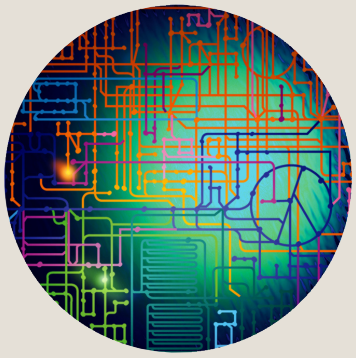
2.0. Missions Overview: The Role of Microbial Systems in Energy Production, Environmental Remediation, and Carbon Cycling and Sequestration

To accelerate GTL research in the key mission areas of energy, environment, and climate, the Department of Energy Office of Science has revised its planned facilities from technology centers to vertically integrated centers focused on mission problems. The centers will have comprehensive suites of capabilities designed specifically for the mission areas described in this roadmap (pp. 101-196). The first centers will focus on bioenergy research, to overcome the biological barriers to the industrial production of biofuels from biomass and on other potential energy sources. For more information, see Missions Overview (pp. 22-40) and Appendix A. Energy Security (pp. 198-214) in this roadmap. A more detailed plan is in Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda, DOE/SC-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy (http://genomicsgtl.energy.gov/biofuels/).



Missions Overview: The Role of Microbial Systems in Energy Production, Environmental Remediation, and Carbon Cycling and Sequestration

The Department of Energy's overarching missions are to advance the national, economic, and energy security of the United States; promote scientific and technological innovation in support of that mission; and ensure environmental cleanup of the national nuclear weapons complex (*The Department of Energy Strategic Plan,* 2003).

2.1. Introduction to GTL Goals for DOE Missions

The complexity of DOE's missions requires groundbreaking research and integration across multiple disciplines to create new generations of technologies. In the coming decades, bioscience and biotechnology must play an increasing role in informing policy and decision making and providing innovative solutions (see Fig. 1. Grand Challenges for Biology, Payoffs for the Nation, p. 23). The earth's microbial systems are the foundation for life and a potential source of capabilities that we can put to use to meet national challenges; their study forms the core of the GTL program. This chapter links the scope of DOE missions, some potential microbial contributions, and science and technology objectives to achieve timely impacts.

The ultimate GTL scientific goal is to attain a predictive systems-level understanding of microbes. Each mission area has a distinct technical endpoint and set of subsidiary science goals as described in this chapter. These goals define a unique set of research challenges that collectively will require new capabilities and a large body of integrated knowledge on every aspect of microbial systems behavior.

2.2. GTL Research Analyzing Mission-Relevant Systems

In the first phase of the GTL program, research projects are focusing on basic biological studies relating to mission-relevant systems. The goals are to understand scientific issues and challenges, begin to use new generations of research technologies, learn how to apply computation and modeling, and work in a multidisciplinary team environment (see 3.3. Highlights of Research in Progress to Accomplish Milestones, p. 55, and Appendix E. GTL-Funded Projects, p. 245). DOE BER has sequenced the genomes of nearly 200 microbes with wide-ranging biochemical capabilities (see Appendix G. Microbial Genomes Sequenced or in Process by DOE, p. 253). Some of the microbes and microbial communities being studied in GTL have potential for stabilizing toxic metals and radionuclides, degrading organic pollutants, producing energy feedstocks including biofuels and hydrogen, sequestering carbon, and playing a critical role in cycling ocean carbon and other elements.

2.2.1. Engineered Systems

Biology will be transformed into a quantitative and model-based science to allow the kind of systems engineering that has typified material- and chemical-based mission technologies. We need to understand the molecular mechanisms of microbial processes well enough to reliably redesign systems for new applications in unique engineered environments. This scientific research requires multidisciplinary teams focused on substantial goals.

2.2.2. Natural Ecosystems

For climate and environmental applications, GTL will develop the capability to understand the molecular mechanistic processes and the global responses of microbes in ecosystems. Metagenomics, a new field of culture-independent genomic analysis of microbial communities (Schloss and Handelsman 2003), is revealing that microbes in oceans and soils are substantially more genetically and potentially more biochemically diverse than expected. One recent experiment in the Sargasso Sea has uncovered more than a million genes, resulting in doubling the total amassed set of sequenced genes in the world (Venter et al. 2004; Meyer 2004). Since fully 40% of genes encountered are of unknown function and even homology-assigned functions often are uncertain, we must devise means to analyze very large numbers of genes in the laboratory without expressing them in their native hosts. In addition, the wide genetic diversity of hydrogenases and bacteriorhodopsins, for example, calls for analyses to determine the functional significance of gene variations. Specifically, working from genomic sequence, we can create and characterize proteins to estimate function. Ultimately, we will measure the molecular responses of cultured cells or cells in their natural environments (e.g., transcriptomes, proteomes, metabolomes). Mining the global gene pool also presents an opportunity to discover new genes, processes, and species that could point the way for biotechnology applications for DOE missions.

2.2.3. Shortening the Missions Technology Cycle

GTL knowledge, experimental capabilities, and facilities coupled with the GTL computational biology environment will form a bridge between science and applications. Comprehensive data and models will allow scientific discoveries at molecular-level time and spatial scales to be incorporated into larger models and simulations. These will cover the large process, spatial, and time scales used in mission applications for systems engineering of application technologies (e.g., biofuel production and bioremediation) and policy-support products (e.g., climate models, economic models, and integrated assessments). These capabilities will contribute to a dramatic shortening of the technology cycle, allowing frontier science to be incorporated more directly into useful systems and reducing time and costs between discovery and use.

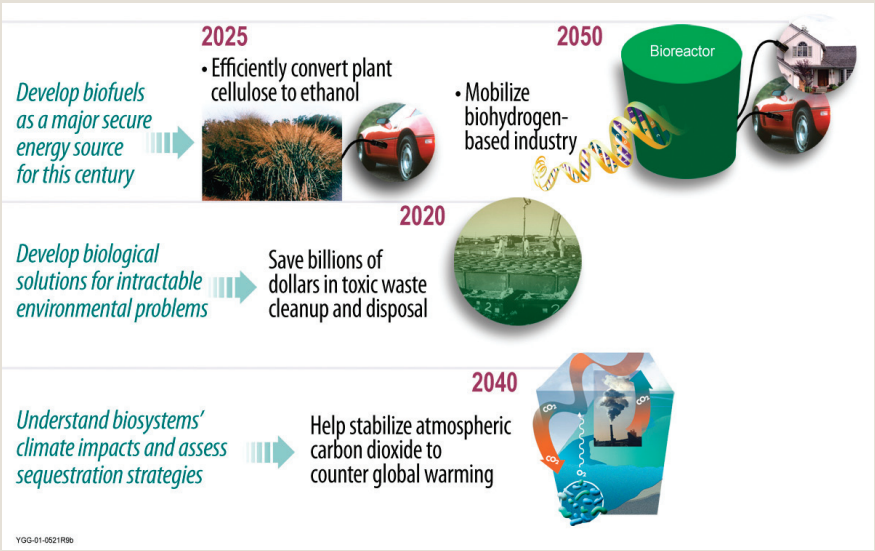


Fig. 1. Grand Challenges for Biology, Payoffs for the Nation.

2.3. Basic Energy Research: Develop Biofuels as a Major Secure Energy Source

Global energy demand is projected to rise rapidly in this century due to population growth and increasing worldwide gross domestic products, standards of living, and the energy intensity of developing economies (see sidebar, The Framework for DOE Missions, this page, and Appendix H. Programs Complementary to GTL Research, p. 265).

The national energy strategy's central tenet is that technology development will enable deployment of necessary energy resources and greenhouse gas (GHG) abatement as world economies build out the energy infrastructure to meet increased demand. Only a small part of the global energy infrastructure required by the end of the century exists today, and much of that will require replacement in the next 50 years. Without new energy technologies and sources, this situation will result in raising GHG emission levels significantly and increasing the strain on global energy supply lines and their security and on economic growth. Numerous technology options are required, and biotechnology is projected to have a substantial role in this buildout (Abraham 2004; Pacala and Socolow 2004; Socolow 2005).

