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U.S. DEPARTMENT OF ENERGY

Office of Science

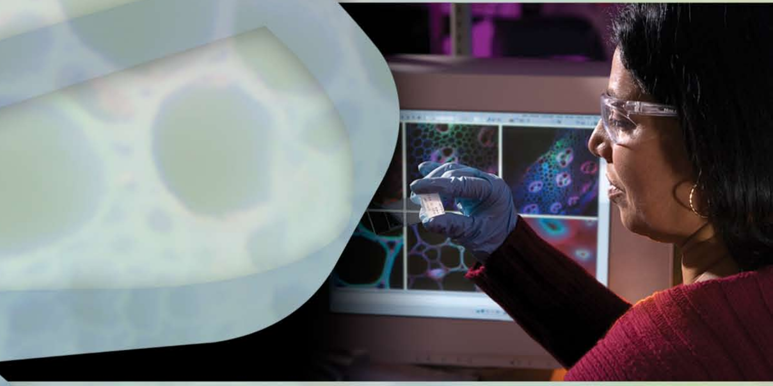
Office of Biological and Environmental Research

U.S. DEPARTMENT OF ENERGY'S

Bioenergy Research Centers

An Overview of the Science

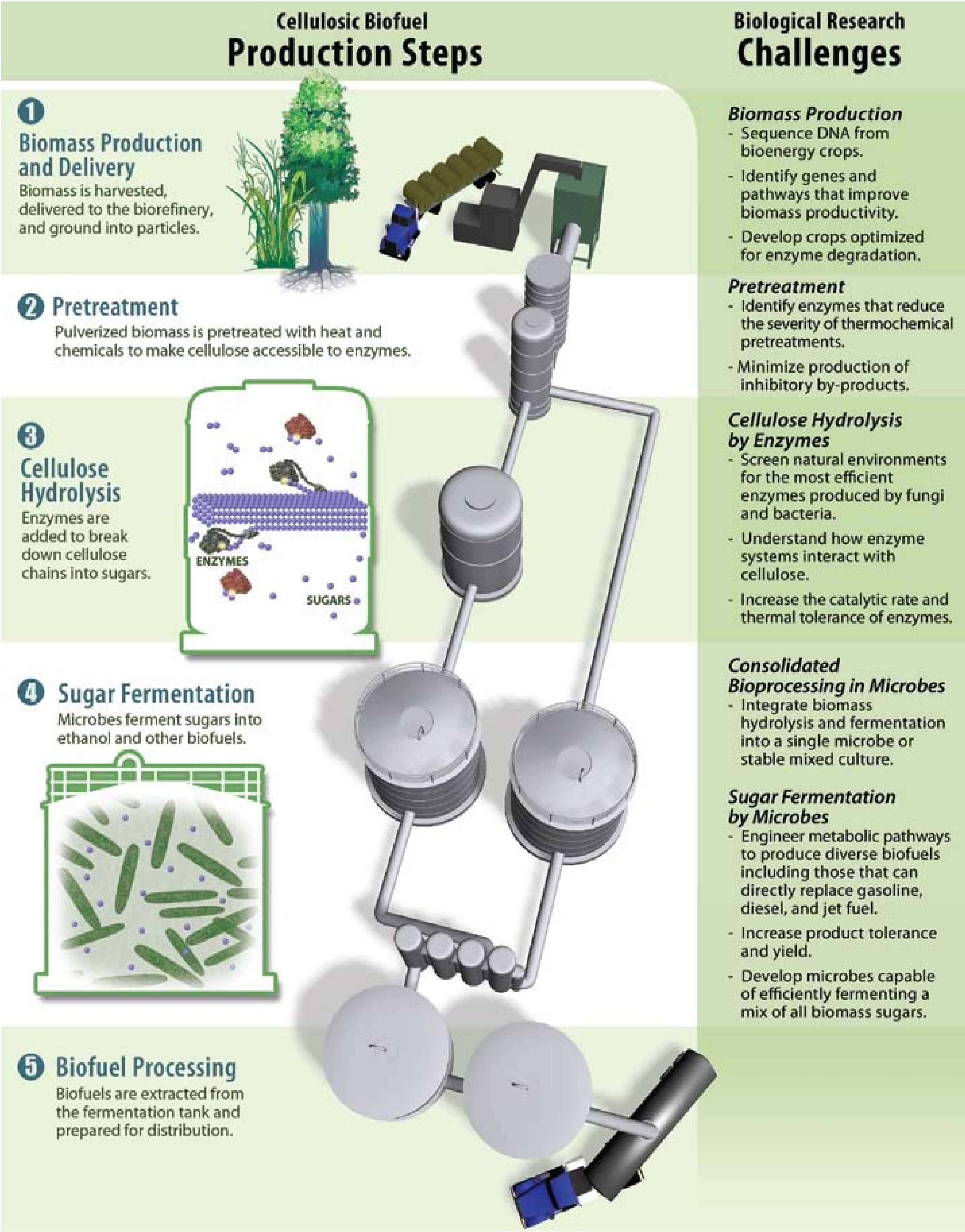
Great Lakes Bioenergy Research Center



Joint BioEnergy Institute

BioEnergy Science Center

July 2009



Cellulosic Biofuel Production Steps and Biological Research Challenges. This figure depicts some key processing steps in an artist's conception of a future large-scale facility for transforming cellulosic biomass (plant fibers) into biofuels. Three areas where focused biological research can lead to much lower costs and increased productivity include developing crops dedicated to biofuel production (see step 1), engineering enzymes that deconstruct cellulosic biomass (see steps 2 and 3), and engineering microbes and developing new microbial enzyme systems for industrial-scale conversion of biomass sugars into ethanol and other biofuels or bioproducts (see step 4). Biological research challenges associated with each production step are summarized in the right portion of the figure.

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Sources for cover images: Great Lakes Bioenergy Research Center photo by Kurt Stepnitz, Michigan State University. Joint BioEnergy Institute photo by Randy Wong, Sandia National Laboratories. BioEnergy Science Center photo by Jason Richards, Oak Ridge National Laboratory.

Contact for DOE Bioenergy Research Centers

Sharlene Weatherwax
U.S. Department of Energy
Office of Biological and Environmental Research
Sharlene.Weatherwax@science.doe.gov

URLs for DOE Bioenergy Research Centers

DOE Great Lakes Bioenergy Research Center
glbrc.org

DOE Joint BioEnergy Institute
jbei.org

DOE BioEnergy Science Center
bioenergycenter.org

URL for this Document: genomicsgtl.energy.gov/centers/brcbrochure.pdf

DOE Bioenergy Research Centers

Introduction

Alternative fuels from renewable cellulosic biomass—plant stalks, trunks, stems, and leaves—are expected to significantly reduce U.S. dependence on imported oil while enhancing national energy security and decreasing the environmental impacts of energy use. Ethanol and other advanced biofuels from cellulosic biomass are renewable alternatives that could increase domestic production of transportation fuels, revitalize rural economies, and reduce carbon dioxide and pollutant emissions. According to U.S. Secretary of Energy Steven Chu, "Developing the next generation of biofuels is key to our effort to end our dependence on foreign oil and address the climate crisis while creating millions of new jobs that can't be outsourced."

In the United States, the Energy Independence and Security Act (EISA) of 2007 is an important driver for the sustainable development of renewable biofuels. As part of EISA, the Renewable Fuel Standard mandates that 36 billion gallons of biofuels are to be produced annually by 2022, of which 16 billion gallons are expected to come from cellulosic feedstocks.

