Appendix B. DOE Mission: Environmental Remediation

DOE has intractable contamination challenges at diverse sites around the country, making accurate prediction of contaminant behavior critical in determining the need for restoration and in suggesting stabilization or restoration strategies.

Understanding the complex interactions of microbes with contaminants and the subsurface environment—a GTL goal—will allow such predictions to be based on fundamental biological, geochemical, and hydrological properties of specific environments (see Mission Science Goals and Challenges, below right).

DOE Mission: Environmental Remediation

Develop Biological Solutions for Intractable Environmental Problems

B.1.1. Environmental Remediation Challenge

DOE is committed to remediating the large volumes of soil, sediments, and groundwater contaminated with metals, radionuclides, and a variety of organics at diverse defense production facilities and sites across the nation (see sidebar, A Legacy of Hazardous Waste, p. 217).

As an example of the problem's scope, about 5700 individual contaminant plumes, some quite extensive, are known to exist on DOE land (Linking Legacies 1997). Contaminated soils and sediments at the Nevada Test Site and Fernald, for example, are 1.5 and 0.71 million m^3, respectively. One plume at the Savannah River site extends over 7.8 km^2, and a plume of 18 km^2 exists at the Hanford site. In addition, unknown quantities of waste are buried at numerous sites. Without major breakthroughs in technology, projected costs for locating and characterizing contamination, restoring these sites, and disposing of wastes over the next 35 years are $142 billion (Closure Planning Guidance 2004). Although DOE has the goal of completing the remediation of 108 of 114 contaminated sites by 2025 (DOE Strategic Plan 2003), the 6 sites remaining to be addressed are the most challenging, and successful remediation will require development and deployment of innovative methods (see Table 1. Bioremediation: Goals and Impacts, p. 217).

Mission Science Goals and Challenges

Mission Science Goals: Understand the processes by which microbes function in the earth's subsurface, mechanisms by which they impact the fate and transport of contaminants, and the scientific principles of bioremediation based on native microbial populations and their interactions with the environment. Develop methods to relate genome-based understanding of molecular processes to long-term conceptual and predictive models for simulating contaminant fate and transport and development of remediation strategies.

Challenges: Bioremediation will require understanding biogeochemical processes from the fundamental-molecular to community levels to describe contaminant-transformation processes coinciding with simulated changes in microbial-community composition and structure.

B.1.2. The Role of Microbial Systems in Remediation

Microbes found in the contaminated subsurface and other environments often have the metabolic capability to degrade or otherwise transform contaminants of concern to DOE. Currently, DOE environmental restoration is targeting soluble forms of toxic metals and radionuclides in soils and sediments that move through the groundwater. Subsurface microbes, through their interactions with each other and the geochemical environment, play a role in modifying the geochemistry of these subsurface environments, thereby affecting their chemical form and movement. Microbes can directly, or indirectly through their influence on sediment geochemistry, provide a potential cost-effective bioremediation strategy to immobilize contaminants. *Shewanella* and *Geobacter*, for example, are two types of microbes that can enzymatically transform toxic species such as Uranium(VI), which is soluble and moves in groundwater, to Uranium(IV), which is insoluble and precipitates as UO_2 (uraninite) (see sidebars, Microbial Transformation of Toxic Metals, p. 218 and BER Research Advancing the Science of Bioremediation, p. 219).

A Legacy of Hazardous Waste

For more than 50 years, the United States created a vast network of facilities for research and development, manufacture, and testing of nuclear weapons and materials. The result is subsurface contamination on more than 7000 sites at over 100 facilities across the nation, more than half of which contain metals or radionuclides and many with chlorinated hydrocarbons. Biologically based techniques can provide cost-effective restoration strategies for many of these sites.

A Legacy of Hazardous Waste

Reactor

Tanks
- Cobalt
- Strontium
- Cesium
- Technetium
- Iodine
- Uranium
- Cyanide
- Nitrates
- Chromium

Direct injection
- Strontium
- Cesium
- Plutonium

Liquids to ground
Ponds
Cribs
Trenches
- Carbon Tetrachloride
- Iodine
- Tritium
- Uranium
- Technetium
- Cobalt
- Chromium
- Chloroform
- Cyanide
- Nitrate

Solids to ground
Pits
Burial trenches
Landfills
- Strontium
- Cesium
- Uranium
- Plutonium
- Carbon
- Tritium
- Americium

Water table

Waste plume

Table 1. Bioremediation: Goals and Impacts

- Understand and incorporate the effects of biological processes into computer models describing the fate and transport of contaminants in the environment. This knowledge could result in billions of dollars of savings by supporting decisions to take advantage of natural attenuation alternatives, use bioremediation for previously intractable problems, or improve the efficiency of conventional technologies.