The Framework for DOE Missions

DOE's Energy-Production and Climate-Change Strategies. DOE pursues its energy technology and climate science goals under the multiagency framework defined by the Climate Change Science Program (CCSP) and the Climate Change Technology Program (CCTP)—both founded on a technology-development strategy. The programs seek to understand climate change, reduce greenhouse gas (GHG) emissions, provide growing global economies with adequate and inexpensive energy, and improve GHG emission monitoring (see Appendix F. Strategic Planning for CCSP and CCTP, p. 249).

DOE Environmental Remediation Commitment. Agreements among DOE, the Environmental Protection Agency, and affected states have committed them to cleaning up the legacy of defense-related nuclear activities (i.e., large volumes of soil, sediments, and groundwater contaminated with metals, radionuclides, and a variety of organics). DOE estimates that, without major technical breakthroughs, cleanup will take about 35 years at a cost up to $142 billion (Closure Planning Guidance 2004).

By 2100, biotechnology-based energy use could equal all global fossil energy use today (see Fig. 2. Filling the Technology Gap, p. 25). Biologically derived fuels are renewable and expandable to meet the growing demand. They are domestically and globally available for energy security, with most being carbon neutral—or potentially carbon negative (if coupled with sequestration)—and supportable within the current agricultural infrastructure.

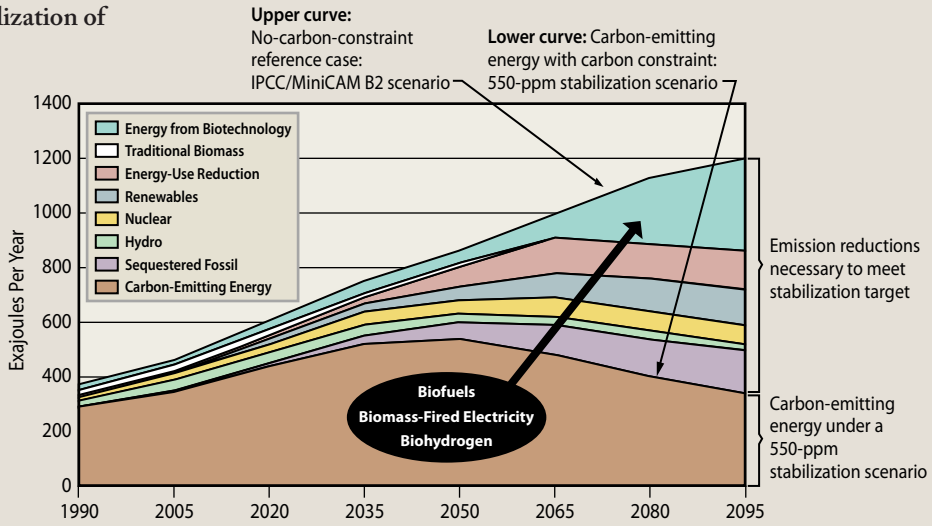
Two example biofuels discussed here are cellulose-derived ethanol and biophotolytic hydrogen. Cellulosic ethanol, a carbon-neutral fuel, is usable with the existing energy infrastructure. Hydrogen is the ultimate carbon-free energy carrier that can be converted efficiently to energy in fuel cells, with water as the only chemical by-product. Other potential biofuels include lipids, biodiesel, ammonia, methane, and methanol, each with multiple production options. Other future energy systems might include fuel cells based on biological processes.

The technology endpoint for energy systems is global deployment of engineered biological or biobased processes. This application requires a science base for molecular and systems redesign of numerous proteins, pathways, and full cellular systems. Biofuels could be produced using plants, microbes, and enzymatic solutions. Understanding the entities involved in these processes and the principles that govern biological mechanisms will allow scientists and technologists to design novel biofuel-production strategies such as engineered nanobiostructures (see sidebar, Synthetic Nanostructures, p. 25).

2.3.1. Ethanol Production from Cellulose

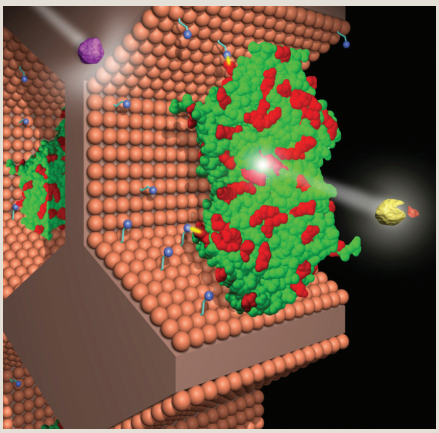
Biofuels such as cellulosic ethanol can provide alternatives to oil, displacing it as a transportation fuel with security, economic, and environmental benefits. Cellulosic ethanol can be cost competitive with oil-based gasoline and can reduce net CO_2 emissions from the transportation sector (roughly one-third of U.S. emissions) by more than 80% at no extra cost (Greene et al. 2004; Mann 2004). The cellulosic ethanol option would allow us to invest our energy dollars domestically, providing a profitable crop for farmers. In addition to

Fig. 2. Filling the Technology Gap. Based on technical and economic analyses, this figure compares two hypothetical scenarios for energy demand and supply growth over this century: (1) The IPCC/MiniCAM B2 scenario (upper curve), which assumes a relatively unchanged energy mix, is not carbon constrained; (2) A carbon-constrained scenario that stabilizes atmospheric concentrations of CO_2 at 550 ppm (family of curves below) was chosen to illustrate the types of changes in energy mix that might occur. An acceptable level of atmospheric CO_2 is still to be determined. U.S. energy strategy is based on technology development to provide multiple options to fit various national and global contingencies, market forces, and the ultimate stabilization of carbon emissions to near zero. This combination of technologies includes energy-use reduction, new and expanded nonemitting energy sources, and carbon sequestration. To meet these goals in the scenario illustrated here, analysis indicates that by the end of the century biotechnology sources of energy must grow to roughly equal today's fossil-fuel usage.



Synthetic Nanostructures: Putting Microbial Capabilities to Work

Understanding the sophisticated biochemistries of microbes can lead to the discovery of ways to isolate and use their components to carry out some of the functions of living cells. An example in this figure shows the enzyme organophosphorus hydrolase (OPH), which has been embedded in a synthetic nanomembrane (mesoporous silica) that enhances its activity and stability [*J. Am. Chem. Soc.* 124, 11242–43 (2002)]. The OPH transforms toxic substances (purple molecule at left of OPH) to harmless by-products (yellow and red molecules at right). Applications such as this could optimize the functionality of countless enzymes for efficient production of energy, removal or inactivation of contaminants, and sequestration of carbon to mitigate global climate change. The knowledge gained from GTL also could be highly useful in food processing, pharmaceuticals, separations, and the production of industrial chemicals.



E. Ackerman, Pacific Northwest National Laboratory

reducing GHGs, these crops improve air and soil quality, reduce soil erosion, and expand wildlife habitat. GTL research can contribute to making cellulosic ethanol more economical and practical by decreasing the complexity and cost of processing cellulose to ethanol.

2.3.1.1. Science and Technology Objectives

The first step in increasing the economic viability of biofuels and biochemicals, including ethanol, is to use cellulose and other such biomass constituents as hemicellulose and lignin instead of the ultimately limited food starch that predominates today. Ethanol from cornstarch has a 14% energy yield (i.e., net energy content of the feedstock converted to energy in ethanol), whereas cellulose can have a 37% yield (see Table 1, Cellulosic Ethanol Goals and Impacts, p. 26) resulting from improved process efficiencies

(Smith et al. 2004). Use of waste cellulose can provide an energy source equal to 10% of current gasoline usage; to achieve greater impacts, energy crops must be used (Mann 2004). Cellulose, a carbohydrate polymer that makes up plant cell walls, is the most abundant biological material (see sidebar, Cellulose: Microbes Process it into Ethanol-Convertible Sugars, p. 27). The strong, rigid, water-insoluble nature of cellulose and the cell's other structural materials, however, makes them resistant to degradation into sugars and difficult to process into ethanol. The complex multistep process for commercially converting cellulose into ethanol currently combines thermochemical and biological methods in large centralized processing plants. With improved enzyme systems, we can replace expensive thermochemical processes. The ultimate innovation, integrated processing—combining all key hydrolytic and fermentative steps in one process using either a single microbe or stable mixed culture—would enable smaller-scale and more cost-effective and energy-efficient distributed processing plants.