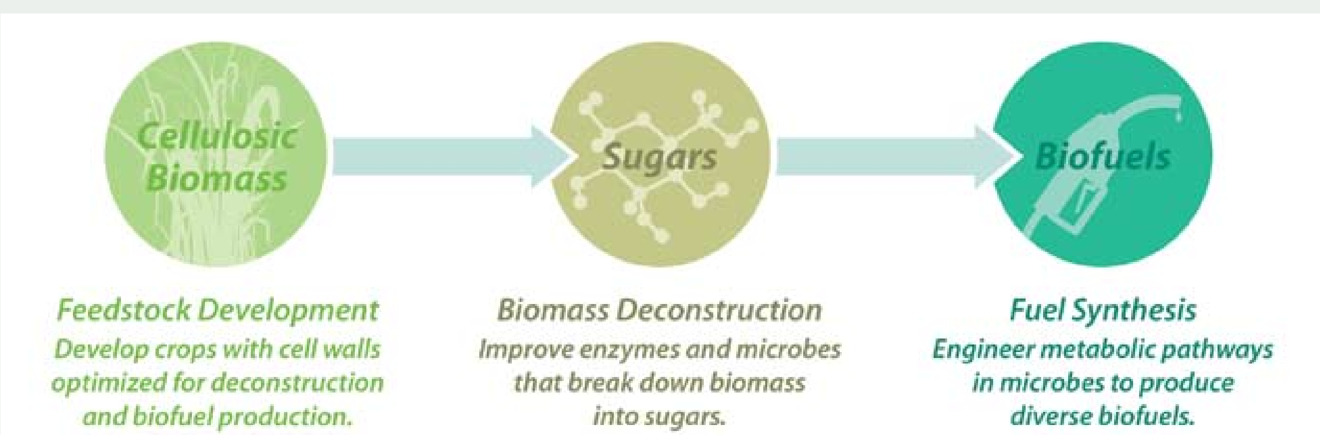
Although cellulosic ethanol production has been demonstrated on a pilot level, developing a cost-effective, commercial-scale cellulosic biofuel industry will require transformational science to significantly streamline current production processes. Woodchips, grasses, cornstalks, and other cellulosic biomass are widely abundant but more difficult to break down into sugars than corn grain—the primary source of U.S. ethanol fuel production today.

Biological research is key to accelerating the deconstruction of cellulosic biomass into sugars that can be converted to biofuels (see illustration on inside front cover, Cellulosic Biofuel Production Steps and Biological Research Challenges; figure below, From Biomass to Biofuels; and sidebar, Plant Cell-Wall Recalcitrance, p. 2).

The Department of Energy (DOE) Office of Science continues to play a major role in inspiring, supporting, and guiding the biotechnology revolution over the past 25 years. The DOE Genomic Science Program is advancing a new generation of research focused on achieving whole-systems understanding for biology. This program is bringing together scientists in diverse fields to understand the complex biology underlying solutions to DOE missions in energy production, environmental remediation, and climate change science. New interdisciplinary research communities are emerging, as are knowledgebases and scientific and computational resources critical to advancing large-scale, genome-based biology. For more information on the Genomic Science Program, see p. 23.

To focus the most advanced biotechnology-based resources on the biological challenges of biofuel production, DOE established three Bioenergy Research Centers (BRCs) in September 2007. Each center is pursuing the basic research underlying a range of high-risk, high-return biological solutions for bioenergy applications. Advances resulting from the BRCs will provide the knowledge needed to develop new biobased products, methods, and tools that the emerging biofuel industry can use. The scientific rationale for these centers and for other fundamental genomic research

From Biomass to Biofuels

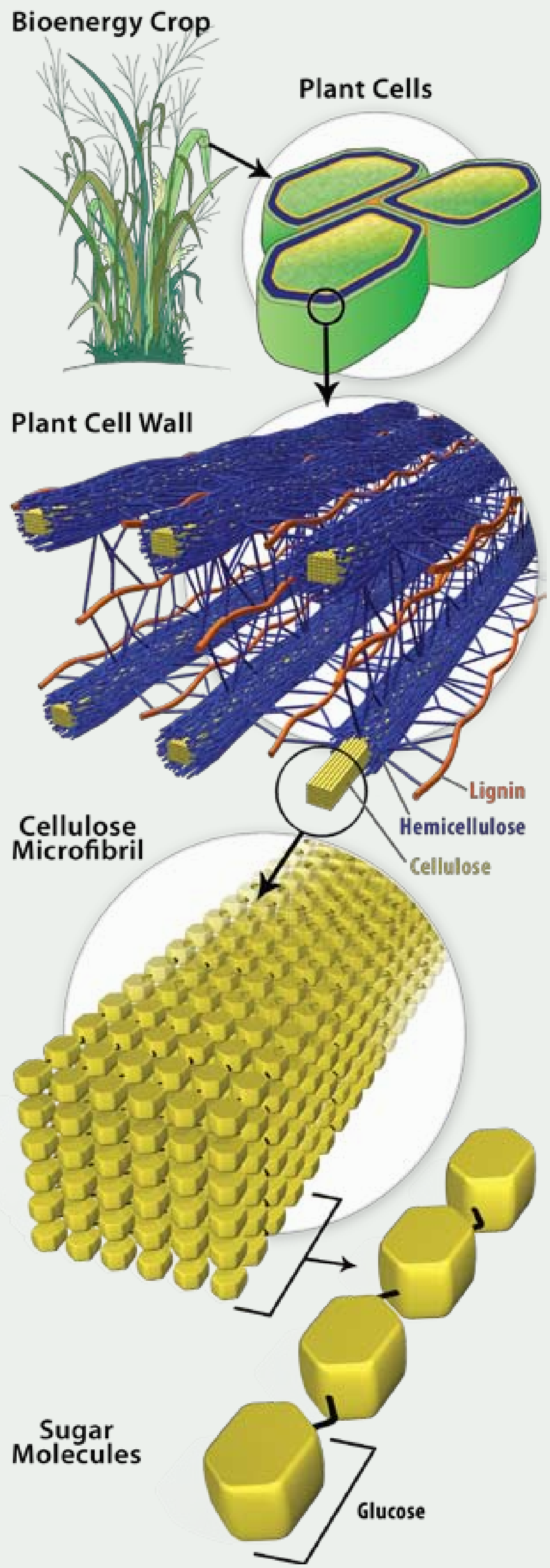


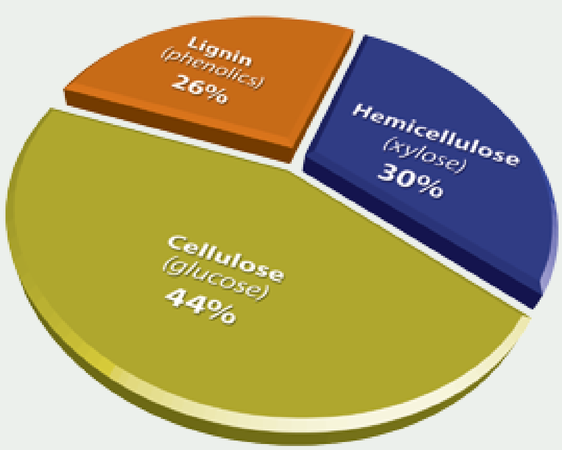
Plant Cell-Wall Recalcitrance: A Key Scientific Challenge

The tough structural materials in plant cell walls form a complex composite exquisitely designed to support plant structure and resist biological and chemical assaults. This natural resistance to degradation is called "recalcitrance" and represents one of the greatest challenges to attaining a viable, cost-effective cellulosic biofuel industry.

A large portion of the plant cell wall contains long chains of sugars (polysaccharides) that can be converted to biofuels. Cellulose—the most abundant biological material on Earth—consists of tightly bound sugar chains organized into strong cable-like structures (microfibrils). Like steel girders stabilizing a skyscraper's structure, microfibrils reinforce plant cell walls. Locked away within the microfibril's sugar chains are thousands of molecules of glucose, a type of six-carbon sugar readily converted into biofuels by microbes. Physically accessing these sugars, however, is difficult.