- Develop new or improved bioremediation strategies and technologies. Potentially billions of dollars could be saved over traditional treatments. Bioremediation may offer solutions in previously intractable cases (i.e., where there was no solution at any price).

- Develop new suites of biosensors and performance assessment and monitoring techniques to track progress of environmental cleanup strategies and optimize operation of current cleanup techniques.

B.1.2.1. Benefits and Impacts

Although comparisons of the cost and effectiveness of metal and radionuclide bioremediation strategies with those of traditional remediation methods are not available, the cost savings for bioremediation of organics are estimated to range from 30 to 90%. In addition, in situ bioremediation, taking advantage of natural microbial populations in the subsurface, has the potential to reduce costs and increase the efficiency of groundwater treatment as compared to conventional pump-and-treat technology. Given that over 1 billion m³ of water and 55 million m³ of solid media at DOE sites in 29 states are contaminated with radionuclides (Linking Legacies 1997), potential savings accrued by use of innovative technologies are likely to amount to billions of dollars (Bioventing 1996; Patrinos 2005; Scott 1998).

Microbial Transformation of Toxic Metals

Naturally occurring, metal-reducing bacteria can influence the mobility of radionuclides such as uranium via enzymatic reduction processes. For example, *Shewanella oneidensis* reduces a wide range of organic compounds, metal ions, and radionuclides. Immobile precipitates are shown on the surface of this cross-section of *S. oneidensis*.

Research is needed to provide useful information to decision makers on whether remediation is necessary and practical, give an accurate prediction of contaminant mobility, and suggest bioremediation strategies. A biotreatment technique that works well at one site may perform poorly at another because we lack understanding of the unique interactions—in these "geologically powered dark ecosystems"—between the microbial community and subsurface geochemistry (Nealson 2005). Characterization and monitoring tools must be developed to gain that understanding. At a few sites of specific interest to DOE, less than 1% of the microorganisms have been collected, cultured, and characterized in any great detail, and only a small fraction of those have had their genomes sequenced. Even less is known regarding the interactions of microorganisms in communities. We have only begun to appreciate the existence of such systems, let alone understand them so we can take advantage of their diverse capabilities (Gold 1992; see The Microbial World, p. 13).
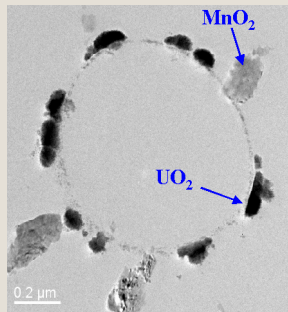
B.1.2.2. Establishing the Link Between Biology and Geochemistry

A key to successfully understanding these systems will lie in establishing the link between biology and geochemistry. An exacerbating challenge in establishing a meaningful science base for future applications is the inherent complexity of the subsurface and the distribution of contaminants. Most contaminated sites have extremely heterogeneous geology, hydrology, and resultant geochemistry. Subsurface processes are difficult to measure, control, and monitor, in part because samples from monitoring wells provide only single time-point data from a large three-dimensional area and because monitoring wells can disturb subtle interactions. Contaminants do not flow uniformly from a source point and can be transformed physically, chemically, or biologically to alter their state and mobility. Microbial and geochemical processes can, for example, both immobilize contaminants through redox (oxidation/reduction) processes and enhance transport through complexation. These natural and induced heterogeneities dramatically affect the distribution and makeup of microbial communities, resulting in countless niche environments and communities that must be understood and for which remediation strategies must account.

