provides DOE-relevant samples contaminated with uranium and other radionuclides or metals to researchers. The FRC is described in more detail in Section V. Additional information about the NABIR program can be accessed from the NABIR Web site at http://www.lbl.gov/NABIR/.

The program elements are highly integrated (Figure 1), and scientific findings are shared among researchers at informal workshops and annual PI meet-

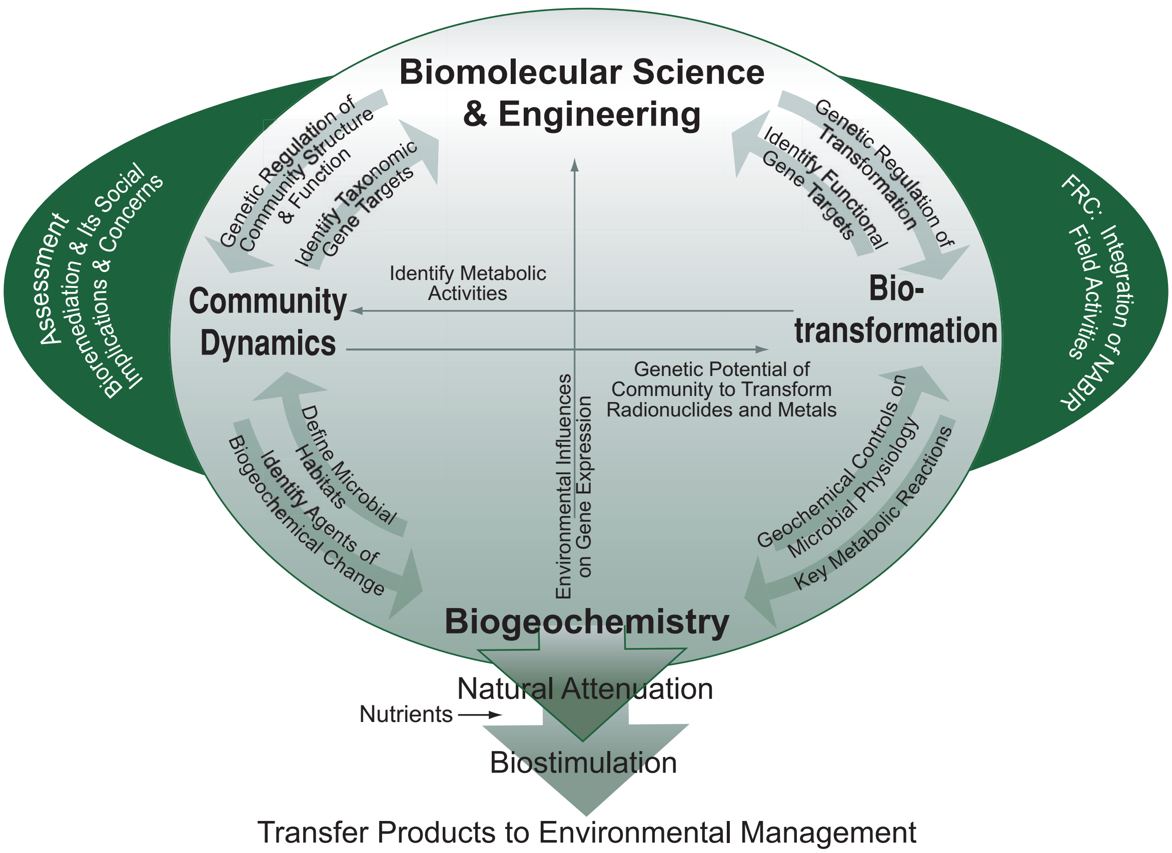

Figure 1. Integration of NABIR program elements. Process level understanding is shared among the four Science Elements (Biomolecular Science and Engineering, Community Dynamics, Biotransformation, and Biogeochemistry). Assessment and BASIC serve as cross-cutting elements. The Field Research Center (FRC) provides a focal point for field research). NABIR will provide the scientific basis for natural attenuation and biostimulation strategies.

ings. The highly interdisciplinary nature of the program has resulted in most researchers being involved in multiple program elements. Scientific disciplines represented in the program include microbial physiology and ecology, molecular biology, geochemistry, hydrology, and mathematical modeling. The Science Elements provide the basis for understanding intrinsic bioremediation of metals and radionuclides, and for understanding the potential for accelerated bioremediation through biostimulation. NABIR research proposals are evaluated by a process involving a competitive peer review for scientific merit and a review for programmatic relevance by NABIR program managers.