Biofuel Development

- **Mission Science Goals:** Understand the principles underlying the structural and functional design of microbial and molecular systems, and develop the capability to model, predict, and engineer optimized enzymes and microorganisms for the production of such biofuels as ethanol and hydrogen.
- **Challenges:** Analyze thousands of natural and modified variants of such processes as cellulose degradation, fermentative production of ethanol or other liquid fuels, and biophotolytic hydrogen production.

Biomass-degrading microbes and fungi are sources of enzymes that can improve wood preprocessing and cellulase enzymes that break down cellulose into fermentable sugars (see Fig. 3. Converting Cellulose to Sugars, p. 27). Large numbers of cellulase-producing organisms and potentially thousands of bacteria and yeast species can convert simple sugars to ethanol. An important part of GTL science will be to analyze and screen different microbes, fungi, and natural microbial communities to increase the number of enzymes that can be examined. The DOE Joint Genome Institute has determined the genome sequence of white-rot

Table 1. Cellulosic Ethanol Goals and Impacts

Factors	Today	Interim	Long-Term*
Billion gallons Fossil fuel displaced** CO_2 reduced	4 2% 1.8%	20 10% 9%	30 to 200 15 to 100%*** 14 to 90%
Feedstock****	Starch (14% energy yield)	Waste cellulose	Cellulosic energy crops (>37% energy yield)
Process	Starch fermentation Little cellulose processing	Acid decrystallization: Transition to enzymes Cellulases Single-sugar metabolism Multiple microbes Some energy crops	Enzyme decrystallization and depolymerization Cellulase and other glycosyl hydrolases Sugar transporters High-temperature functioning Multisugar metabolism Integrated processing Designer cellulosic energy crops Carbon sequestration through plant partitioning
Deployment	Large, central processing	Large, central processing	Distributed or centralized, efficient processing plants
Other impacts: Energy dollars spent at home, third crop for agriculture, land revitalization and stabilization, habitat, soil carbon sequestration, yield per acre roughly tripled (cellulose over corn starch).			

*Enabled by GTL.
**Current U.S. consumption of gasoline is about 137 billion gallons per year, which corresponds to about 200 billion gallons of ethanol (Greene et al. 2004) because a gallon of ethanol has 2/3 the energy content of a gallon of gasoline.
***Assumes improvements in feedstocks, processes, and vehicle fuel efficiency.
****Adapted from Smith et al. 2004.

Cellulose: Microbes Process It into Ethanol-Convertible Sugars

Cellulose, the main structural component of plant cell walls, is a linear polymer consisting of thousands of glucose residues arranged in a rigid, crystalline structure. Layers upon layers of cellulose-containing microfibrils give plant cell walls their remarkable strength. Each microfibril consists of a crystalline cellulose core encased within a complex outer layer of amorphous polysaccharides known as hemicellulose. The crystallinity of cellulose and its association with hemicellulose and other structural polymers such as lignin are two key challenges that prevent the efficient breakdown of cellulose into glucose molecules that can be converted to ethanol. Adding to the difficulty is the diverse mix of simple sugar molecules generated from the hydrolysis of cellulose and hemicellulose. Fermentative microorganisms prefer to use six-carbon sugars (e.g., glucose) as substrates for producing ethanol; however, hemicellulose is composed of a variety of five-carbon sugars that are not efficiently converted into ethanol by microorganisms. [Microfibril structure adapted from J. K. C. Rose and A. B. Bennett, "Cooperative Disassembly of the Cellulose-Xyloglucan Network of Plant Cell Walls: Parallels Between Cell Expansion and Fruit Ripening," *Trends Plant Sci.* 4, 176–83 (1999).]

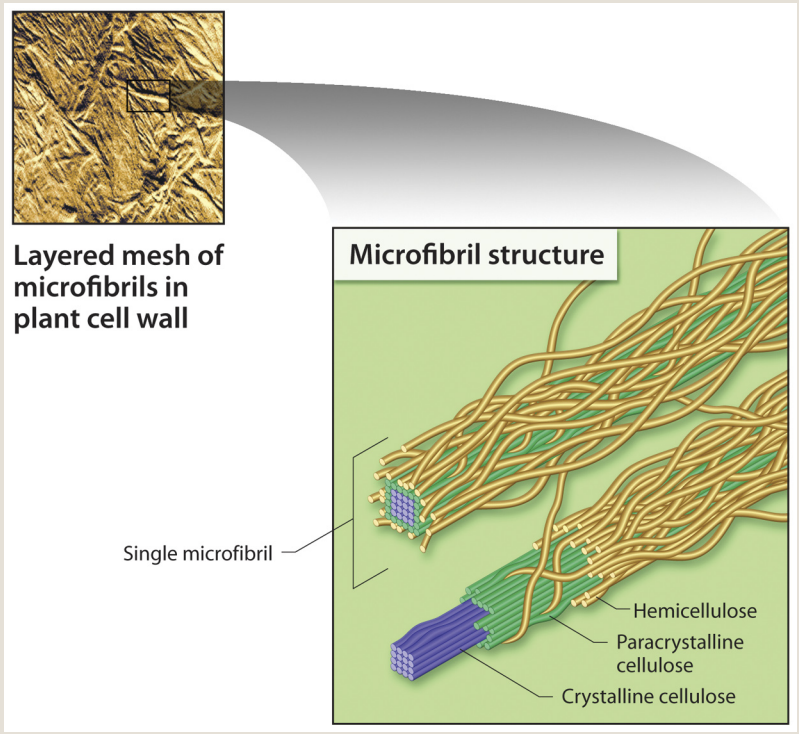


Fig. 3. Converting Cellulose to Sugars. Cellulases include a mix of enzymes that break down cellulose into simple sugars that can be fermented by microorganisms to ethanol. Three general classes of cellulases—endoglucanases, exoglucanases, and cellobiases—work together in a coordinated fashion to hydrolyze cellulose. Endoglucanases internally cleave a cellulose chain, and exoglucanases bind the cleaved ends of the cellulose chain and feed the chain into its active site where it is broken down into double glucose molecules called cellobiose. Cellobiases split cellobiose to yield two glucose molecules. The cellulase pictured is an exoglucanase whose binding domain on the right extracts a cellulose chain. At the active site in the larger catalytic domain on the left, the cellulose chain is hydrolyzed to yield cellobiose subunits. [Image from M. Himmel et al., "Cellulase Animation," run time 11 min., National Renewable Energy Laboratory (2000).]

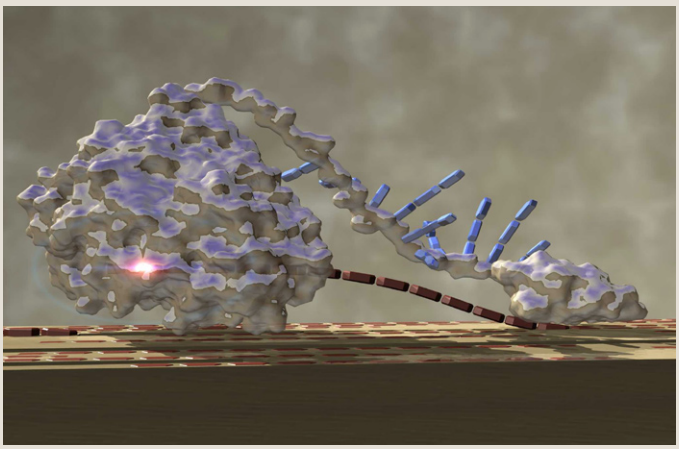


Fig. 4. White-Rot Fungus, this page). Such enzymes as cellulase and other glycosyl hydrolases are capable of hydrolyzing biomass polymers. Because these hydrolases are much slower in their intrinsic turnover rates (the number of molecules hydrolyzed per second) than most other enzymes, one goal is to improve their efficiency. To increase understanding of these multisubunit complexes and derive the principles of their function, large numbers of natural and modified cellulases and other molecular machines must be analyzed (see Table 2. Cellulosic Ethanol Challenges, Scale, and Complexity, this page).