B.1.3. Using Genome Sequences as a Launch Point to Understand Communities

In this complex venue, we first must define the genomic potential of microbial communities. GTL uses genome sequences as a launch point for detailed, mechanistic investigations into microbial metabolism and other cellular subsystems to achieve a systems-level understanding. Having complete genome sequences for microbes known to catalyze important contaminant-transformation reactions provides an unprecedented

BER Research Advancing the Science of Bioremediation
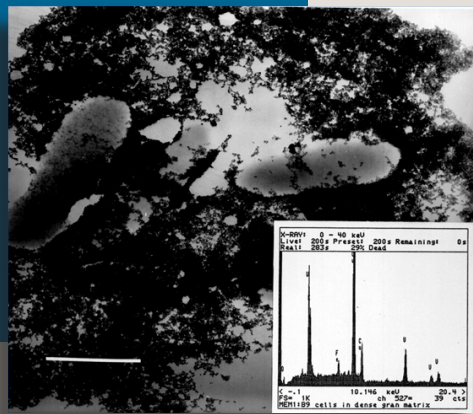## *Genome-Enabled Techniques Contribute to Model Development*

Geobacter species can transform uranium from a soluble to an insoluble form, effectively removing it from groundwater and preventing its further mobility. Environmental Remediation Sciences Division (ERSD) investigations demonstrated that this reaction is coupled to growth, indicating that the process is part of cellular respiration. Further exploration into *Geobacter's* metabolic pathways showed that uranium reduction is linked to the oxidation of organic carbon compounds such as acetate. In ERSD programs, adding acetate over a 3-month period to a site where *Geobacter* and uranium were present resulted in the *Geobacter*-mediated precipitation of uranium. After 50 days, the responsible *Geobacter* species became a minor component of the entire microbial community, demonstrating a need to better optimize strategies for long-term bioremediation by these microbes.

The ultimate goal is for GTL environmental-remediation research to develop in silico models that can be used before initiating bioremediation to accurately predict the metabolic behavior of microorganisms involved. Such models will enable evaluation of multiple potential bioremediation strategies before resources and time are committed to field work. GTL and ERSD researchers have made substantial progress toward this goal. An in silico model of *G. sulfurreducens* developed from its genome sequence has accurately predicted its metabolic response to a variety of environmental conditions. With further development, a more generalized in silico model of the *Geobacter* species that predominate during in situ uranium bioremediation will be able to guide optimization of uranium cleanup at a wide range of DOE sites.
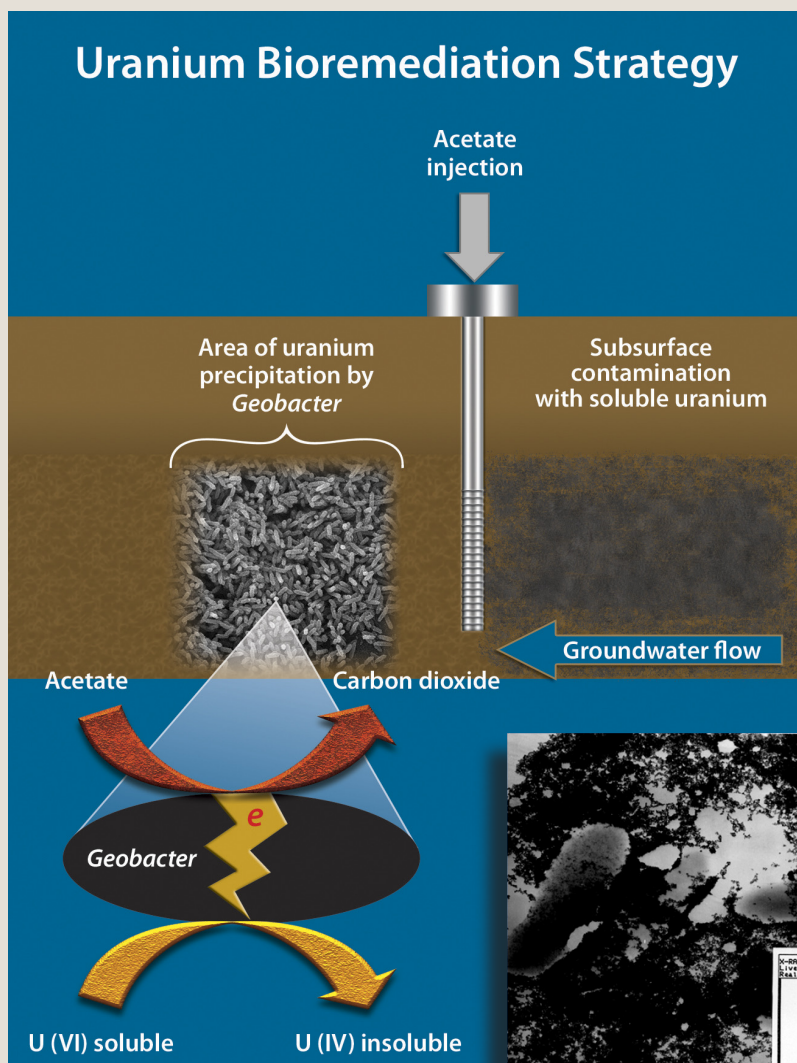
Reference

R.T. Anderson et al., "Stimulating the In Situ Activity of *Geobacter* Species to Remove Uranium from the Groundwater of a Uranium-Contaminated Aquifer," *Appl. Environ. Microbiol.* **69**, 5584–91 (2003).