Cellulose microfibrils are embedded within a matrix of other polymers (hemicellulose and lignin). Hemicellulose, a mix of branched polysaccharides made up of both five- and six-carbon sugars, links to a rigid noncarbohydrate polymer called lignin, which forms a protective coating to shield cellulose and hemicellulose from enzymatic attack. In addition to serving as a physical barrier to enzymes and microbes, lignin also is a source of chemical by-products that can inhibit sugar conversion to biofuels.





Approximate distribution of the three primary components of plant cell walls.

critical to the biofuel industry was established at a DOE workshop involving members of the research community (see sidebar, Biofuel Research Plan, below). The DOE BRCs have developed automated, high-throughput analysis pipelines that will accelerate scientific discovery for biology-based biofuel research (see sidebar, BRC High-Throughput Technologies, p. 4).

The three centers, which were selected through a scientific peer-review process, are based in geographically diverse locations—the Southeast, the Midwest, and the West Coast—with partners across the nation (see U.S. map, DOE Bioenergy Research Centers and Partners, on back cover). DOE's Oak Ridge National Laboratory leads the BioEnergy Science Center (BESC) in Tennessee; the University of Wisconsin–Madison leads the Great Lakes Bioenergy Research Center (GLBRC); and DOE's Lawrence Berkeley National Laboratory leads the DOE Joint BioEnergy Institute (JBEI) in California. Each center represents a multidisciplinary partnership with expertise spanning the physical and biological sciences, including genomics, microbial and plant biology, analytical chemistry, computational biology and bioinformatics, and engineering. Institutional partners include DOE national laboratories, universities, private companies, and nonprofit organizations.

Biofuel Research Plan

In June 2006, DOE released *Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda*. This report provides a detailed roadmap for surmounting biology-based research challenges in cellulosic feedstocks, biomass deconstruction, and sugar fermentation to produce biofuels. The research needs identified in this roadmap determined the scientific rationale underlying the development of the DOE Bioenergy Research Centers and helped establish funding opportunities for the larger research community. The roadmap was jointly supported by the Genomics:GTL systems biology program in the DOE Office of Science's Office of Biological and Environmental Research and by the Biomass Program in the department's Office of Energy Efficiency and Renewable Energy, which conducts applied research. It is available on the Web (genomicsgtl.energy.gov/biofuels/b2bworkshop.shtml).

Biofuels: Grand Challenges for Biology

The ultimate goal for the three DOE Bioenergy Research Centers is to better understand the biological mechanisms underlying biofuel production so that those mechanisms can be redesigned, improved, and used to develop novel, efficient bioenergy strategies that can be replicated on a mass scale. New strategies and findings emanating from the centers' fundamental research ultimately will benefit all biological investigations and will create the knowledge underlying three grand challenges at the frontiers of biology:

- Development of next-generation bioenergy crops
- Discovery and design of enzymes and microbes with novel biomass-degrading capabilities
- Development of transformational microbe-mediated strategies for biofuel production

The extremely complex science needed to solve these challenges requires multiple interdisciplinary teams that approach the same problems from different directions to accelerate scientific progress (see table, DOE Bioenergy Research Center Strategies at a Glance, p. 5). The following sections explain some scientific issues related to these challenges.

1. Development of Next-Generation Bioenergy Crops

Bioenergy crops include grasses, trees, and other plants grown specifically for energy production. These crops and other forms of cellulosic biomass provide the raw material for bioenergy. The raw material comes from cellulose and other complex cell-wall compounds, such as lignin, that strengthen and support plant structure (see sidebar, p. 2). The primary constituents of many plant cell-wall compounds are simple sugars amenable to fermentation, producing ethanol, other biofuels, and chemicals. The cell walls are so complex that several thousand genes are thought to be involved in their synthesis and maintenance. Few of these genes have been identified, and little is known about their function.

By understanding the genes and mechanisms that control cell-wall synthesis in plants, scientists could develop new energy crops with altered biomass composition or modified links within and between cell-wall components. These

BRC High-Throughput Technologies

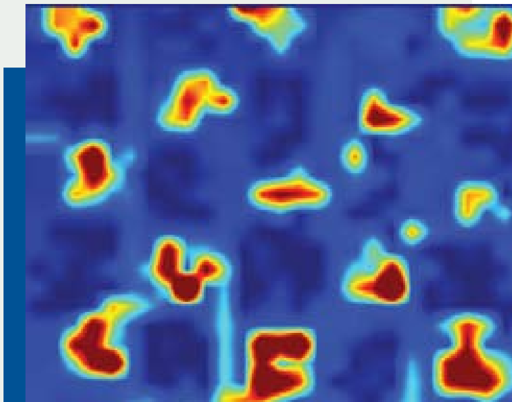
Accelerating Discovery by Increasing the Scale of Cellulosic Biofuel Research

The large-scale research conducted at each DOE Bioenergy Research Center promotes efficiencies that are not likely to be achieved with smaller projects. The large scope and multidisciplinary complexity of the challenges being addressed by the BRCs allow plant and microbial researchers to work together with technology-development specialists and others to design automated pipelines that streamline workflow and increase research efficiencies. Each BRC has established high-throughput systems for processing and screening hundreds to thousands of biological samples in rapid succession.

Great Lakes Bioenergy Research Center (GLBRC). The GLBRC conducts large-scale screening of hundreds of microbial strains for traits beneficial to biofuel production (see figure, GLBRC Microbial Screening). A small subset of microbial samples with favorable traits is selected from this initial screening to undergo reactor-scale fermentation studies that provide data used in a mathematical model for predicting ethanol yield and behavior of the microbial strains. The GLBRC also has established a biomass analysis robot called iWall that can grind, weigh, and dispense 350 samples of plant material per day. This robot allows GLBRC researchers to examine the molecules present in specific amounts of plant material with increased speed and accuracy.

GLBRC Microbial Screening. Wesley D. Marner, II, works with highly instrumented bioreactors to study experimental fermentation and microbial production of biofuels at cellular and reactor scales. [Photo by Wolfgang Hoffmann, University of Wisconsin–Madison]

Joint BioEnergy Institute (JBEI). One high-throughput technology developed by JBEI is a microfluidic chip for the automated characterization of glycans (sugar molecules) and the enzymes that make or break them. Unlike other current high-throughput techniques for glycan and enzyme studies, this microfluidic chip is compatible with JBEI research on ionic liquid pretreatment. Another high-throughput development is a glyco-chip used to rapidly screen large numbers of enzymes for sugar hydrolysis activities in laboratory-grown or environmental microbial samples (see figure, JBEI Microchip for Screening Enzymes). This glyco-chip is based on JBEI's "Nimzyme" technology, which uses fluorine chemistry to immobilize enzymes on a mass spectrometry surface.



JBEI Microchip for Screening Enzymes. This image shows a high-throughput Nimzyme-based glyco-chip used to simultaneously measure the activity of multiple cellulose-degrading enzymes. Areas of high enzyme activity are shown in crimson across a section of the chip.

BioEnergy Science Center (BESC). To determine the chemical, structural, and genetic features that make cellulosic biomass recalcitrant, BESC has developed a high-throughput characterization pipeline that integrates advanced technologies for compositional analysis, pretreatment, and enzyme digestibility studies (see figure, BESC Biomass Characterization Pipeline). BESC researchers now can compare hundreds of samples to see how turning different plant genes on or off can cause cell-wall compositional and structural changes that affect enzyme deconstruction. BESC analysis of thousands of poplar samples with this system has led to the discovery of new links between genes and recalcitrant biomass characteristics (see BESC Research Highlights, p. 21).