GTL research can help by producing and characterizing complex cellulase structures and their functions, by analyzing naturally occurring and modified protein and molecular machine variants of essential pathways, understanding the synergistic activity of multiple cellulases, and resolving temperature-sensitivity issues that prevent optimal functioning of cellulase enzymes at fermentative temperatures. Other challenges include characterizing the structures and functions of membrane-bound molecular machines that deliver sugars to the metabolic pathways of fermentative organisms; understanding the inefficiencies in conversion of different sugars to ethanol; maintaining large-scale mixed cultures; and improving disease resistance. Finally, understanding cell regulatory processes will be central to incorporating multiple functionalities into a single organism or a microbial consortium and enabling optimized overexpression in many related processes.

For more information, refer to Summary Table. GTL Science Roadmap for DOE Missions, p. 40, and 5.0. Facilities Overview, p. 101.

2.3.1.2. Other Commercial Products

These grand challenges to biology posed by DOE missions will provide the foundation for countless new commercial bioproducts and bioprocesses. A strategy for introducing these technologies to the marketplace could be integrated biorefineries capable of producing a suite of products as substitutes for chemical-based fossil feedstocks.

Fig. 4. White-Rot Fungus. In this image of a longitudinal section of *Phanerochaete chrysosporium* colonizing aspen, hyphae are visible throughout and in the vessel pit on the right. This June 2004 issue of *Nature Biotechnology* reports the full genome sequence of the white-rot fungus *P. chrysosporium* (Martinez et al. 2004). [Cover and caption used by permission from *Nat. Biotechnol.*, www.nature.com/nbt/]

Table 2. Cellulosic Ethanol Challenges, Scale, and Complexity

Research and Analytical Challenges	Scale and Complexity
• Screening of databases for natural variants of cellulases (generally glycosyl hydrolases) and other enzymes or molecular machines in metabolic networks; and characterization of variants • Analysis of modified variants to establish design principles and functional optimization • Modeling and simulation of cellulase, sugar transport, and multiple sugar-fermentation processes and systems • Integration of processing steps into single microbes or stable cultures	• Thousands of variants of all enzymes; screening of millions of genes, thousands of unique species and functions • Production and functional analysis of potentially thousands of modified enzymes, hundreds of regulatory processes and interactions • Models at the molecular, cellular, and community levels incorporating signaling, sensing, regulation metabolism, transport, biofilm, and other phenomenology and using massive databases in GTL Knowledgebase • Incorporation of complete cellulose-degradation and sugar-fermentation processes into microbes or consortia—hundreds of metabolic, regulatory, and other interconnected pathways

Market demand for these high-value alternatives can generate financial returns that make biorefineries commercially competitive, providing a viable base for lower-value products such as transportation fuels. An example is the polylactic acid now being produced by the Dow-Cargill venture, providing a biodegradable polymer that can be used in a variety of applications from carpets to clothes (Littlehales 2004; www.natureworkspla.com). After meeting market opportunities for high-value products, other lower-value products now derived from fossil fuel would be produced, ultimately leading to mass marketing of fuels from biomass resources. In developing microbial systems for supporting energy applications, a useful consideration would be systems that can produce, for example, ethylene, benzene, vinyl chloride, adipic acid, and, ultimately, the full suite of industrial chemicals derived from fossil fuels.

For more information, see www.bioproducts-bioenergy.gov/pdfs/BioProductsOpportunitiesReportFinal.pdf, www.metabolix.com, www.eere.energy.gov, and www.bio.org.

2.3.2. Biophotolytic Hydrogen Production

The importance of microbial systems in developing biological solutions to the energy challenge has been recognized by the National Research Council and the National Academy of Engineering (NAE). In a report on the hydrogen economy, the NAE Committee on Alternatives and Strategies for Future Hydrogen Production and Use recommended that DOE "refocus its biobased program on more fundamental research on photosynthetic microbial systems to produce hydrogen from water at high rate and efficiency" and "make use of important breakthroughs in molecular, genomic, and bioengineering research." [*The Hydrogen Economy: Opportunities, Costs, Barriers, and R&D Needs,* NAE (2004)] (See also Fig. 1, p. 23; Fig. 5. Two Approaches of GTL Research, this page; and Table 3. Biophotolytic Hydrogen: Goals and Impacts, p. 30.)

2.3.2.1. Science and Technology Objectives

As the planet's dominant photosynthetic organisms, microbes are capable of using solar energy to drive the direct conversion of water to hydrogen and oxygen (biophotolysis). One technology option for deploying biophotolytic hydrogen-production systems would involve the use of living organisms. Extensive farms of sealed enclosures (photobioreactors) containing photosynthetic microbes would split water to produce hydrogen for collection; oxygen would be released as the only by-product. Another deployment option would involve engineering artificial systems that would use natural or designed enzyme catalysts to yield

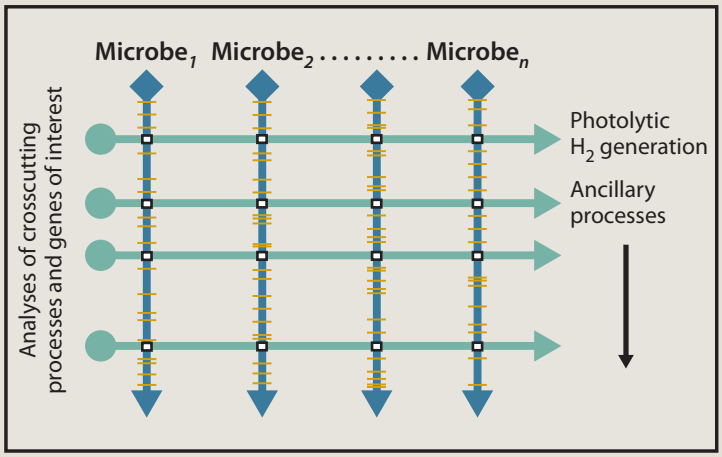


Fig. 5. Two Approaches of GTL Research. As outlined in Tables 2, 4, 6, and 8, research on GTL problems will take two general forms. The example illustrated above depicts analysis of the functionality of microbes and microbial communities. A vertically integrated approach will examine all relevant processes with the goal of consolidating the data to achieve a predictive whole-systems understanding.

To analyze such processes as hydrogen production, biomass conversion, or contaminant transformation, families of individual molecular assemblies and pathways will be studied horizontally across a wide set of natural variations. This approach will achieve insights into design principles and the range of variations. In addition to the primary process, all relevant ancillary processes (e.g., signaling, transport, and regulation) will require similar analyses. The eventual goal is to optimize these processes, either in living systems or captured in, for example, inorganic nanosystems.

hydrogen in vitro. Enzymes that split water are fixed to a synthetic nanostructure that maintains optimal conditions for hydrogen production. In addition to biophotolytic hydrogen production, other microbial processes that generate hydrogen (e.g., fermentation of biomass and nitrogen fixation) should be developed.

Photolytic production of hydrogen uses metabolic steps that are part of the photosynthetic process of microbes. Biological systems are designed for growth, functioning, and survival—producing biomass for structure, energy, and function. We must learn how to convert such a process to one that generates hydrogen in continuous water splitting—thus subverting the microbe's natural goal—to achieve our energy objective. To accomplish this, molecular and process models will be developed for enzyme, pathway, and whole-systems design (see Table 4. Biophotolytic Hydrogen Production Challenges, Scale, and Complexity, this page).