Uranium Bioremediation Strategy

Acetate injection

Area of uranium precipitation by *Geobacter*

Subsurface contamination with soluble uranium

Groundwater flow

Acetate

Carbon dioxide

e

Geobacter

U (VI) soluble

U (IV) insoluble

D. Lovley, Univ. of Mass., Amherst

opportunity to describe these processes, from molecules through systems. Genome sequences enable investigations of the identities and functions of individual genes that code for important contaminant-transformation reactions, and their expression can be related to field-scale models of transformation processes in the environment—an important advance from earlier, more qualitative descriptions. Whereas historically our studies have been limited to microbes that can be cultured in the laboratory, the combination of metagenomics with the production and characterization of proteins from genes allows new insights into microbial function.

Most current research focuses on understanding single microbial species having potential for environmental remediation and stabilization (e.g., *Shewanella, Desulfovibrio,* and *Geobacter*) in laboratory-based cultivation or field studies. Microbially mediated environmental processes, however, rarely are due to the activity of a single group of organisms—microbes, even those in contaminated environments, typically live in diverse communities. Little is known of the overall dynamics of these communities, and the complexity and spatial structure of energy-transfer reactions that occur across the microbe-mineral interface have only begun to be revealed (see sidebars, BER Research Advancing the Science of Bioremediation, p. 219; *Geobacter*, p. 74; and *Shewanella*, p. 70). Characterization of microbial communities generally has been investigated using single-gene (16S rRNA) sequencing surveys to gain phylogenetic insights. Only a few organisms (notably metal and sulfate reducers) have been sequenced and characterized to any extent. Obtaining detailed links between genome sequence and molecular mechanistic function will require the use of the most robust metagenomic techniques to assess the makeup of communities and their genomic potential.

B.1.3.1. Modeling Microbial Metabolic Activities

Mechanistic models of microbial communities are key requirements in constructing field-scale contaminant fate and transport models that extrapolate over very long time periods. These models must treat such aspects of microbial metabolism as reactions to growth, stress, and nutrient limitation (among many others) that can directly affect gene expression. Understanding changes in all these metabolic activities along the migration pathway or with respect to time requires mathematical models. These models accurately simulate microbial metabolism and are supported by data from biogeochemical and environmental measurements to viably reflect the dynamic interplay of microbes and environment (see sidebar, A Revolutionary Whole-Genome Perspective, p. 221). Results will form the basis for evaluating and modeling pathways of such cellular processes as signaling, regulation, and response to contaminants. Key elements of modeling scenarios include microbe-mineral interactions and resulting molecular structural and charge-transfer responses; microbial-community responses (e.g., signaling, motility, biofilm formation, and other structural responses); and ensuing community functionality.

B.1.3.2. Merging Metabolic and Field-Scale Models

Eventually, GTL in silico genome-based microbial-metabolism models must be merged with Environmental Remediation Sciences Division (ERSD) field-scale models of contaminant fate and transport (see sidebar, Microbe-Mineral Interface in Contaminated Environments, p. 222). Some of these techniques already are being generated within environmental-restoration programs and GTL. GTL currently is focusing on quantitatively deciphering the molecular and biochemical pathways of several model microbes that catalyze contaminant-transformation reactions of interest to DOE. Additionally, complementary research within ERSD focuses on understanding the biogeochemical potential of subsurface microbes (see sidebar, Environmental Remediation Sciences Division Activities Complementary to GTL, p. 223). [For more information, see *Bioremediation of Metals and Radionuclides: What it is and How it Works*; 2nd ed., 2003).] To accomplish this linkage, more trained scientists are needed to determine the makeup of subsurface microbial communities and their interactions with the geochemical environment.