BESC Biomass Characterization Pipeline. Processing more than 1,000 biomass samples per week, the BESC pipeline's high-throughput assays for enzyme deconstruction are used to assess recalcitrant features of biomass samples and screen for more effective enzymes.

"designer" bioenergy crops would retain robust growth in the field but could be triggered to break down rapidly in a biorefinery. Besides modification of plant cell walls, another approach to improving bioenergy crops is to increase the accumulation of starches and oils in plant tissues. Starches and oils can be converted into biofuels much more easily than cellulose.

Altering biomass composition is one approach to developing better bioenergy crops, but other important improvements include increasing biomass productivity per acre, increasing resistance to pests and drought, and decreasing applications of fertilizers and other inputs. Many potential energy crops are grasses or fast-growing trees that have not

DOE Bioenergy Research Center Strategies at a Glance

The complexity of the three biological grand challenges that must be overcome to achieve industrial-scale bioenergy production requires the coordinated pursuit of numerous research approaches to ensure timely success. Collectively, the DOE Bioenergy Research Centers* provide a portfolio of diverse and complementary scientific strategies that address these challenges on a scale far greater than any effort to date. Some strategies are listed briefly in the table below.

Grand Challenge: Development of Next-Generation Bioenergy Crops	
Center Strategies	• **GLBRC** – Engineer "model" plants and potential energy crops to produce new forms of lignin and more starches and oils, which are more easily processed into fuels. • **JBEI** – Enhance lignin degradation in "model" plants by changing cross-links among lignin subunits; improve deconstruction and subsequent fermentation by altering linkages between lignin and other cell-wall components; translate genetic developments to switchgrass. • **BESC** – Decrease or eliminate harsh chemical pretreatments by engineering plant cell walls in poplar and switchgrass to be less recalcitrant; simultaneously increase total biomass produced per acre.
Grand Challenge: Discovery and Design of Enzymes and Microbes with Novel Biomass-Degrading Capabilities	
Center Strategies	• **GLBRC** – Identify combinations of enzymes and pretreatment needed to digest specific biomass types; express biomass-degrading enzymes in the stems and leaves of corn and other plants. • **JBEI** – Develop new ionic liquid pretreatments that can completely solubilize and fractionate biomass components; improve performance and stability of enzymes obtained from the rainforest floor and other environments; engineer, through directed evolution, highly efficient cellulase enzymes. • **BESC** – Screen natural thermal springs to identify enzymes and microbes that effectively break down and convert biomass at high temperatures; understand and engineer cellulosomes (multifunctional enzyme complexes for degrading cellulose).
Grand Challenge: Development of Transformational Microbe-Mediated Strategies for Biofuel Production	
Center Strategies	• **GLBRC** – Start with an ethanol-producing microbe and add lignocellulose-degrading capabilities to substantially reduce costs. • **JBEI** – Connect diverse biological parts and pathways to create entirely new organisms that produce fuels other than ethanol; engineer organisms to produce and withstand high concentrations of biofuels; derive useful chemical products from lignin degradation. • **BESC** – Start with a lignocellulose-degrading microbe and add ethanol-producing capabilities to substantially reduce costs; develop a knowledgebase and pathway analysis tools to aid this manipulation.

*BESC: BioEnergy Science Center; GLBRC: Great Lakes Bioenergy Research Center; JBEI: Joint BioEnergy Institute.

benefited from the years of agricultural research devoted to breeding traditional crops such as corn or wheat. Availability of more plant-genome sequence information can accelerate the development of DNA markers used to identify and isolate the many genes associated with traits that can improve energy crop yield, degradability, and sustainability. Having DNA markers and other new biological tools could significantly reduce the time required to identify desired genetic variants and produce new energy crops.

2. Discovery and Design of Enzymes and Microbes with Novel Biomass-Degrading Capabilities

Nature uses both enzymes and multienzyme complexes, including those called “cellulosomes,” to break down cellulosic biomass (see sidebar, Tapping Nature’s Strategies for Biomass Degradation, p. 7). The biomass-degrading enzymes and cellulosomes studied thus far function slowly enough that scientists are optimistic that their activity and effectiveness can be improved significantly. Several factors—the nearly impenetrable architecture of plant cell walls, chemical and physical changes to biomass during pretreatment, and structural features of the enzymes—collectively contribute to the inefficiency of current biomass deconstruction approaches. Therefore, multiple strategies must be studied simultaneously and systematically at each DOE Bioenergy Research Center to illuminate the various biological and chemical processes at work.

Certain fungi and bacteria specialize in producing enzymes that degrade biological materials in natural environments. Discovering, harnessing, and enhancing the best biomass-degrading enzymes and microbes in nature ultimately will have a significant impact on increasing the efficiency and reducing the cost of cellulosic biofuel production. Scientists are just beginning to explore the staggering diversity of enzymes in environments such as the termite gut and cow rumen, and the vast majority of natural habitats are yet to be investigated. To accelerate the discovery of novel enzymes and microbes and to understand how their degradative processes work synergistically, each center is searching diverse biomass-degrading environments, from hot springs to rainforests to the guts of plant-eating insects.

Discovering new biomass-degrading capabilities in nature is only part of the challenge. Molecular-level understanding of how enzymes and cellulosomes degrade biomass is a prerequisite to designing improved processes. Because no single research approach can provide this understanding, each center is integrating different combinations of methodologies. These include high-throughput screens for proteins and metabolites, chemical analyses, state-of-the-art imaging technologies, and computational modeling to identify and characterize important factors influencing the rapid deconstruction of plant materials into sugars and other energy-rich components that can be converted to biofuels.

3. Development of Transformational Microbe-Mediated Strategies for Biofuel Production

In addition to cellulose, other carbohydrates (collectively called hemicelluloses) in plant cell walls are broken down into fermentable sugars when biomass is pretreated with heat and chemicals. Although cellulose is made of one type of six-carbon sugar (glucose) that is readily converted into ethanol and other products, microbial fermentation of the five- and six-carbon sugar mix from hemicelluloses is less efficient and thus is a key area for improvement.

En route to the fermentation tank, biomass currently is subjected to physical, chemical, and enzymatic processing steps that can create by-products and conditions that might inhibit microbial conversion of sugars into biofuels. Ethanol and other biofuel products also inhibit microbial fermentation at high concentrations. Consequently, another important research area is developing microbes robust enough to withstand the stresses of industrial processing and tolerate higher ethanol concentrations.

An additional research target that could radically simplify the entire production process is consolidated bioprocessing (CBP). This scientific strategy combines cellulose deconstruction and sugar fermentation into a single step mediated by a “multitalented” microbe or mixed culture of microbes. CBP requires a redesign of microbial systems far more extensive than conventional genetic engineering approaches. For example, genetically engineering the microbial production of a single drug or other biochemical product might involve the modifications of only a few genes. A successful CBP microbe or specially designed microbial consortium may be required to produce a variety of biomass-degrading enzymes; produce only minimal amounts of molecules that inhibit the overall process; ferment both five- and six-carbon sugars; and thrive in industrial reactors with high temperature, low pH, and high concentrations of biofuel products. Simultaneously incorporating so many different capabilities into a single microbe or consortium requires an unprecedented understanding of microbial systems.

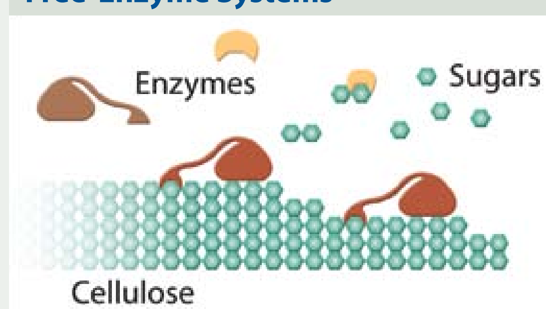
To accelerate development of the next generation of high-energy biofuels, the DOE Bioenergy Research Centers also are designing novel microbial systems that can produce biofuels other than ethanol. Some of these new fuels may be oily, petroleum-like products that are easily extracted from the watery solutions in biorefinery reactors and thus less inhibitory to biofuel-synthesizing microbes. These new biofuels also would be compatible with existing motor vehicles and fuel transportation infrastructure and contain as much energy per unit volume as gasoline or diesel.