Screening of natural environments, particularly those under extreme conditions where hydrogen production might contribute to the success of microbial communities, may identify novel enzymes with desirable properties and new metabolic pathways that generate hydrogen. We must learn what makes biophotolysis possible in oxygenic photosynthetic microbes (e.g., algae and cyanobacteria that split water to generate oxygen); understand the principles underlying the natural range of hydrogenase properties, relevant metabolic processes and pathways involved in hydrogen production, regulatory processes that inhibit hydrogen overproduction, electron transfer-rate limitations, and competing pathways. Reducing the oxygen sensitivity of hydrogenases is a needed breakthrough that will require the redesign of multiple metabolic-network elements. Other phenomena to be understood include reverse reactions, efficiency of light utilization, and ways in which the organism or its component processes can be manipulated to increase the efficiency and yield of hydrogen production. Thousands of natural and modified hydrogenases and supporting pathways will be analyzed for relevant mechanisms, desirable properties, and insight into design principles. These capabilities will enable production systems engineering and allow frontier science to be imported quickly into technologies.

For more information, refer to Summary Table, p. 40; 5.0. Facilities Overview, p. 101; and Appendix A. DOE Mission: Energy Security, p. 197.

Table 3. Biophotolytic Hydrogen: Goals and Impacts

- Sunlight and water, two resources in virtually limitless supply, can be used to produce the ultimate fuel and energy carrier, hydrogen. High-efficiency use of hydrogen in fuel cells can generate electricity directly with water as the by-product.
- This energy cycle is carbon free and can be developed as the complement to the electric grid for all energy applications—industrial, transportation, and residential.
- Development of biological photolytic processes to produce hydrogen at high rates and efficiency will enable the establishment of a hydrogen-economy strategy based on a renewable source.

Table 4. Biophotolytic Hydrogen Production Challenges, Scale, and Complexity

Research and Analytical Challenges	Scale and Complexity
• Database screening for and characterizing of natural variants of hydrogenases and other enzymes and molecular machines in the entire set of pathways that underlie this process • Analysis of modified variants to establish design principles for functional optimization of the overall process including oxygen sensitivity, reverse reactions, transport, light capture, and conversion efficiency • Modeling and simulation of photolytic systems to support systems design and optimization	• Screening of millions of genes, thousands of unique species and functions, and thousands of variants of all enzymes • Production and functional analysis of potentially thousands of modified enzymes, hundreds of regulatory processes and interactions • Models at the molecular, cellular, and community levels incorporating signaling, sensing, regulation, metabolism, transport, and other phenomenology and using massive databases in GTL Knowledgebase

2.4. Environmental Remediation: Develop Biological Solutions for Intractable Environmental Problems

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 facilities and sites across the nation (see Table 5. Bioremediation: Goals and Impacts, this page). As an example of the problem's scope, about 5700 individual contaminant plumes, some quite extensive, are known to be present at DOE sites (Linking Legacies 1997). One plume at Savannah River extends over 7.8 km^2, and an 18-km^2 plume exists at Hanford. Examples of the volume of contaminated soils and sediments at the Nevada Test Site and Fernald alone are 1.5 and 0.71 million m^3, respectively. Additionally, unknown quantities of waste are buried at numerous places. Projected costs for restoring these sites and disposing of wastes is $142B (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 remaining to be addressed after 2025 are the most challenging, and successful remediation will require development and deployment of innovative methods (see Fig. 6. A Legacy of Hazardous Waste, this page).

Although comparisons of the cost and effectiveness of metal and radionuclide bioremediation (the focus of the DOE effort) with traditional methods are not available, costs savings for bioremediation of organics are

Table 5. 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 savings in the billions of dollars 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 to save potentially billions of dollars 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.

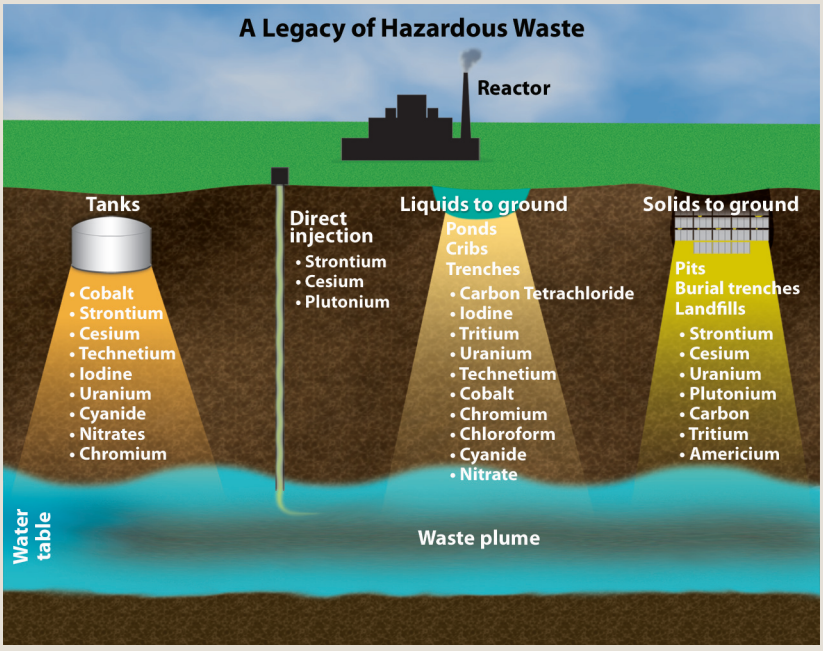


Fig. 6. A Legacy of Hazardous Waste. For more than 50 years, the United States created a vast network of facilities for research and development, manufacture, and testing of nuclear weapons and materials. The result is subsurface contamination on more than 7000 sites at over 100 facilities across the nation, more than half of which contain metals or radionuclides and most with chlorinated hydrocarbons. Biologically based techniques can provide cost-effective restoration strategies for many of these sites.

estimated to range from 30 to 95%. In addition, in situ bioremediation, taking advantage of natural microbial populations in the subsurface, has the potential for reducing costs and increasing the efficiency of groundwater treatment as compared to conventional pump-and-treat technology. Given that over 1 billion m^3 of water and 55 million m^3 of solid media at DOE sites in 29 states are contaminated with radionuclides (Linking Legacies 1997), potential savings accrued by use of innovative biotechnologies are likely to amount to billions of dollars (Bioventing Performance 1996; Patrinos 2005; Scott 1998).

2.4.1. Science and Technology Objectives

In conjunction with the capabilities of other science programs, GTL science will facilitate detailed, large-scale discovery and investigation of microbes with important contaminant-transformation capabilities. DOE bioremediation strategies and biogeochemistry research focus on using natural microbial communities to reduce the mobility and toxicity of metals and radionuclides. The interdependent metabolic survival strategies used by microbial communities can directly or indirectly remove contaminants from groundwater or transform toxic contaminants into benign chemical products. For example, *Shewanella* and *Geobacter*, two model microbes currently being studied in GTL and BER's Environmental Research Sciences Division (ERSD) projects, can enzymatically reduce certain toxic contaminants. This capability transforms, for example, Uranium(VI), which is soluble and moves in groundwater, to Uranium(IV), which is insoluble and precipitates out of the groundwater as a biologically unavailable solid (see 3.3.4. Sidebars Illustrating Details of Specific Research, p. 58). Studying these model microbes is a first step in expanding our understanding of the structure, function, metabolic activity, and dynamic nature of microbial communities and their role in influencing subsurface geochemistry. This knowledge is needed to predict microbe-mediated contaminant fate and transport and to develop efficient bioremediation strategies (Fredrickson and Balkwill 2005; Croal et al. 2004; Madsen 2005; Ben-Ari 2002; Gold 1992; Spear et al. 2005; Nealson 2005).

A biotreatment technique that works well at one site may perform poorly at another because microbial communities, geochemical properties, and flow regimes frequently differ markedly between sites. We often lack understanding of how microbial processes are coupled to other processes influential in contaminant behavior and are scaled in heterogeneous environments. In addition, we need new tools for measuring key microbial, geochemical, hydrological, and geological properties and processes in these systems. Less than 1% of all microorganisms collected at only a few sites have been cultured and characterized in any great detail, and only a small fraction of those have been sequenced. Even less is known regarding the interactions of microorganisms in communities.