Meshing GTL's Approach to ERSD's Challenges
A Revolutionary Whole-Genome Perspective

Genomic information on cellular metabolism can be incorporated as physiological modules into computer (in silico) models to better understand metabolism, predict cell responses under different environmental conditions, and improve contaminant fate and transport models. Microbes already sequenced by DOE and under intense analysis in GTL have been detected in Environmental Remediation Sciences Division (ERSD) studies of in situ immobilization techniques in the subsurface of uranium-contaminated aquifers. These organisms are closely related to the GTL organisms on which existing physiological modules are based. As additional sequenced organisms become available, similar in silico models could be developed to more accurately model multispecies phenomena. These include syntrophic relationships, anaerobic degradation consortia, and shifts in the dominant terminal electron accepting process (called TEAP) observed in sediments. Although progress is being made, many challenges remain in placing fundamental physiological knowledge in the context of the dynamic flow and transport regimes characteristic of DOE sites.
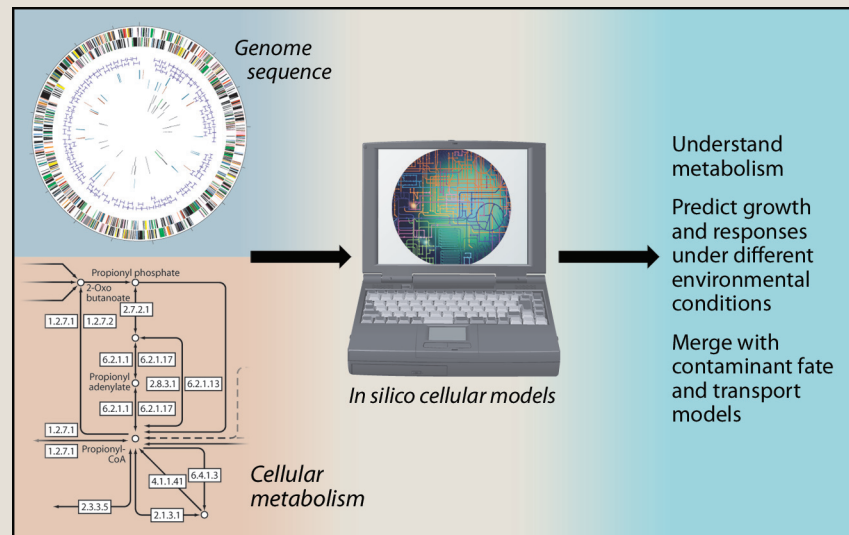
B.1.4. GTL's Vision for Environmental Remediation and Restoration

GTL science will facilitate detailed, large-scale discovery and investigation of microbes and microbial ecosystems with important contaminant-transformation capabilities. These studies will expand knowledge about structure, function, metabolic activity, and the dynamic nature of microbial communities and their interaction with the geochemical environment. The information will aid in the prediction of microbe-mediated contaminant fate and transport by providing more reliable, science-based information upon which to base remediation decisions. Capabilities and information established by GTL in conjunction with ERSD programs also will enable the integration of predictive microbiology with remediation science, leading to more effective and reliable applications.

B.1.4.1. Gaps in Scientific Understanding

Details underlying successful field-scale models ultimately focus on dynamic microbe and microbial-system geochemical interactions and functionality but have their foundations in the molecular interactions and processes that GTL seeks to understand. The following outlines, in broad terms, the science required over the next 15 years to deliver an integrated physiology and genomics-based understanding of microbial metabolism to support the detailed modeling of microbially catalyzed contaminant transformation in the environment. Key questions that we must be able to answer include the following (see also 5.4.1.1. Probing Mixed Microbial Populations and Communities, p. 175):

- What is the makeup of microbial communities? We need to learn who is there, their physiological states, their individual contributions, how they relate to each other and the environment, and how metagenomic DNA sequence can be used to predict the function, behavior, and evolutionary trajectory of microbial communities.

- How do microbes identify, access, and modify their local geochemical environments to gain energy and nutrients and meet other metabolic requirements?

- What physicochemical environmental interactions control the dynamic makeup, structure, and function of microbial communities in the subsurface, and what is the resultant impact on contaminant transformation?

- How and why do contaminants impact microbial communities, and what are potential indicators of these impacts?

- How do molecular mechanistic processes in microbes and communities relate to macroscopic behaviors in field environments?

B.1.4.2. Scientific and Technological Capabilities Required to Achieve Milestones

Key science and technology milestones for GTL and partner programs over the next 15 years are discussed in sections 1.4.2.1–1.4.2.4. Table 2. Bioremediation Challenges, Scale, and Complexity, p. 223, lists some research and analytical challenges with the scale and complexity of their scope.