Tapping Nature's Strategies for Biomass Degradation

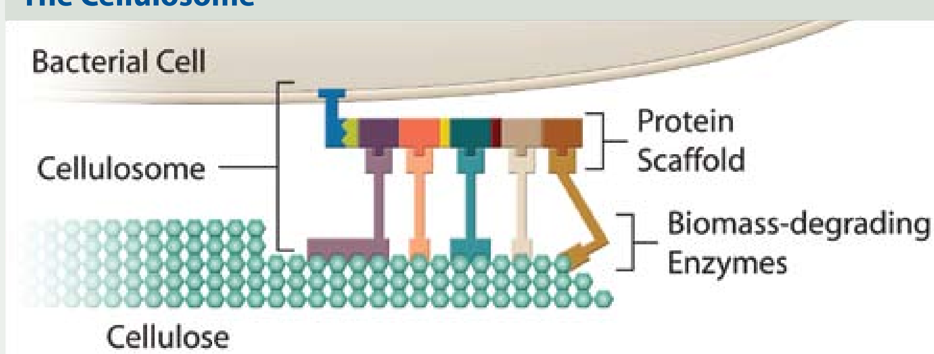
Nature has devised two types of enzyme-based strategies for breaking down plant cell walls. One strategy observed in oxygen-rich environments involves fungi and bacteria that secrete individual enzymes with complementary functions for degrading cell walls. These free enzymes work as a team to deconstruct plant cell-wall carbohydrates into simple sugars (see figure, Free-Enzyme Systems). Some of the most-studied free enzymes are cellulases secreted by *Trichoderma reesei*, a canvas-eating fungus isolated during World War II from tents and uniforms damaged by jungle rot. The DOE Bioenergy Research Centers are exploring natural environments to identify and produce new mixes of biomass-degrading enzymes that can substantially increase the efficiency of cellulosic biofuel production.

A second strategy, occurring in oxygen-free environments, is exhibited by bacteria that produce cellulosomes—large multiprotein complexes that degrade the cell wall by assembling several different enzymes into a single structure. Cellulosomes protrude from bacterial surfaces, latch onto plant cell walls, and tear carbohydrates into simple sugars. *Clostridium thermocellum*, a model bacterium for studying cellulosomes, can produce more than 25 different cell wall–degrading enzymes that it can "plug and play" into its large protein scaffold (see figure, The Cellulosome). By sensing the surrounding environment, *C. thermocellum* can modify the functionality of its cellulosomes on demand by assembling different combinations of enzymes to attack various compounds in the plant cell wall. The LEGO-like arrangement of enzymes in cellulosomes offers a unique opportunity to engineer "designer" multienzyme complexes targeted to specific biomass types or different stages of biomass deconstruction.

Free-Enzyme Systems



The Cellulosome



The DOE Great Lakes Bioenergy Research Center (GLBRC) is led by the University of Wisconsin–Madison, in close partnership with Michigan State University (see box, GLBRC Partners, p. 9). Located in the world's most productive agricultural region, the GLBRC is exploring scientifically diverse approaches to converting sunlight and various plant feedstocks—agricultural residues, wood chips, and grasses—into biofuels. In addition to its broad range of research projects, the GLBRC is collaborating with agricultural researchers and producers to help develop the most economically viable and environmentally sustainable practices for bioenergy production. A new facility is being designed to house GLBRC and other UW–Madison bioenergy programs.

The GLBRC scientific portfolio is organized into four core discovery programs: (1) Improving Plant Biomass, (2) Improving Biomass Processing, (3) Improving Biomass Conversion to Biofuels, and (4) Creating Sustainable Bioenergy Practices. Each core discovery program has a targeted set of mutually supportive goals designed to develop biofuel technologies and transfer them to industry. The GLBRC's sustainability projects span both basic science and applications. The center's research activities are integrated so that data and models generated in one area inform research and technology development by the other core discovery programs. Research support activities that cut across all four discovery areas include the development of enabling technologies such as automated screens for genes and proteins in plants and microbes, the creation and management of informatics and information technology tools, and education and outreach. Some recent highlights and successes of GLBRC research are featured on p. 11.

Research Strategy

1. Improving Plant Biomass

In addition to investigating how genes affect cell-wall digestibility in model plants, cornstalks, and switchgrass, GLBRC researchers are using information from model and agronomic systems to breed plants that produce more or altered hemicelluloses, starches, oils, or new forms of lignin that are easier to process into fuels. Plant oils have twice the energy content of carbohydrates and require little energy to extract and convert into biodiesel. GLBRC researchers aim

GLBRC
Great Lakes Bioenergy Research Center

"We are proud of the pieces of the scientific puzzle we assembled during our first year. We have brought together more than 275 hand-picked scientists and staff and outfitted core facilities for analyzing bioenergy microbes and plants in the lab or the field. The productivity of this team is evident in dozens of peer-reviewed publications and patentable technologies that can serve as a foundation for a new renewable bioenergy future." – Tim Donohue

Tim Donohue
GLBRC Director

Tim Donohue is the GLBRC principal investigator and director as well as a professor of bacteriology at the University of Wisconsin–Madison. He is an expert in applying the latest genomic and systems biology approaches to understanding how genetic pathways and networks in microorganisms are used to generate cell biomass or biofuels from sunlight.

GLBRC Facility. University of Wisconsin–Madison's new Microbial Sciences Building houses GLBRC administration and scientific research. [Photo by Wolfgang Hoffmann, University of Wisconsin–Madison] to increase the energy density of grasses and other nontraditional oil crops by understanding and manipulating the metabolic and genetic circuits that control accumulation of oils and other easily digestible, energy-rich compounds in plant tissues (see figure, Improving Plant Biomass Research at GLBRC, below).

2. Improving Biomass Processing

Located at the intersection of America's agricultural heartland and its abundant northern forest biomass, the GLBRC has access to a rich diversity of raw biomass for study. GLBRC biomass-processing research focuses on finding and improving natural cellulose-degrading enzymes extracted from diverse environments. Improved enzymes created by the GLBRC protein-production pipeline are tested with a range of plant materials and pretreatment conditions to identify the best combination of enzymes, chemicals, and physical processing for enhancing the digestibility of specific biomass sources. GLBRC researchers identify and quantify small molecules generated by different pretreatment methods and examine how these molecules impact biofuel yield.

To decrease the costs of producing and using enzymes to break down cellulose in plants, scientists in this discovery program are working with plant-biomass researchers. They are expressing biomass-degrading enzymes in the stems and leaves of corn and other plants—essentially designing plants to "self-destruct" on cue in a biofuel production facility.

GLBRC Partners

University of Wisconsin–Madison (lead institution): The GLBRC's lead partner provides expertise in genome-enabled analysis of plant and microbial pathways, networks, and systems; computational analysis of bioenergy proteins, organisms, and ecosystems; biosynthesis and chemistry of lignin and cell-wall cross-linking; and discovery, production, and improvement of bioenergy enzymes.

Michigan State University (MSU), East Lansing: MSU researchers are experts in the breakdown and biosynthesis of plant cell walls, oils, and other polymers; the breakdown of cellulose in plant stems, stalks, and leaves, including grasses, trees, and other woody plants; and the development of biofuel production practices that are environmentally and economically sustainable.