Program Phases

The NABIR program is planned as a 10-to-12-year program, consisting of three phases and an optional fourth phase:

Phase I: The goal of the first three years of NABIR was to develop a broad science base for bioremediation of metals and radionuclides, taking advantage of laboratory-based studies and "sites of opportunity" for collection of field samples. This science base included the four science elements and the two cross-cutting elements (Assessment and BASIC), as well as research in bacterial transport, data management, and systems integration. Sites of opportunity included a chromium-contaminated Superfund site at Cannelton, Michigan; Uranium Mill Tailing Remedial Action sites at Shiprock, New Mexico, and Gunnison, Colorado; and a pristine site at Oyster, Virginia, for bacterial transport studies. The Field Research Center (FRC) was selected after extensive peer review and completion of an environmental assessment and finding of no significant impact under the National Environmental Policy Act (NEPA).

Phase II: The Program has now entered Phase II. The goal of Phase II is to conduct hypothesis-driven research to understand biostimulation at the field scale, taking advantage of the FRC at Oak Ridge, Tennessee. NABIR investigators are utilizing samples from the FRC site (or other DOE-relevant sites) for laboratory-based studies on microbial communities in high-uranium/high-nitrate subsurface environments. In addition, several large-scale field experiments are planned, including push-pull and nutrient addition studies. In-depth characterization of the microbiology, geochemistry, and hydrology of the FRC site is in progress.

Phase III: In this phase, synthesis and modeling of data from field experiments at the FRC site will be a priority. Key findings will be applied to related sites, and opportunities to transition NABIR science to EM and other customers, including jointly funded research projects, will be pursued aggressively.

Phase IV (optional): If biostimulation does not effectively immobilize metals and radionuclides in the field, and if data suggest this failure is due to the lack of key microbial populations, a bioaugmentation experiment will be planned at the FRC. If necessary to ensure the survival of introduced microorganisms, bioaugmentation would be combined with biostimulation. Such a bioaugmentation experiment would take advantage of previous NABIR-funded studies on bacterial transport at Oyster, Virginia.

A. BIOTRANSFORMATION

Goal

The objective of this element is to understand the mechanisms of microbially mediated transformation of metals and radionuclides in subsurface environments leading to in situ immobilization and long-term stability. Physiological studies of the transformation of metals and radionuclides by key subsurface microorganisms and microbial consortia will provide the knowledge base needed to understand intrinsic bioremediation and to stimulate biotransformation in situ.

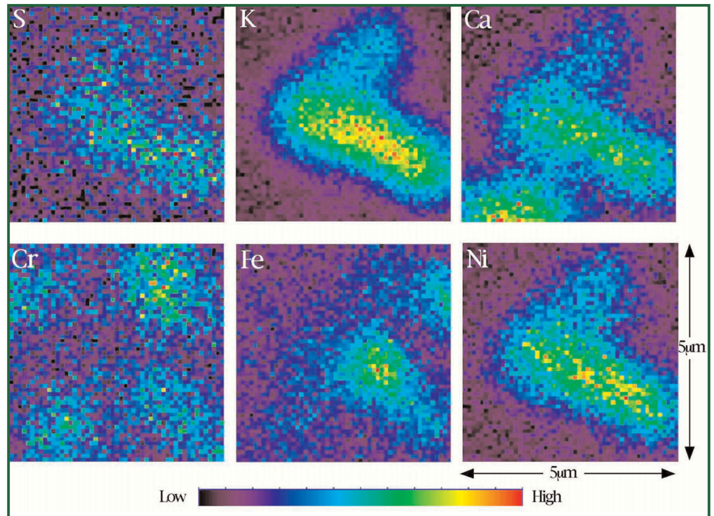

Figure 2. Elemental map of hydrated microorganism treated with Cr+6 solution. (K. M. Kemner, Argonne National Laboratory, Argonne, IL). Tools such as the Advanced Photon Source provide valuable information about microbe-metal interactions at the cellular level.