Mission Challenge of Environmental Remediation Sciences Division

Microbe-Mineral Interface in Contaminated Environments

Biogeochemical processes driven by interactions at the microbe-mineral interface in soils and sediments influence contaminant behavior. These reactions occur at the level of the individual cell or groups of cells, forming an architecture assembled against the mineral surface. The cells interact with each other and the mineral surface, creating a dynamic, microscale domain that controls the kinetics of biogeochemically mediated reactions.

Before large-scale restoration strategies can be implemented, such processes must be better understood to predict contaminant transport in situ under natural and induced conditions. ("Induced conditions" refers to the addition of nutrients, oxygen, or other electron donors and acceptors to increase microbial activity.) Achieving this level of knowledge requires new tools to characterize and resolve fundamental phenomena at the molecular, cellular, and community levels. Microbial information must be integrated with the subsurface strata's geochemical characterization and the system's hydrological properties at the sediment mineral and pore scales.
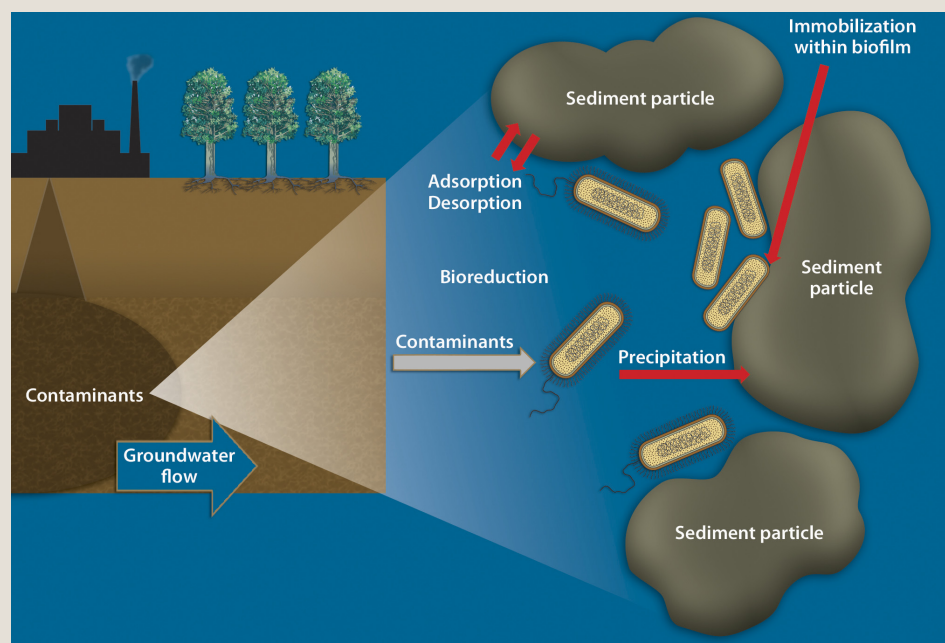
B.1.4.2.1. Defining Microbial Communities and Their Potential

- **Metagenomic methods tailored to unique sub-surface communities and environments to assess their general and specific makeup.** These studies will identify biochemical potential where possible and determine unknown genes through protein production and characterization and **other methods. Improving high-throughput** sequencing is allowing recovery of the genetic potential of single-strain microbes and whole communities (Venter et al. 2004; Tyson et al. 2004).

- **Refinement of the growing genome sequence databases and bioinformatics tools to identify and analyze genes found in environmental organisms.** New and faster methods of genome annotation ("super annotation") are required to capture fully the genetic potential of sequenced organisms.

- **Modeling and experimental capabilities to explore the physiology of full systems.** Current genomic descriptions of microbial metabolism for sequenced species are based on genes and the proteins they encode of known function or inferred from similar gene sequences found in other organisms such as *E. coli*. Genes of unknown function, which constitute a significant portion of most sequenced genomes, cannot be modeled. We must be able to produce and characterize these unknown proteins on demand. The sheer number of novel gene sequences to be investigated dwarfs currently available investigative techniques (such as knockout mutant characterization).

Environmental Remediation Sciences Division Activities Complementary to GTL

The Environmental Remediation Sciences Division (ERSD) of DOE's Office of Science seeks to understand microbial function in diverse environments and how these functions can be harnessed for restoration of contaminated DOE sites. Field-scale models for predicting contaminant fate and transport and designing remedial measures in complex heterogeneous environments depend on understanding biogeochemical reactions occurring in the subsurface at much smaller scales. This knowledge must be both spatially and temporally extrapolated. ERSD is positioned to identify microorganisms and processes by taking advantage of the genomic and proteomic systems biology tools offered by GTL. The goal is to use microbial capabilities for improving our understanding of the complex processes operating in the subsurface, placing them in the context of other simultaneous chemical and physical processes, and scaling the results to the field using advanced conceptual and mathematical models.