Illinois State University, Normal: Researchers at Illinois State University work on the genetic and molecular analysis of switchgrass.

Iowa State University (ISU), Ames: ISU researchers are experts in constructing economic models of biomass practices.

Lucigen Corporation, Middleton, Wisconsin: Lucigen provides valuable expertise in functional screening for cellulases and other enzymes, isolation and growth of thermophiles, ethanol production, and molecular cloning techniques.

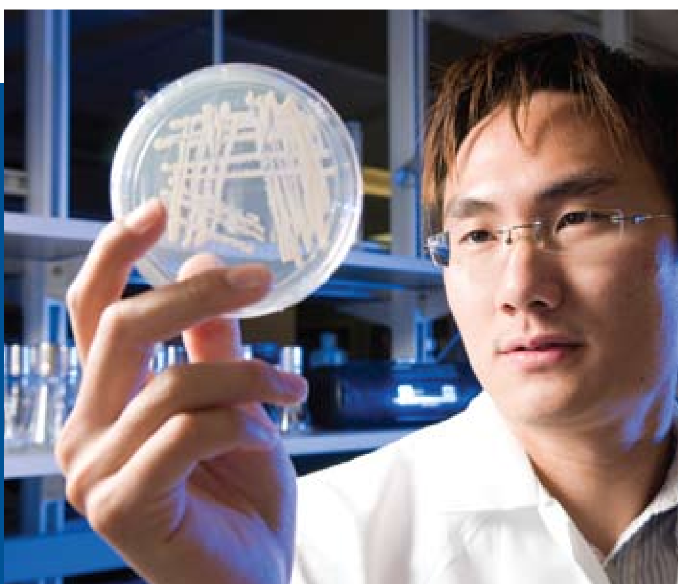
DOE's Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee: ORNL will enable the GLBRC to evaluate biomass sustainability by modeling ecosystem changes that could result from the biofuel production cycle.

DOE's Pacific Northwest National Laboratory (PNNL), Richland, Washington: PNNL enables the GLBRC to complete high-throughput analysis of bioenergy proteins and organisms and analyze the entire life cycle of bioenergy practices.

Improving Plant Biomass Research at GLBRC. Michigan State University researcher, Sanjaya, has developed a transformation procedure in which rutabaga plants are engineered to convert carbohydrates into oil in their beet-like storage organ. [Photo by Kurt Stepnitz, Michigan State University]

3. Improving Biomass Conversion to Biofuels

GLBRC biomass-conversion research is driven by the need to increase the quantity, diversity, and efficiency of energy products derived from plant biomass. Cellulosic ethanol is a major focus for GLBRC research (see figure, Improving Biomass Conversion at GLBRC, below), but the center also aims to improve both biological and chemical methods for converting plant material into intermediate chemicals that can be used to produce ethanol and other transportation fuels. In addition to producing new generations of cellulose-derived liquid transportation fuels, GLBRC researchers are improving the processes by which microbes directly convert a combination of biomass and sunlight into hydrogen or electricity. Another target is to develop a microbe capable of carrying out all biologically mediated biofuel production steps. The GLBRC strategy to reach this target is to start with efficient ethanol-producing microbes and enable them to produce enzymes and pathways for breaking down cellulose.



Improving Biomass Conversion at GLBRC. Ming W. Lau at Michigan State University examines a transgenic yeast strain capable of cofermenting ethanol from glucose and xylose. Using AFEX-treated corn stover, this strain can grow and produce ethanol without additional nutrient supplementation or detoxification. [Photo by Kurt Stepnitz, Michigan State University]

4. Creating Sustainable Bioenergy Practices

For the emerging cellulosic biofuel industry to have a positive impact on the United States, complex issues involving agricultural, industrial, and ecological systems as well as factors affecting human decision making and behaviors must be addressed. To create a better understanding of the larger context that ultimately influences the direction and acceptance of new biotechnologies, GLBRC scientists are examining the environmental and socioeconomic dimensions of converting biomass to biofuel.

To determine the best practices for biofuel production, GLBRC researchers are analyzing the impacts of issues such as minimizing energy and chemical inputs for bioenergy crop production and reducing greenhouse gas emissions from the entire biofuel production life cycle. They also are seeking to understand the environmental impacts of removing leftover stalks, stems, and leaves from food crops. Data from these and other studies will allow GLBRC scientists to make predictions on the social and financial incentives needed to promote the adoption of more environmentally beneficial practices.

Education and Outreach

The staff and partners of the GLBRC Education and Outreach area inform a variety of audiences about biofuels research, energy concerns, and sustainability issues affecting our planet. Their goal is to broaden the understanding of current issues in bioenergy for the general public as well as students and educators at the K–12, undergraduate, and graduate levels. A strong emphasis is placed on using critical thinking, quantitative reasoning, and systems-based logic in the development of bioenergy-related K–12 classroom materials and other informational resources. Because bioenergy research and development are important contemporary issues, Education and Outreach members participate in various programs and events to present research from GLBRC labs in a way that is accessible and interesting to a broad array of audiences. Summer research experiences for undergraduates at UW–Madison and Michigan State University and other Education and Outreach projects are described in detail at glbrc.org/education/.

GLBRC Research Highlights

GLBRC Sustainable Biofuel Landscapes. At Michigan State University's W. K. Kellogg Biological Station, GLBRC researchers are evaluating the performance of a variety of novel bioenergy crop production systems for crop yield and quality, impacts on microbial-plant interactions, biogeochemical and biodiversity responses, and water use. [Photo by Kurt Stepnitz, Michigan State University]

Using 2D NMR to Understand Plant Cell-Wall Composition and Function. Characterization of the composition and structure of the cell wall's entire polymer complex is difficult because of the amorphous nature and complexity of the molecules. Solution-state nuclear magnetic resonance (NMR) instruments at the GLBRC provide high-resolution profiling (called "fingerprinting") of cell-wall composition and structure. These methods have been used to provide deeper insight into processes such as the increase in cellulose deposition in poplar tension woods, fungal degradation mechanisms, and the beneficial changes that can occur in a plant when cell-wall gene expression is increased or decreased. Preliminary trials suggest that multivariate methods applied to the two-dimensional (2D) NMR fingerprint of cell walls will provide a useful tool for relating their profiles to other analytical or process data—presumably, for any process that depends on the composition and structure of the wall. The 2D NMR fingerprint then can be used to inform research on optimal feedstock selection, allow biomass conversion efficiencies to be predicted, and aid in pretreatment and process optimization.

Enzymes for Biomass Pretreated with AFEX. GLBRC proteomics experts characterized protein sources rich in a large variety of enzymes that degrade biomass. Results led to the identification of a minimal enzyme set needed to deconstruct corn stover that has been treated with an ammonia fiber expansion (AFEX) process. This finding will help reduce the number and amount of enzymes required for lignocellulase biomass processing and thus the cost of producing cellulosic ethanol.

Two-Step Chemical Method for Converting Untreated Biomass to Biofuel. Taking a chemical approach, GLBRC scientists developed a two-step method to convert raw, untreated biomass into a promising biofuel. The key to the new process is the first step in the conversion of cellulose into a "platform" chemical called 5-hydroxymethylfurfural (HMF) from which a variety of valuable commodity chemicals can be made. The special mix of solvents and additives, for which a patent is pending, is effective because it has the capacity to slip between lignin molecules, which are notoriously hard to break apart. Once the solvent moves past the lignin, it is able to dissolve cellulose. In the second step, HMF is converted to the promising biofuel 2,5-dimethylfuran.