Subsurface microbial communities can be quite distinct from soil and ocean communities, with far lower microbial densities and unique genetic traits. The metabolic processes observed in the subsurface are often the result of unique interactions—in these "geologically powered dark ecosystems"—between the microbial community and subsurface geochemistry (Nealson 2005). We have only begun to appreciate the existence of such systems, let alone understand them so that we can take advantage of their diverse capabilities (Gold 1992; see sidebar, The Microbial World, p. 13).

In this complex venue, we first must define the genomic potential of microbial communities (see Table 6. Bioremediation Challenges, Scale, and Complexity, p. 33). 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 now allows culture-independent insights into microbial function. Though difficult to obtain, if environmental or cultured samples are available, then functional determination can include information on microbial responses to environmental stimuli as captured in, for example, gene and protein expression and metabolite analyses. In addition, new imaging techniques augmented by fluorescent probes with molecular resolving power will allow the analysis of individual cells and processes in complicated community and geochemical venues.

These results will form the basis for evaluating and modeling pathways of such cellular processes as signaling, growth, and response to contaminants. Other processes of importance in modifying contaminant trans-

port and form and in development of bioremediation strategies 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. The mechanistic linking of metabolism to contaminant transformation will represent an important advance from previous contaminant-fate models.

To accomplish this linkage, we first need a cohort of trained scientists to determine the makeup of subsurface microbial communities and their interactions with the geochemical environment. Methods also must be developed for incorporating GTL genome-based microbial knowledge into meaningful field-scale models. Some of these techniques already are being generated within BER ERSD environmental-restoration programs and GTL (www.science.doe.gov/ober/ERSD_top.html). For more information, refer to Table 5, p. 31; Table 6, this page; Summary Table, p. 40; 5.0. Facilities Overview, p. 101; and Appendix B. DOE Mission: Environmental Remediation, p. 215.

Environmental Remediation

- **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 (see 3.0. GTL Research Program, p. 41).**
- **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.**

2.5. Microbial Roles in Carbon Cycling and Sequestration: Understand Biosystems' Climate Impacts and Assess Sequestration Strategies

Marine and terrestrial ecosystems each play major roles in the global cycling of carbon. Microbes are essential to maintaining the planet's ability to sustain life, including recycling most of earth's biomass, both assimilating and respiring large amounts of carbon dioxide—many times that of anthropogenic CO_2 emissions (Doney et al. 2004; Falkowski et al. 2000; Field et al. 1998; Johnston et al. 2004; Hess 2004; see Fig. 7. Simplified Global Carbon Cycle, p. 34, and 2.6. Summing Up the Challenges, p. 37). Small changes in these natural fluxes induced by climate change or natural processes could overwhelm any attempts at mitigation we might make within global energy systems, some of which might be very costly.

Table 6. 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 macromodels for fate and transport

These global ecosystems are affected by climate change (Climate Change Science Program 2003). Ascertaining relationships among such natural and managed carbon pools as agricultural soils, forests, and atmospheric CO_2 and determining the resultant climate effects will make important new contributions to climate models. Such models will aid in understanding the long-term sequestration capacities of these pools. Microbes as primary mediators of earth's elemental cycling also can serve as indicators of ecosystem health and change as we monitor the impacts of climate (see Table 7. Carbon Cycling and Sequestration: Goals and Impacts, this page; sidebar Ocean Monitors, p. 234; and Tringe et al. 2005).

Table 7. Carbon Cycling and Sequestration: Goals and Impacts

- Improved understanding of key feedbacks and sensitivities of biological and ecological systems and accelerated incorporation into climate models will reduce uncertainties in assessments of climate change.
- Knowledge of the carbon cycle will allow evaluation of carbon-sequestration strategies and alternative response options.
- Development of sensors and monitoring techniques and protocols will allow use of these sensitive ecosystems as sentinels for the effects of climate change.

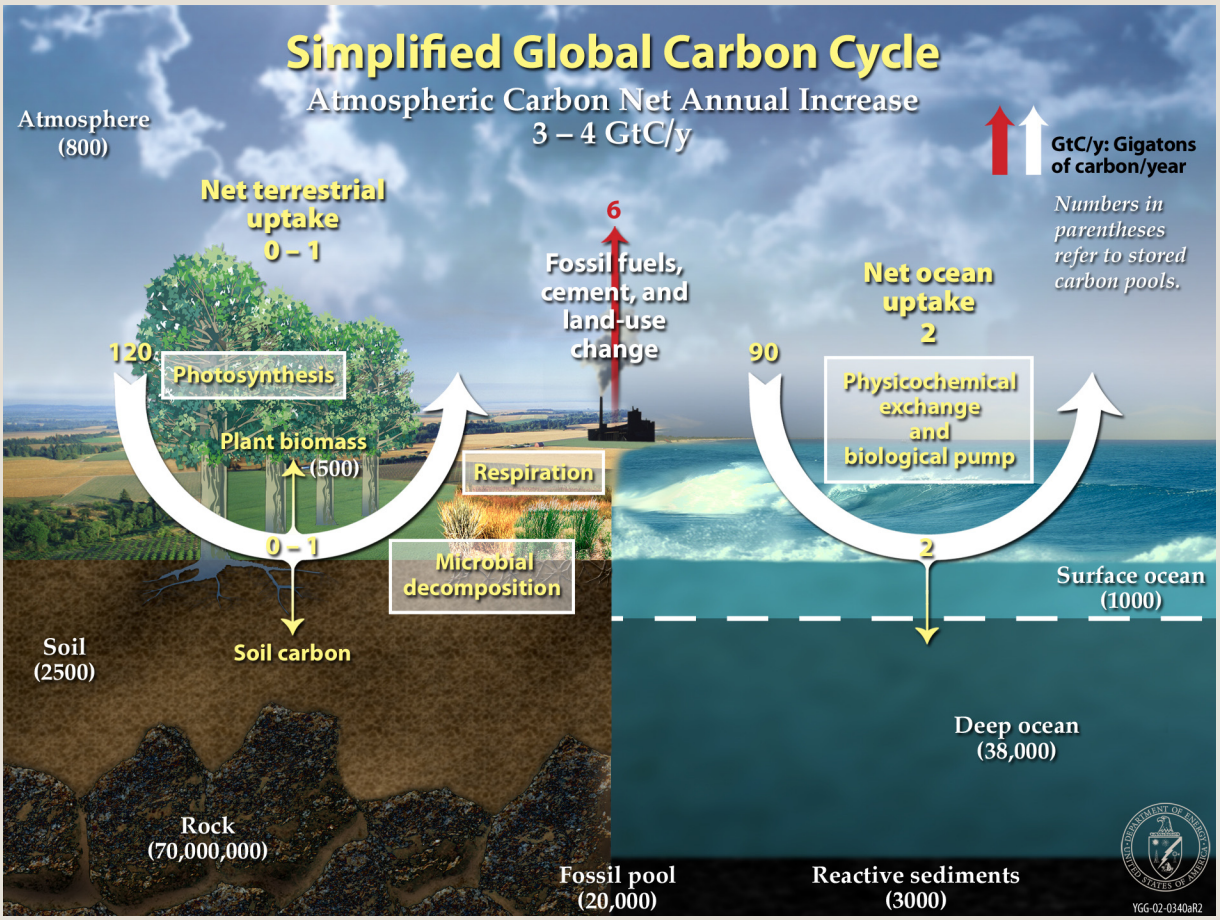


Fig. 7. Simplified Representation of the Global Carbon Cycle. The illustration depicts human-induced changes relative to the total cycle. [Graphic adapted from *Carbon Sequestration Research and Development* (1999).]

2.5.1. Science and Technology Objectives

The science undertaken by the GTL program will provide a systems-level understanding of microbial processes essential to carbon cycling in ocean and terrestrial environments, as well as the cycling of such other elements as nitrogen, phosphorous, sulfur, oxygen, and metals. Detailed knowledge revealed by GTL about the mechanistics and functions of microbial processes and communities will be incorporated into global climate-change models to provide a robust science base for evaluating potential impacts of proposed carbon-management strategies. Moreover, GTL ultimately will help determine ways in which microbial environments might be used or manipulated to enhance carbon residence time in ocean and soil ecosystems without harming those ecosystems.