ERSD funds numerous laboratory- and field-based projects to evaluate the potential for subsurface microorganisms to immobilize or remobilize contaminant metals (including radionuclides) in situ. Significant progress has been made in detecting subsurface microorganisms associated with this process and in describing and modeling biogeochemical reactions mediated by microbes. A more complete understanding of microbial metabolism and community behavior will help determine the impact of microorganisms on contaminant fate and transport.

Table 2. Bioremediation Challenges, Scale, and Complexity

Research and Analytical Challenges	Scale and Complexity
• Analysis of microbial communities and their metabolic activities that impact the fate and transport of contaminants • Analysis of geochemical changes in subsurface environments due to microbial or chemical activity • Accurate conceptual and quantitative models for coupling and scaling microbial processes to complex heterogeneous environments	• Hundreds of different sites, millions of genes, thousands of unique species and functions • Functional analysis of potentially thousands of enzymes involved in microbe-mineral interactions; hundreds of regulatory processes and interactions; spatially resolved community formation, structure, and function; influence on contaminant fate • Models at the molecular, cellular, and community levels incorporating signaling, sensing, metabolism, transport, biofilm, cell-mineral interactions; incorporated into macro-models for fate and transport

- **Genome annotation to include functional genomics information such as cell response to environmental stimuli using functional gene arrays or expressed protein analyses.** These results will develop the basis for identifying and modeling biochemical pathways and regulatory networks within cells, including growth, stress, and metabolic responses to potential contaminants and other environmental factors.

- **Additional genome sequence generated for microbes and microbial communities and increased computing power to enable more intricate comparative genomics studies.** Also, more extensive protein structural analysis, protein networks analysis, and fold-recognition applications will be possible.

- **Method refinements to monitor growth and activities of microbial communities within the subsurface.** GTL will develop and standardize technologies for extraction of mRNA and protein from environmental samples. Information provided by these analyses forms crucial links between results obtained from current GTL research and the microbial processes as they occur in the environment.

- **Improved cultivation methods such as the microdroplet technique (Zengler et al. 2002; Keller and Zengler 2004) and others to capture a greater proportion of microbes associated with environmental samples.** These capabilities will enable us to study samples in the laboratory and compare them to the more thoroughly studied model organisms.

B.1.4.2.2. Measuring Microbial Processes and Responses

Breakthrough capabilities and technologies are needed to support measuring and modeling of microbially mediated contaminant-transformation processes and microbe-mineral interactions. These tools should permit a more complete mechanistic understanding of microbially mediated processes within the environment and provide solutions for microbiological and geochemical scaling issues needed for field-scale descriptions. Capabilities will be developed for modeling and measuring communities and single cells, both in isolation and within communities, to understand microbial systems. Examples include the following:

- **New, sensitive methods to measure the proteome, metabolome, and transcriptome of populations and communities of organisms.** Such measurements will involve significant developments in the area of high-throughput gene sequencing, protein identification and production, multivariable cultivation techniques, controlled cultivation and physiological analyses, and enzymatic analysis.

- **Methods to determine the biochemical basis for intracellular and intercellular interactions that contribute to the functionality of biofilms and other structured communities.**

- **Multifunctional imaging techniques to monitor biological, chemical, and physical changes simultaneously within environmental samples.**

- **Methods to investigate subsurface biogeochemical processes.** These require further development because most remain focused on either the geochemical or biological aspects of subsurface processes, to better integrate mechanistic aspects of both. As examples,

  - Several microspectroscopic techniques afford detailed analyses of mineral and biological composition and structure at high resolution and small scales and can be further developed for use with live biological analyses.

  - Kinetic data gathered from nutrient-enriched cultures grown in the lab often do not reflect processes observed in the field. Computer models of contaminant fate and transport within the environment must be developed to include robust biogeochemical modules. New techniques and capabilities are needed to apply laboratory data to complex field environments.

- **Techniques to examine and identify the composition of natural organic matter for exploring the metabolism of naturally derived substrates in environmental samples.** Only a subset of this material currently can be identified.