Sustainable Biofuel Landscapes. GLBRC sustainability researchers found that growing more corn to produce ethanol—which results in less diverse landscapes—reduces the ability of beneficial insects to control pests. As land is converted to corn, the population of lady beetles declines, resulting in a population growth of soybean aphids, the most significant soybean pest. The calculated loss for this effect is about $58 million per year in the four states studied (Michigan, Iowa, Minnesota, and Wisconsin). Research is under way to collect data and apply these techniques to cellulosic feedstocks (see figure above, GLBRC Sustainable Biofuel Landscapes).

Industry Partnerships

The GLBRC employs a systems-driven, genome-informed, basic science approach within a project-management environment. Thus, the center operates primarily in the early research and development arena. Once technology is developed, the center works closely with industry partners through technology-transfer mechanisms or collaboratively to achieve commercial implementation. The GLBRC's Tech Transfer Working Committee is poised to rapidly disseminate resulting technologies by protecting intellectual property and providing a one-stop shop for industry partners. More information about technology-transfer opportunities from GLBRC research is available from the Wisconsin Alumni Research Foundation (www.warf.org).

The DOE Joint BioEnergy Institute (JBEI) is a six-institution partnership led by Lawrence Berkeley National Laboratory (Berkeley Lab). It is based in the San Francisco Bay Area, which is fast becoming a hub of renewable energy research and development, and is headquartered in a new facility in Emeryville, close to its partner institutions (see box, JBEI Partners, p. 15). JBEI researchers are engineering microbes and enzymes to process the complex sugars of lignocellulosic biomass into biofuels that can directly replace gasoline. Among the strategies they employ to produce these next-generation biofuels are the tools of synthetic biology. By developing new bioenergy crops, JBEI researchers will improve the fermentable content of biomass and transform lignin into a source of valuable new products.

JBEI's research revolves around four interdependent efforts that focus on (1) developing new bioenergy crops, (2) enhancing biomass deconstruction, (3) producing new biofuels through synthetic biology, and (4) creating technologies that advance biofuel research. Some recent highlights of JBEI research are featured on p. 16.

Research Strategy

1. Developing New Bioenergy Crops

To increase our understanding of genes and enzymes involved in the synthesis and modification of plant cell walls, JBEI researchers are using well-characterized genomes and genetic-engineering tools established for rice and *Arabidopsis* (a small flowering plant related to mustard). These two model systems are ideal for research because their development from seed to mature plant takes only weeks or months, rather than the year or more required for energy crops such as switchgrass and poplar. Genetic insights from rice (a model for grasses) and *Arabidopsis* (a model for trees) will accelerate the development of new energy crops such as switchgrass (see figure, Bioenergy Crop Research at JBEI, p. 14).

In addition, JBEI scientists are investigating metabolic pathways involved in lignin biosynthesis. The research may lead to development of plants that can be deconstructed more easily. This unique basic research program also could help transform lignin into a valuable source of chemicals

jbei
Joint BioEnergy Institute

"DOE JBEI is designed to be an engine of ingenuity, dynamically organized with all the scientific teams working together in a single location to enable researchers to share ideas and address cellulosic biomass problems at a systems level. Within 60 miles of JBEI, we have available some of the world's foremost authorities on energy, plant biology, systems and synthetic biology, imaging, nanoscience, and computation, plus the highest concentration of national laboratories and research universities in the nation." – Jay Keasling

Jay Keasling
JBEI Chief Executive Officer

Jay Keasling is the JBEI Chief Executive Officer, director of Berkeley Lab's Physical Biosciences Division, and a University of California, Berkeley professor of chemical engineering. He also is an award-winning scientific researcher and one of the world's leading authorities on synthetic biology.

JBEI Research Facility. JBEI researchers are located at a single site in Emeryville, California. [Photo by Roy Kaltschmidt, Berkeley Lab] and polymers, while improving the economics of converting cellulosic biomass into fuels.

2. Enhancing Biomass Deconstruction

Scientists at JBEI are developing new pretreatment approaches and enzymes that enhance cellulose conversion to sugars and minimize the formation of toxic by-products. A large focus is on the use of ionic liquids, salts that are liquid rather than crystalline at room or near-room temperatures. JBEI researchers are investigating both the effects of ionic liquids on biomass and the recovery of sugars from the liquid product through the use of solvents. They also are exploring a broad range of environments, from rainforests to compost, to discover and isolate new enzymes that more efficiently degrade cellulose and lignin. JBEI studies of the mechanisms of biomass deconstruction at the molecular level will enable new insights and approaches for the efficient conversion of all plant components to useful products.

3. Producing New Biofuels Through Synthetic Biology

JBEI researchers are applying synthetic biology techniques and mathematical models of metabolism and gene regulation to engineer microorganisms that convert the sugars released from biomass deconstruction into advanced biofuels, such as alcohols (e.g., butanol) and alkanes (see figure, Fuels Synthesis at JBEI). These next-generation biofuels will yield almost as much energy per volume as gasoline and will be transportable through existing fuel pipelines (see sidebar, Synthetic Biology, p. 17). Biologically produced alkanes and other oil-like hydrocarbons could replace gasoline in today's cars on a gallon-for-gallon basis.

Bioenergy Crop Research at JBEI. Lignocellulose in the cell walls of potential bioenergy crops like switchgrass (pictured) or other plant material has the potential to provide biofuels that yield the same energy as gasoline and can be easily distributed through the existing pipeline and gas station infrastructure, provided it can be efficiently broken down into its constituent sugars. [Photo by Roy Kaltschmidt, Berkeley Lab]

Fuels Synthesis at JBEI. In JBEI's Fuels Synthesis Division, researchers are using the tools of synthetic biology to engineer new microbes that can quickly and efficiently ferment cellulosic sugars into transportation fuels and other valuable chemical products. [Photo by Roy Kaltschmidt, Berkeley Lab]

4. Creating Technologies that Advance Biofuel Research

JBEI scientists are creating new, broadly applicable technologies to advance research that will speed biofuel development. Among these technologies is a novel chip-based system that can be used to identify new enzymes with cellulose- and lignin-degrading activities. In addition, the researchers are constructing automated microfluidic platforms that can screen hundreds of enzymatic reactions simultaneously to help identify the best enzymes for biomass deconstruction. Technologies also are being developed for rapid high-resolution imaging to visualize and characterize the effects of pretreatment protocols on plant biomass. These and other enabling technologies are expected to generate large volumes of data that will be collected and catalogued in a centralized database and then analyzed using new bioinformatic tools.

Industry Partnerships

To promote the transfer of JBEI inventions to private industry for commercial development that can benefit the nation, JBEI has established collaborations with companies

JBEI Partners

DOE's Lawrence Berkeley National Laboratory (Berkeley Lab), Berkeley, California (lead institution): Berkeley Lab, a multidisciplinary national laboratory, is home to the Advanced Light Source, the Molecular Foundry, the National Center for Electron Microscopy, and the National Energy Research Scientific Computing Center. It also is a founding partner of the DOE Joint Genome Institute, one of the world's largest and most productive DNA sequencing centers. Berkeley Lab provides expertise in bioinformatics, data management, and biological modeling, in addition to being a world leader in advanced imaging, nanoscale biology, artificial photosynthesis, and technologies focused on energy efficiency and conservation.

DOE's Sandia National Laboratories, Albuquerque, New Mexico, and Livermore, California: Sandia National Laboratories consist of multidisciplinary research institutes that provide expertise in systems engineering and integration function, microfluidics, computation, and robotics and materials development, as well as experience in manufacturing technologies.

University of California, Berkeley: UC Berkeley, ranked first for distinguished scholarship by the National Research Council, is home to the Energy Biosciences Institute, a partnership that includes Berkeley Lab and the University of Illinois, whose research complements that of JBEI. UC Berkeley provides a broad range of expertise in JBEI-related fields, including molecular and cellular biology, molecular genetics, proteomics, and environmental sciences.