R&D Challenges

DOE subsurface sites encompass a wide range of redox environments where contaminants such as uranium are present. The first challenge is to understand the impact of these environments on microbial physiological processes involved in the transformation of radionuclides and metals to an immobilized form. The second challenge is to accelerate the rates of these physiological processes in situ, in complex "real world" environments where multiple contaminants are common.

R&D Initiatives: Current Status

The focus of the current research in this element is on the effects of dominant redox processes on microbial transformation of metals and radionuclides. Microcosm studies utilizing consortia of naturally occurring microbial communities from subsurface environments, serve as laboratory-scale models of intrinsic bioremediation and biostimulation. These studies include the examination of a range of terminal electron acceptors such as oxygen, nitrate, iron, manganese, sulfate, chlorate, and humics. Model subsurface organisms such as *Shewanella*, *Geobacter*, and *Desulfovibrio* are being utilized to study in detail the physiological processes of metal reduction and immobilization. Microbe-metal or microbe-radionuclide interactions, including microscopic and submicroscopic characterization of the cellular microenvironment, are being studied using state-of-the-art tools such as the advanced light sources at DOE laboratories (See Figure 2). The biotransformation of organic-metal/radionuclide complexes also is being studied, because transport of radionuclides and metals is profoundly affected by complexing agents and chelators that commonly occur at DOE sites.

R&D Initiative: 3 Year Targets

Within three years, physiological processes studied at the laboratory scale will begin to be scaled to the field. Biotransformation researchers will take advantage of the FRC as a key resource, both for field-scale hypothesis testing and for obtaining DOE-relevant sample material. Quantitation of in situ biotransformation kinetics will be a strong emphasis. Potential in situ inhibitors of microbial biotransformation of metals and radionuclides at the FRC will be identified. The in situ production of extracellular polymers for immobilization of metals and radionuclides will be examined.

R&D Initiatives: 7-10 Year Targets

Within seven to ten years, biostimulation strategies to accelerate intrinsic processes for immobilization of metals and radionuclides will be developed and tested in the field. Opportunities for combining biostimulation approaches developed in NABIR with existing chemical approaches for in situ immobilization will be explored. Mechanistic understanding of in situ biotransformation will be incorporated into numerical models (along with biogeochemical data) for predicting rates of immobilization and long-term stability.

B. BIOGEOCHEMISTRY

Goal

The goal of the Biogeochemistry Element is to understand the fundamental biogeochemical reactions that will lead to long-term immobilization of metal and radionuclide contaminants in the subsurface. The focus is on reactions that govern the concentration, chemical speciation, and distribution of metals and radionuclides in both the aqueous and solid phases.

R&D Challenges

Contaminated subsurface environments are complex. Biogeochemical reactions in subsurface environments are influenced by a wide variety of factors, including the availability of electron donors and acceptors, the nature of the microbial community, the chemical species or form of the contaminants, the hydrology, and the nature of the environmental matrix. Often several competing redox reactions make the prediction of the substrates, products, and reaction kinetics difficult. Microbial processes directly and indirectly influence mobility of metals and radionuclides, (See Figure 3) including alteration of pH and redox conditions, complexation and changes in partial pressure of gases, such as carbon dioxide. The biogeochemical reactions are further complicated by the sorption of contaminants to mineral surfaces and the presence of natural organic matter and co-contaminants. The research challenge is to identify and prioritize the key biogeochemical reactions needed to predict the rate and extent of reactions leading to the immobilization of radionuclides and metals for long-term stability. This fundamental knowledge will also help us to accelerate those processes through biostimulation.