Carbon Cycling and Sequestration

- **Mission Science Goals: Understand the microbial mechanisms of carbon cycling in the earth's ocean and terrestrial ecosystems, the roles they play in carbon sequestration, and how these processes respond to and impact climate change. Develop methods to relate genome-based microbial ecophysiology (functionality) to the assessment of global carbon-sequestration strategies and climate impacts (see 3.0. GTL Research Program, p. 41, and Appendix C. DOE Mission: Carbon Cycling and Sequestration, p. 227).**
- **Challenges: We are just beginning to understand the genetic and functional diversity of ocean and terrestrial ecosystems. They potentially contain millions of microbial species organized in extensive communities. We must understand both the global and molecular mechanistic behaviors of these large systems.**

Because they have been transforming the planet for some 3.5 billion years, microbes play a dominant role in ecosystem processes and are key mediators of energy transfer and materials cycling in the biosphere. Microbes cycle immense volumes of carbon: they can fix CO_2 by light-driven (photoautotrophy) and geochemically driven (lithoautotrophy) reactions, generate methane, produce CO_2 during the decomposition of organic matter, precipitate carbonate minerals, and catalyze the polymerization of plant polymers into recalcitrant pools of soil organic matter. Microbes perform all their activities in dynamic communities—in the upper ocean layer where photosynthesis occurs, removing carbon from and largely returning it to the atmosphere; and in terrestrial communities where microbes and fungi cycle nutrients containing carbon and other elements and decompose biomass. In these systems, we do not envision any redesign of microbes; rather, we seek an understanding of natural processes to enable predictions about microbial evolution and to support wise management practices, including the addition of nutrients where appropriate. As with the subsurface modeling of contaminant transport and fate, this problem involves the very complex linking of the time and length scales of molecular and microbial processes to the millennial and global scales important for carbon sequestration and climate models.

2.5.2. Marine Microbial Communities

In the oceans, microbes are the primary photosynthetic organisms, producing most oceanic organic materials and constituting the foundation of the marine food chain. The photosynthesis of such phytoplankton as diatoms, dinoflagellates, and cyanobacteria converts about as much atmospheric carbon to organic carbon as does plant photosynthesis on land (Fuhrman 2003). Large oscillations in phytoplankton abundance, therefore, can impact greatly the ocean's ability to take up atmospheric carbon. Although most organic matter produced in surface waters is consumed by other microorganisms and returned rapidly to the atmosphere as carbon dioxide, diatoms are capable of synthesizing organically complexed carbon that can be carried to the ocean depths. Because carbon cycling is considerably slower in the deep oceans than in surface waters (thousands to millions of years), this carbon is effectively sequestered. In this way, diatoms may sequester more carbon than all the earth's rainforests combined (Armbrust 2004). In addition to understanding photosynthetic assimilation of atmospheric carbon carried out by diatoms and other phytoplankton, GTL and other BER carbon-cycle research will provide understanding of microbial processes that degrade organic matter in the ocean's depths and ultimately return carbon to the atmosphere.

2.5.2.1. Specific Scientific Needs for Marine Microbial Communities

GTL studies, based on genomic and metagenomic sequences of a broad range of microbes from diverse marine environments, must ascertain the molecular design principles of photosynthetic and related nutrient and metabolic systems. Paramount to understanding the dynamics of carbon-assimilation pathways, these principles will provide the foundation for new hypotheses about the types and diversity of pathways and capabilities of individual species. A key element of these analyses is to determine whether the very broad genetic diversity translates into a commensurate range in function and the environmental significance of that range.

The fact that many environmental microbes remain uncultivated and that we lack good experimental models for most microbes requires the development of high-throughput capabilities for ultimately gaining this information from genome sequence alone. Investigating the natural dynamics of relationships among microbial, biogeochemical, and physical processes requires new high-throughput sampling and analysis tools. Capabilities for explorations of critical biological reactions, including photosynthetic modes and various metabolic pathways, also must be established. The resultant molecular understanding can lead to the development of increasingly sophisticated microsensors that can detect changes in the levels of biomolecules (DNA, mRNA, proteins, metabolites) and serve as indicators of microbial-community response to environmental stressors. Detailed mathematical models that encompass full-systems phenomenology will form the basis for including these processes in climate and integrated-assessment models to inform policy and technology decisions (see sidebar, Integrated Assessment Program, p. 236).

2.5.3. Terrestrial Microbial Communities

Terrestrial ecosystems fix CO_2 directly from the atmosphere into biomass, mainly via plant photosynthesis. Carbon in terrestrial ecosystems can be stored in plant biomass or soils. Microbial communities can influence both areas of terrestrial carbon storage in a variety of ways. In the narrow zone of soil surrounding the root (the rhizosphere), microbial interactions with sugars, amino acids, enzymes, fatty acids, and other organic compounds exuded from roots can significantly impact plant growth and development. Microbes can enhance plant growth by providing nutrients such as phosphorous and nitrogen or by suppressing plant pathogens in the soil. Some microbial populations are beneficial to plant growth, while others have neutral and even harmful effects. Identifying metabolic requirements and environmental factors that can give an advantage to beneficial microbes, therefore, is important. A better understanding is needed of molecular mechanisms that enable microbes to colonize root surfaces, interact with plant exudates, and compete or cooperate with other soil organisms.

In addition to promoting plant growth, microbes can return CO_2 rapidly to the atmosphere as carbon dioxide or transform root exudates and decaying plant materials into humic acids with varying degrees of recalcitrance to degradation. Some of the most stable soil organic compounds have carbon-turnover times of hundreds of years.

Shifts in the microbial decomposition of organic matter to CO_2 can occur during environmental stresses such as climate change (King et al. 2001). For example, plant death due to temperature shift, water stress, or disease can promote microbial decomposition, seriously complicating the use of standing biomass or soil organic matter for reducing the amount of atmospheric CO_2. Although forest clearing and farmland tillage have significantly reduced soil carbon content, the amount currently in soils accounts for 75% of the carbon contained in the terrestrial biosphere. Carbon-depleted soils represent a large potential reservoir (50-Gt one-time gain) that could be used to mitigate atmospheric carbon emissions (Rosenberg, Izaurralde, and Malone 1999).

2.5.3.1. Specific Scientific Needs for Terrestrial Microbial Communities

Systems biology will support a biological understanding of interactions among terrestrial ecosystems and changes in atmospheric composition and the climate system (see Table 8. Carbon Cycling and Sequestration

Table 8. Carbon Cycling and Sequestration Challenges, Scale, and Complexity

Research and Analytical Challenges	Scale and Complexity
• Analysis of ocean and terrestrial microbial-community makeup and genomic potential • Analysis of carbon and other cycling processes » Photosynthesis and respiration in oceans » Storage and decomposition in soil: Microbial, fungal, and plant communities • Modeling and simulation of microbe biogeochemical systems	• Thousands of samples from different sites consisting of millions of genes, thousands of unique species and functions • Functional analysis of enzymes involved—potentially tens of thousands; hundreds of regulatory processes and interactions; spatially resolved community formation, structure, and function • Models at the molecular, cellular, and community levels incorporating signaling, sensing, metabolism, transport, biofilm, and other phenomenology into macroecosystem models

Challenges, Scale, and Complexity, this page). We must elucidate microbial contributions to carbon transformation in soils and assess the potential for sequestering meaningful amounts of carbon (gigatons per year) in more stable forms. We seek to understand the genomic-mechanistic basis for adaptations made by microbial species to climate change. We also must determine biological feedbacks to the climatic system brought about through the terrestrial carbon cycle and microbial contributions to carbon transformation in soils. This knowledge will enable us to design and assess sequestration strategies and make crucial contributions to overall climate-modeling efforts (King et al. 2001). GTL technical requirements for analyses of ocean and terrestrial systems are very similar.