- **Data to characterize natural communities, including the following:**

  - Microbe-microbe interactions such as cell signaling, materials and energy transfer, gene transfer, and syntrophy to study phenomena occurring in biofilms. They remain poorly understood at a mechanistic level.

  - Functional microbial-community analysis to understand microbial positioning, including the potential for biofilm and other structured community development; their structure and relationship to function; and the molecular basis for microbial motility, competition, and niche exploitation. Little is known about the molecular basis for changes in community structure resulting from shifts in environmental conditions such as the introduction of contaminants. Detailed information about the impact of environmental changes on microbial-community structure leads to understanding of the community's functional stability and the net metabolic flux of electron donors and acceptors, including contaminant transformation. This knowledge will lead to better, more environmentally relevant descriptions of microbe-mediated processes.

B.1.4.2.3. Microbe-Mineral Interactions

Techniques will be developed for evaluating electron transfer and growth at mineral surfaces. Many microbes of interest to DOE respire metals (i.e., iron and manganese) that typically exist in solid-phase minerals. These organisms oxidize organic compounds and hydrogen and ultimately pass these electrons onto mineral surfaces, capturing energy for growth during the process. A crucial component of investigation is the ability to image and quantify processes occurring at the microbe-mineral interface, including the following:

- **The molecular basis for changes in cell-membrane structure to understand attachment, electron transfer, and mineral chemistry at the microbe-mineral interface.** More detailed analyses at the microbe-mineral interface will include molecular-level analyses of cell-membrane composition and dynamics and the interactions between membrane proteins and potential contaminants and mineral phases that can serve as electron acceptors.

- **Methods to measure microbial-community and environmental chemical and physical structures using methods that combine, for example, nuclear magnetic resonance (NMR) and optical imaging technologies.**

- **Further development of tomographic and spectromicroscopic techniques to provide information about chemical changes occurring on and, to a certain extent, within mineral surfaces, as well as about metals associated with microbial cells.**

- **Application of established and novel microscopies in combination with new sample-preparation and spectroscopic techniques to observe bacteria attaching to and growing on mineral surfaces in a noninvasive, real-time fashion.** These new techniques, in addition to infrared and micro-NMR imaging, will be needed to evaluate microbe-mineral associations under noninvasive conditions and in real time, a significant challenge.

- **Imaging abilities coupled with probes designed to evaluate metabolic processes as they occur within cells and communities of cells at a mineral surface in real time.** Coupled visual measurements of biological and geochemical parameters at the cellular scale will aid development of temporal and structural descriptions of microbial communities at mineral interfaces.

- **New suites of biosensors or probes to measure a variety of metabolic and geochemical processes down to the microbe-mineral scale.** These tools must be specific enough to relate biochemical changes to geochemical processes at high spatial resolution and extremely small scale. The sensors, along with required advances in cultivation techniques, will permit the investigation of microbially mediated processes, not only under controlled laboratory conditions but also within the environment. Once these processes can be measured and evaluated, new methods of kinetics analysis will be required for accurate testing and evaluation.

B.1.4.2.4. Modeling and Simulation Capabilities and Data Management

- **Novel analysis techniques to mate the results of GTL in silico models of microbial metabolism with field-scale contaminant fate and transport models created within DOE ERSD.** This is part of a classic problem in relating the temporal, spatial, and process scales of molecular mechanisms to the scales of macrosystems such as contaminant plumes that function over years and kilometers.

- **Computational techniques to correlate spatial information with geochemical properties and reactions at meaningful scales.**

- **Improved modeling techniques to enable incorporation of many more variables, particularly when attempting to simulate molecular processes at larger scales or under a variety of environmental conditions.** Current modeling capabilities and analysis tools are geared to mathematical representations that are too simple or too small (<10-state variables).

- **Significant improvements in data management and interpretation to use and distribute the enormous amounts of data generated by genome sequencing and associated systems biology.** Current bioinformatics techniques are limited primarily to sequence and structure analyses of genetic information. The vast amount of biological and environmental data presents equally pressing challenges for storing and managing access to these very large data sets. Organization is key, and centralization is envisioned in which input of and access to data can be standardized to common QA/QC protocols and routines. Metadata definitions and databases must be formalized and managed in a similar fashion. (For a more detailed discussion, see 4.0. Creating an Integrated Computational Environment for Biology, p. 81.)

- **Significant computer power and efficient computational methods to support visualization techniques, particularly important for protein structure and 3D simulation of processes in both the laboratory and environment.**