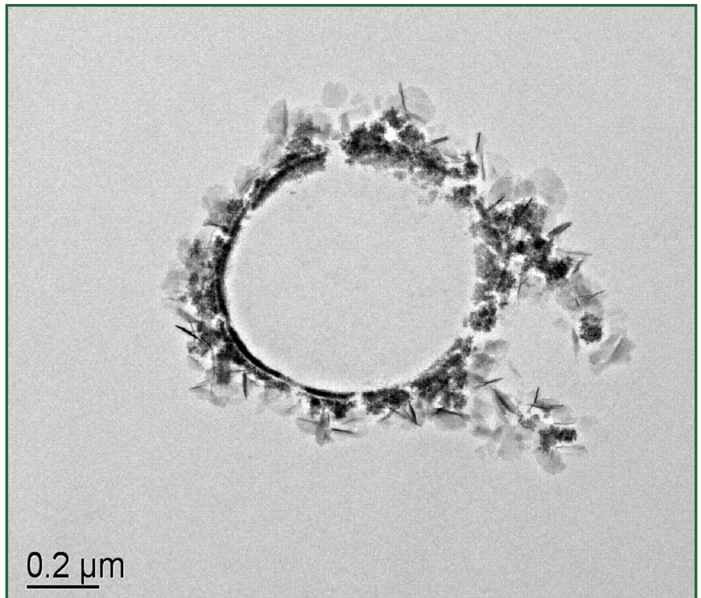

Figure 3. Cross section of *Shewanella* with uraninite precipitated on the cell surface (S.E. Fendorf, Stanford Univ., Stanford, CA). Microbes can play an important role in immobilizing radionuclides.

R&D Initiatives: Current Status

Current research in this element focuses on three areas: 1) the relative importance of abiotic and biotic redox reactions, and the kinetics of those reactions; 2) the impact of reactive sediment surface chemistries, such as iron oxide crusts, on the mobility of radionuclides and metals; and 3) the influence of redox reactions on the mobility and stability of radionuclides and metals.

R&D Initiative: 3 Year Targets

Within three years, some of the key chemical pathways of redox reactions involved in metal and radionuclide transformations will be determined in laboratory-based experiments. The technical approaches will emphasize natural geological matrices. Field investigations on biogeochemical dynamics will have begun at the FRC and will include the influence of co-contaminants such as nitrate on biogeochemical processes leading to the immobilization of radionuclides and metals.

R&D Initiatives: 7-10 Year Targets

Within seven to ten years, experiments will focus on coupled systems (biological, geochemical, hydrological) to accelerate immobilization by biostimulation and on the long-term chemical stability of immobilized metals and radionuclides in subsurface environments. Information on biogeochemical processes at the FRC will be integrated with biological processes (from the Biotransformation Element) and hydrological processes (from the FRC site characterization) into numerical models.

C. COMMUNITY DYNAMICS/MICROBIAL ECOLOGY

Goal

The goal of this element is to determine the potential for natural microbial communities to immobilize radionuclides and metals. In particular, research focuses on: 1) understanding the structure and function of microbial communities in the subsurface at DOE sites contaminated with metals and radionuclides; and 2) identifying and optimizing the in situ growth of microorganisms that transform radionuclides and metals. This research will enhance our ability to predict the effectiveness of intrinsic bioremediation and to optimize microbial communities for in situ immobilization of these contaminants.

R&D Challenges

Diverse microbial communities can be found in subsurface environments. These communities represent an untapped catalytic potential for transformation of radionuclides and metals. Most of these microbes, however, are as yet uncultured using current methods. One challenge is to use modern molecular methods to characterize these communities without the need for culturing individual organisms. In particular, scientists need to determine if sufficient genotypic and/or phenotypic potential exists to support natural attenuation or biostimulation. A second challenge is to optimize the community structure and activity for immobilization of radionuclides and metals, and to determine the long-term stability of such communities. Knowledge of microbial community structure and function may allow the stimulation and control of subsurface communities capable of immobilizing radionuclides and metals.