- Defining communities and their collective genetic functional potential requires single-cell and community sequencing (in situ and in vitro), systems biology studies, and the ability to relate microbial activities to soil processes.
- We need to understand processes that impact production of GHGs (carbon dioxide, methane, nitrous oxide, dimethyl sulfide); correlate biomolecular inventories with environmental conditions; characterize microbial system interactions with soils, rhizosphere, and plants; and functionally image microbial activities (e.g., proteome and metabolome) at cellular and community levels.
- We need the ability to predict microbial responses to manipulations of plant inputs to the carbon cycle, to human inputs to soils, and to other environmental changes.
- GTL seeks to employ microbes as sentinels of change in environmental conditions; measures of change include microbial responses (biomarkers) including an array of biological components (e.g., RNAs, proteins, metabolites, and signaling); community genomic makeup; functional assays; and environmental conditions including carbon and nutrient inventories.

For more details see Appendix C. DOE Mission: Carbon Cycling and Sequestration, p. 227.

2.6. Summing Up the Challenges

Each of these mission areas poses grand scientific and technical challenges for biology. Each is confronted with the tremendous genetic and functional diversity of microbial systems in widely varying environments (note scale and complexity in Tables 2, 4, 6, 8; see sidebar, Deciphering the Scale and Complexity of Global Microbial Communities, p. 39, including Table 9. Microbial Community Characteristics in Diverse Earth Environments, p. 39). Each also requires an unprecedented level of understanding to be able to predict systems behaviors or to engineer systems for energy or other applications. The Summary Table, p. 40, presents a capsule summary of systems being studied, mission goals that drive the analysis, generalized science roadmaps, and outputs to DOE missions. Science capabilities that GTL will use to implement these roadmaps and achieve a systems-level understanding of microbial processes are described in the following chapters and in the three mission appendices beginning on p. 197.

MISSIONS OVERVIEW

Genomics has opened the door to the study of these complex natural systems and processes, but the science also has revealed an unforeseen diversity, bringing the realization that we know the functions of only a small fraction of newly discovered genes. Clearly, we must develop the capabilities to move beyond the parts list provided by genomics to understand microbes so well that we can predict their behavior. While each of the systems presents a daunting challenge, the fact that they all obey the same set of principles and share enduring genetic and functional traits means that studying them together will have a synergistic effect. Researchers can use the same set of tools and concepts to study all these systems, and the knowledgebase amassed by GTL will inform new investigations in all realms. But to achieve timely impacts in mission problems, to deal with the complexities of microbial systems, and to practice "systems biology," we must develop technological and computing capabilities with dramatically improved performance, throughput, quality, and cost. The following chapters in this roadmap describe the science and technology goals and milestones to achieve these gains including computing, technologies, and facilities.

Deciphering the Scale and Complexity of Global Microbial Communities

At the frontier of modern biology, microbes in their vast natural communities are immensely important to our planet's future. DOE missions lead us to the study of ocean, terrestrial, and subsurface microbial communities whose existence in disparate environments, genetic diversity, and complexity of function have been appreciated only recently. With the advent of genomics and systems biology, the myriad capabilities of these organisms now can be revealed by deciphering the interactions of millions of genes with physicochemical variables. Understanding their specialized biochemical capabilities and their contributions to nutrient cycling, to the global carbon cycle, and to overall ecosystem function could lead to new energy sources and cost-effective strategies for remediating the environment and sequestrating carbon.

Although the three environments and resulting microbial biochemistries differ greatly, the basic processes by which microbes operate (based on DNA) are the same and can be investigated using the same set of core principles and technologies. Studying a broad range of microbial systems in one program at a scale commensurate with practical national and global needs will enhance the richness of the science and its impact on our understanding of all living systems. The table below compares and contrasts these microbial communities in their different ecosystems, their metabolic processes and structures, and other characteristics that make studying microbes one of the great challenges and opportunities of 21st Century science.

Table 9. Microbial Community Characteristics in Diverse Earth Environments

Topic	Oceans (water column)	Terrestrial (surface soils)	Deep Subsurface (ocean, terrestrial)
Energy Sources and Ecologies	Primary photosynthesis (i.e., microbes at base of food chain)	Plant photosynthesis, plant-rhizosphere-microbe symbiosis (microbes are decomposers)	Reduced inorganic compounds, simple communities, lack of predators, minimal gene exchange
Energy and Materials Storage	Rapid carbon and nutrient turnover, carbon exported to depths	High resident carbon (plant roots, microbes, soil organic matter)	Minerals, microbial biomass, and fossil organic carbon
Key Processes	Ocean carbon biological pump, C, N, P, O cycles	Soil carbon cycle, symbiosis, C, N, P, O cycles	Redox manipulation of subsurface to transform contaminants
Rates of Cell Division (and Metabolism)	Relatively rapid (hours to weeks)	Relatively long (months to 100s of yrs)	Very long (decades to 100s of yrs)
Microbial Population Size	Medium density	High density	Low density
Key Environmental Variables	pH, light, nutrients (e.g. P, Fe), dissolved organic matter, mixing, temperature, currents	Plant community, organic matter content and composition, mineral composition, temperature, moisture	Mineralogy, hydrology, trace elements, gas composition, groundwater geochemistry, temperature
Science Goals	Understand microbes and communities and flow of carbon and nutrients (C, N, P, S, O, metals)	Understand microbe-rhizosphere symbioses, carbon flows and lifetimes, and nutrient cycles (C, N, P, S, O, metals)	Mechanistic understanding of electron-transfer processes, utilization of solid-phase nutrients (minerals, rocks)
Application Goals	Predict responses of ocean C cycling communities to climate scenarios	Predict responses of soil C cycling communities to perturbations and manipulations	Prediction of geomicrobial fate of metals and radionuclides in the subsurface

Summary Table. GTL Science Roadmap for DOE Missions

GTL Science Roadmap for DOE Missions

		DOE Mission Goals		GTL Science Roadmaps
Selected Processes	Biofuels	**Processes to convert cellulose to fuels** • Understanding and improving cellulase activity • Improving sugar transportation and fermentation to alcohols • Integrated processing **Microbial processes to convert sunlight to hydrogen fuels** • Understanding photolytic fuel production • Designing photosynthetic biofuel systems	Science Objectives	▸ **Characterize genes, proteins, machines, pathways, and systems** • Conducting genomic surveys and comparisons • Mining natural systems for new functions • Producing and characterizing proteins • Analyzing interactions, complexes, and machines ▸ **Understand functions and regulation** • Measuring molecular responses: Inventories • Performing functional assays ▸ **Develop predictive mechanistic models** • Conducting experimental design • Designing and manipulating molecules • Using cellular and cell-free systems
	Environmental Remediation	**Microbial processes to reduce toxic metals** • Understanding microbe-mineral interactions • Devising restoration processes	Mission Outputs	**Systems engineering** • System-design strategies for deployment • Living and extracellular systems • Validation and verification analyses
Natural Systems' Behavior	Carbon Cycling and Sequestration	**Subsurface microbial communities' role in transport and fate of contaminants** • Understanding fate and effects • Supporting remediation decisions **Ocean microbial communities' role in the biological CO_2 pump** • Understanding C, N, P, O, and S cycles • Predicting climate responses • Assessing impacts of sequestration **Terrestrial microbial communities' role in global carbon cycle** • Understanding C, N, P, O, and S cycles • Predicting carbon inventories and climate responses • Assessing sequestration concepts	Science Objectives	▸ **Analyze communities and their genomic potential** • Sequencing and comparing genomes • Screening natural systems for processes • Producing and characterizing proteins ▸ **Understand community responses, regulation** • Comparing CO_2, nutrients, biogeochemistry cycles • Producing cellular and community molecular inventories • Performing community functional assays ▸ **Predict responses and impacts** • Building interactive and predictive models • Applying natural and manipulated scenarios
			ission utputs	**Robust science base for policy and engineering** • Model ecosystem response to natural events • Efficacy and impacts of intervention strategies **Sensor development**

A capsule summary of systems being studied, mission goals that drive the analysis, generalized science roadmaps, and outputs to DOE missions. To elucidate design principles, each of these goals entails the examination of thousands of natural primary and ancillary pathways, variants, and functions, as well as large numbers of experimental mutations.