R&D Initiatives: Current Status

Researchers in this element are developing and applying molecular and biochemical methods to characterize the structure, activity, distribution, abundance, and diversity of microbial communities at contaminated DOE sites. They are determining ways to identify and quantify key bioremediative populations within these communities (See Figure 4). For example, specific gene probes are being developed to identify populations of radionuclide and metal reducing microbes.

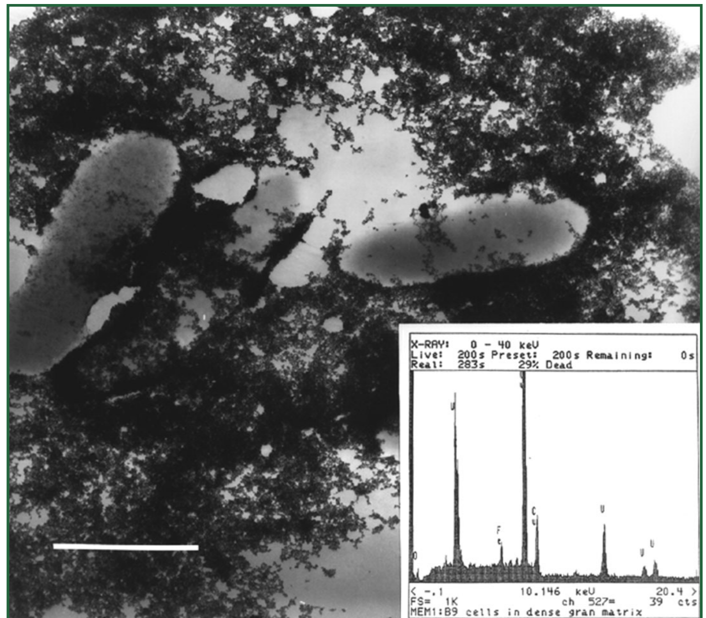

Figure 4. ***Geobacter*****, a microbe that can precipitate uranium, is commonly found in subsurface microbial communities. (D. Lovley, Univ. Massachusetts).**

R&D Initiative: 3 Year Targets

The role of consortial interactions will be determined in contaminated subsurface environments and comparable uncontaminated sites at the FRC and at other DOE-relevant sites. Competition among microorganisms for substrate and terminal electron acceptors, including metals and radionuclides, will be studied. The role of environmental factors (such as pH and interfacial chemistry) in regulating community structure and function will be examined. In addition, the potential importance of gene transfer at the level of microbial communities at contaminated subsurface sites will be examined to determine whether genes that transform metals and radionuclides or that protect cells from these contaminants can spread among bacteria in situ.

R&D Initiatives: 7-10 Year Targets

Growth and metabolism of key populations (such as metal reducers) will be optimized within natural microbial communities to enhance bioremediation of radionuclides and metals. Research will focus on controlling the stability (structure/function) of bioremediative communities for long-term site stewardship.

D. BIOMOLECULAR SCIENCES AND ENGINEERING

Goal

Research in the Biomolecular Sciences and Engineering element will provide a knowledge base, at the biomolecular level, of the processes leading to the immobilization of metals and radionuclides by subsurface microorganisms. The primary goal is to understand the genetic, biochemical, and regulatory processes that mediate biotransformation of metals and radionuclides. Characterization of genes, gene products, and genetic regulatory networks associated with these biotransformations is key to this understanding. Secondary goals include 1) understanding, at a molecular level, the process of lateral transfers between microbes of genes involved in biotransformation of metals and radionuclides; 2) developing novel technologies to provide insights into biomolecular mechanisms of metal and radionuclide biotransformation (See Figure 5); and 3) developing approaches to manipulate pathways and enzyme systems that mediate these transformations. The last goal focuses on the potential to engineer microbes at a genetic level to improve their ability to immobilize metals and radionuclides. This research will be important should the option of bioaugmentation be pursued.

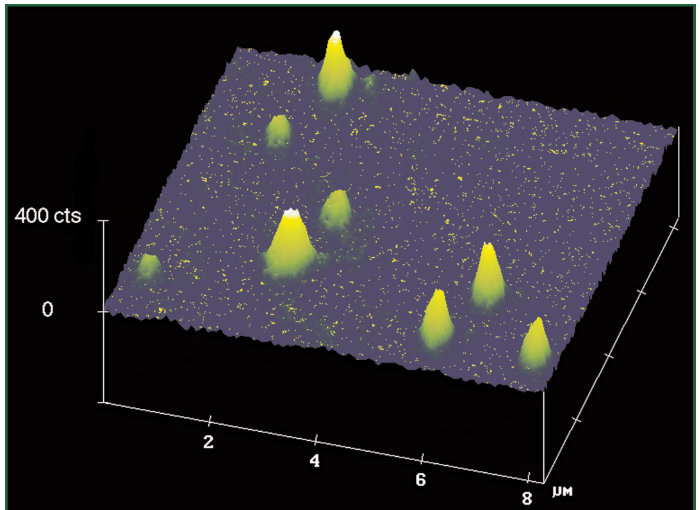

Figure 5. **Fluorescence image of single enzyme molecules (X.S. Xie, Harvard Univ., Cambridge, MA). Advances in spectroscopy provide a molecular level understanding of enzymatic kinetics of biodegradation of chelating agents found with radionuclides.**

R&D Challenges

DOE subsurface sites encompass a wide range of environments, with a diversity of microbial communities, consortia, and contaminants. One of the first challenges in this element is to select microbes for biomolecular studies that are, in fact, active members of subsurface microbial communities. A second, even more difficult challenge is to extrapolate laboratory findings on pure cultures under controlled laboratory conditions to complex in situ environmental conditions. This extrapolation is especially critical in studying gene expression, which may be modified by changes in local cellular environments in the subsurface. A third challenge for scientists in this element is to take full advantage of genomic and other data, derived from the DOE Microbial Genome Program, that is now becoming available through the DOE on subsurface microorganisms such as *Geobacter, Shewanella*, and *Desulfovibrio*.

R&D Initiatives: Current Status

Research is underway to identify and characterize novel genes, gene clusters, promoters, proteins, and pathways that are 1) involved in the biotransformation of metals and radionuclides, and/or 2) promote survival of microorganisms in the presence of metals and radionuclides. Additionally, research is in progress to identify and characterize the transfer of genes and chromosomal segments between organisms that can influence processes involved in bioremediation of metals and radionuclides. Finally, research is underway to engineer or enhance bioremedial enzyme pathways, using genes from microbes able to survive and compete effectively in environments contaminated by metals and/or radionuclides.

R&D Initiative: 3 Year Targets

Within the next three years, research will focus on understanding the regulation of genes that have been identified to be important in 1) the transformation of metals and radionuclides by naturally occurring microorganisms in contaminated subsurface environments, and 2) the survival of microorganisms in the presence of potentially toxic levels of metals and radionuclides. The effects of key environmental parameters on regulation and expression of these genes will be explored. Studies will also begin to elucidate the biomolecular mechanisms involved in lateral gene transfer in subsurface microbial communities. Such studies will improve the ability to predict and to manipulate the activities of microbes in situ, particularly in an in situ immobilization scenario.

R&D Initiatives: 7-10 Year Targets

Within seven to ten years, enzyme systems and pathways that mediate, regulate, and enhance bioremediation processes in natural microbial communities will be characterized in sufficient detail to permit deliberate in situ manipulation of naturally occurring microorganisms at the FRC. Techniques for assessing the character, frequency, lability, and stability of lateral gene transfers will be sufficiently advanced to allow in situ measurements of natural microbial communities at the NABIR FRC.

BASIC: BIOREMEDIATION AND ITS SOCIETAL IMPLICATIONS AND CONCERNS

The objective of this element is to identify and explore societal issues associated with NABIR. Major focus areas for BASIC research include: 1) identifying and prioritizing societal issues associated with bioremediation of metals and radionuclides in subsurface environments, particularly with strategies for immobilization in place; 2) fostering respect and collaboration between NABIR scientists and stakeholders; and 3) enhancing broad communication of NABIR research, including the development of education materials. BASIC is designed to provide information on issues that might influence the implementation of NABIR science and to involve NABIR scientists in discussions about the societal implications of their bioremediation research (See Figure 6). The BASIC program may also provide an avenue for interaction between NABIR investigators and regulators to identify key issues and sensitivities involved in bioremediation strategies such as immobilization of metals and radionuclides in situ, as a means of long-term stewardship.

Figure 6. The first BASIC workshop, held on July 18 and 19, 1996, at the Airlie Center near Warrenton, Virginia, consisted of 45 participants from government, academia, nonprofit institutions, trade organizations, and the business community who discussed the ethical, legal, and social issues (ELSI) associated with NABIR.

ASSESSMENT: INNOVATIVE METHOD DEVELOPMENT WITHIN NABIR

Development of innovative methods in support of the goals of the Science Elements is an important part of the NABIR program. Researchers are encouraged to identify areas where the science is currently limited by lack of appropriate analytical or field-usable technologies. Such innovations may provide a range of new methods—from development of cDNA-based microarrays for analysis of gene expression, to hand-held, antibody-based sensors of uranium species (See Figure 7), to infrared geophysical tools.

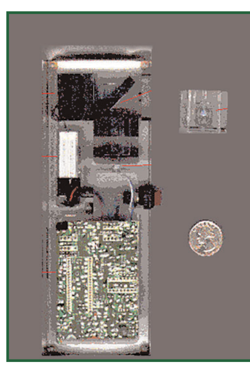

Figure 7. Portable antibody based sensor for uranium detection (D. Blake, Tulane Univ., New Orleans, LA).

The NABIR FRC provides a site for investigators to conduct field-scale research and to obtain DOE-relevant subsurface samples for laboratory-based studies of bioremediation (See Figure 8). The FRC is located on the U.S. Department of Energy Oak Ridge Reservation in Oak Ridge, Tennessee; it is operated by the Environmental Sciences Division of the Oak Ridge National Laboratory. The contaminated and background (uncontaminated control) areas are located in Bear Creek Valley (BCV) within the Y-12 Plant area. See http://www.esd.ornl.gov/nabirfrc for more information on the NABIR FRC.

The contaminated research site is a 98-hectare plot containing uranium, nitrate, technetium, strontium, and cadmium in groundwater, soils, and sediments. To a lesser extent, metals such as mercury, copper, zinc, and lead, and organics such as acetone, methylene chloride, tetrachloroethylene, and toluene are also present. The contaminated area includes the commingled groundwater plumes that originated from a combination of the S-3 Waste Disposal Ponds and the Bone Yard/Burn Yard. Both the background and contaminated areas are well-characterized and well-instrumented, and should be available for a duration of five to ten years. The water table resides between 0 and 3 m below the surface and is readily accessible to multilevel groundwater monitoring wells.

The initial focus of NABIR field research is on in situ biostimulation experiments to promote immobilization of uranium. Understanding natural and stimulated uranium biotransformation in the presence of high nitrate in unconsolidated residuum and fractured rock is one of the biggest challenges at the FRC at Oak Ridge, and at other DOE sites. Two NABIR research projects are underway at the FRC that address in situ immobilization of uranium in the presence of high nitrate. Some of the research questions that are currently being posed include: How can we accurately assess the biotransformation potential in situ ? What are the structure, function, distribution, and activity of microbial communities at the FRC? What nutrients will stimulate bioremediative microbes? How effective will biostimulation be in unconsolidated residuum? In the future, it will be important to also address

Figure 8. Drillers collect subsurface sediment cores at the NABIR Field Research Center, Oak Ridge, TN.

the hydrodynamics of the system and the pore scale, and the role of fluid flux on mobilization and transport of contaminants.

The FRC is a valuable resource through which NABIR researchers can conduct controlled, field-scale hypothesis testing to answer these and other questions. In addition, the FRC is currently providing subsurface samples for 20 laboratory-based NABIR projects. These projects span all NABIR Science Elements as well as the Assessment Element. Site characterization activities are ongoing and will result in a rich database for use by NABIR researchers. The FRC is responsible for data management, systems integration, and fundamental hydrological and geochemical modeling of the contaminated and background sites. The FRC also makes these data and models accessible to all NABIR researchers.

While the FRC provides a major focus for the NABIR program, it is recognized that other sites that represent different hydrogeological regimes at DOE sites will also be valuable to researchers. A large fraction of the national inventory of DOE wastes resides in unconsolidated, porous media in relatively thick, vadose zones and in groundwaters low in soluble organic carbon. For this reason, NABIR investigators are encouraged to take advantage of opportunities to collect and analyze samples from arid western environments that typify the Hanford Reservation and Uranium Mill Tailings Remedial Action (UMTRA) sites.

VI. Linkages to Genomics Programs in Biological and Environmental Research (BER)

NABIR has strong linkages to other BER programs such as the Microbial Genome Program, and other microbiological research programs. Advances in microbial genomics can lead to more reliable management and predictability of microbial processes, including those affecting the fate of contaminants. The NABIR program is taking advantage of the progress in functional genomics, proteomics, and environmental genomics in the Biomolecular Science and Engineering Element and in the Community Dynamics/Microbial Ecology Element. New technologies such as microarray analysis of gene expression and mass spectrometric analyses of cellular proteins are being used to better understand individual microorganisms such as *Geobacter* and *Shewanella* as well as microbial communities at DOE sites. The science of genomics both complements and enhances the basic science in NABIR.

VII. Interface with the DOE Office of Environmental Management

The NABIR program supports the goals of the Office of Environmental Management's (EM) Subsurface Contaminants Focus Area (SCFA). The SCFA is a national technology program whose mission is "to provide environmental stewards with remediation expertise and cost-effective solutions for DOE subsurface contaminant problems." NABIR program managers and scientists work closely with colleagues in EM to refine and focus the scientific research to meet EM needs, and to identify potential customers for transitioning NABIR basic research to applied research and technology development. EM is actively involved in providing input to the NABIR program through participation in panels, reviews, site visits, and FRC selection. NABIR and EM are planning a joint solicitation to encourage partnerships between NABIR scientists and problem holders in the SCFA community. Successful projects, which would be jointly funded by NABIR and EM, would transition more mature basic research into application and testing in the field at DOE sites. NABIR and EM will continue to work together to foster communication of science and technology to key stakeholders.

NABIR is also coordinating with EM on bioremediation research funded by the Environmental Management Science Program (EMSP). This research focuses on biodegradation of organic contaminants at DOE sites, such as chlorinated solvents; thus, it is complementary to NABIR. NABIR also coordinates with EM on research initiatives addressing vadose zone and groundwater contamination.

VIII. Coordination with Other Federal Agencies

The NABIR program is coordinated with bioremediation research by other federal agencies. The Interagency Working Group on Environmental Biotechnology, which is part of the Biotechnology Research Subcommittee of the National Science and Technology Council, is the primary vehicle for interagency coordination. Members include the Department of Defense (DOD), the Strategic Environmental Research and Development Program (SERDP), the National Science Foundation (NSF), the U.S.

Environmental Protection Agency (EPA), the U.S. Department of Agriculture (USDA), and the National Oceanic and Atmospheric Administration (NOAA). DOE is a partner in SERDP with DOD and EPA, and the NABIR team is represented on the Technical Thrust Area Working Group on Cleanup. SERDP serves as another potential transition route for NABIR science to applied research and technology demonstration.

IX. Points of Contact for NABIR Program

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