University of California, Davis: UC Davis oversees the California Biomass Collaborative and is home to the Northern California Nanotechnology Center, the Western Regional Center of the National Institute for Global Environmental Change, and the UC Davis Genome Center. The university provides expertise in plant and environmental sciences, genetics, plant physiology, evolutionary biology, and environmental science.

Carnegie Institution for Science, Palo Alto, California: The Carnegie Institution for Science is a private, nonprofit organization on the campus of Stanford University. It maintains TAIR, a comprehensive database on *Arabidopsis thaliana*, the model organism for plant molecular genetics, and provides expertise in photosynthesis, bioinformatics, and growth and developmental processes that enable plants to survive disease and environmental stress.

DOE's Lawrence Livermore National Laboratory (LLNL), Livermore, California: LLNL is a multidisciplinary national laboratory that hosts the Center for Accelerator Mass Spectrometry and the world's fastest supercomputer. LLNL also is one of the DOE Joint Genome Institute's founding partners and provides expertise in genomics, bioinformatics, experimental protein production, advanced measurement technologies, and high-performance scientific computing.

JBEI Research Highlights

Development of Ionic Liquid Pretreatment. The conversion of cellulosic biomass to biofuels begins with pretreatment—the use of chemical or physical treatments to loosen the tight linkages among cell-wall components, making the biomass easier to degrade. A new development in pretreatment research is the use of ionic liquids—salts that are liquid rather than crystalline near room temperature (see figure, JBEI Ionic Liquid Pretreatment Research). Ionic liquids can dissolve both lignin and cellulose; their use, however, has required large amounts of antisolvent to recover the dissolved cellulose. JBEI researchers have studied solvent extraction technology based on the chemical affinity of boronates to complex sugars and determined optimal pH and temperature conditions for recovering sugars from the ionic liquid–biomass liquor.

JBEI Ionic Liquid Pretreatment Research. Brad Holmes studies ionic liquids—the focus of a new pretreatment approach that has a unique capacity for dissolving lignocellulosic biomass and helping to hydrolyze the resulting liquor into sugars for biofuel production. [Photo by Roy Kaltschmidt, Berkeley Lab]

Re-Engineering Microbes to Produce Novel Biofuels. JBEI researchers have used synthetic biology and metabolic engineering techniques in *Escherichia coli* and *Saccharomyces cerevisiae* (yeast) to produce advanced, "drop-in" fuels that perform better than ethanol. The scientists redirected central metabolic, fatty acid, and cholesterol biosynthetic pathways to produce candidate gasoline, diesel, and jet fuel molecules. JBEI also has developed a new metabolic pathway that potentially could produce both advanced fuels and other molecules (e.g., polymer monomers) that might otherwise be produced from petroleum, paving the way to replace a significant portion of petroleum-based products with sugar-based products.

New Strategy for Faster Microbial Metabolic Studies. JBEI researchers established that *Geobacillus thermoglucosidasius*, a bacterium that thrives in the high temperatures and pressures of petroleum reservoirs, can ferment the major five- and six-carbon sugars in cellulosic biomass and tolerate high concentrations of ethanol. Vital determinations about the microbe's metabolism were made using an unusual experimental route that could greatly accelerate future related research efforts. Scientists normally obtain metabolic information on a microbe through genomic sequencing and subsequent proteomic and physiological studies, a process that can take months or even years to complete. JBEI researchers, however, completed their metabolic studies in less than 2 weeks using a combination of *in vitro* enzyme assays and a unique metabolic flux analysis they developed based on the carbon-13 isotope.

Analysis of Plant Gene Family that Inhibits Deconstruction. JBEI researchers annotated all the glycosyl transferase–type enzymes in rice and identified an *Arabidopsis* gene family involved in polysaccharide acetylation, a chemical modification inhibiting feedstock deconstruction and subsequent fermentation of cell-wall sugars into biofuels. Identifying genes involved in this process provides a tool to develop feedstocks with decreased acetylation.

Phylochip for Rapidly Screening Thousands of Microbial Species. To find new and better enzymes that break down lignocellulose, JBEI researchers analyzed microbial communities in Puerto Rican rainforest soils that boast some of the planet's highest rates of biomass degradation. Scientists used the Phylochip, a credit card–sized microarray developed at Berkeley Lab that can quickly detect the presence of up to 9,000 microbial species in samples. Using bags of switchgrass as "microbe traps," the researchers conducted a census of these soil microbes to identify the most efficient biomass-degrading bacteria and fungi.

Structural Characterization of a Heat- and Acid-Tolerant Enzyme. To help improve enzymes that are active in biorefinery environments optimized for efficient conversion, JBEI researchers determined the crystal structure of an important sugar-depolymerizing enzyme (Cel9A). This enzyme is found in *Alicyclobacillus acidocaldarius*, a bacterium that thrives in high-temperature and acidic environments. From their high-resolution crystallography images, researchers identified an immunoglobulin-like domain within the enzyme that appears to play an important role in cellulose breakdown.

that have relevant scientific and marketing capabilities in energy, agribusiness, and biotechnology. The JBEI Industry Partnership Program provides companies with opportunities to contribute to JBEI and become part of the JBEI community. To further help ensure that its science ultimately will be able to serve national needs, JBEI has established an advisory committee, with representatives from the entire spectrum of the biofuel industry. For more information on JBEI'S collaborations with industry, see jbei.org/for-industry/.

Education and Outreach

Educational efforts at JBEI build on strong undergraduate, graduate, and postdoctoral training programs, plus nationally recognized K–12 and community college science outreach programs already in place at JBEI's member institutions. In addition to starting a new student fellowship program, JBEI is collaborating with the University of California, Berkeley's Management of Technology Program to enable young scientists and engineers to develop biofuel-related business plans. JBEI's own education and outreach programs include internships, scientific academies, seminars, and collaborations with academic and industry-based science institutions. In addition to external education opportunities, JBEI also offers its researchers in-house seminars as resources for ongoing education.

Synthetic Biology

Building Novel Biological Systems for Useful Purposes

Synthetic biologists design and build novel organisms to generate products not made by natural systems. This process may involve constructing entirely new biological systems from a set of standard parts—genes, proteins, and metabolic pathways—or redesigning existing biological systems. The tools of synthetic biology also can be used to study the interior of living cells at the molecular level, providing critical new information and insight into the machinery of life and the natural world. Synthetic biology holds promise for advances in many areas, including the development of renewable, carbon-neutral energy sources; nonpolluting biological routes for the production of chemicals; safer and more effective pharmaceuticals; and better environmental remediation technologies.

At JBEI, researchers are using synthetic biology to develop new platform hosts for producing enzymes and fuels and to create biomolecular parts and devices for constructing new fuel-generating organisms and improved plants. Among other advances, such goals will be achieved through the improved capabilities of fermentative organisms to tolerate processing conditions and inhibit unwanted by-products. Capabilities also will be engineered into fuel-producing organisms to convert five-carbon sugars into fuel and make use of lignin monomers. Following the strategy that biological systems can be revamped more effectively or built from scratch if standardized parts are employed, investigators will assemble a catalog of well-characterized biosynthetic components to help in designing, testing, optimizing, and implementing integrated large-scale biosynthetic units. These tools and principles, used by JBEI Chief Executive Officer Jay Keasling to develop a relatively inexpensive microbial-based alternative for producing the antimalarial drug artemisinin, will aid in developing the next generation of biofuels.

DOE BioEnergy Science Center

The DOE BioEnergy Science Center (BESC), led by Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee, focuses on the fundamental understanding and elimination of biomass recalcitrance—the resistance of cellulosic biomass to enzymatic breakdown into sugars. BESC approaches the problem of biomass recalcitrance from two directions by closely linking (1) plant research to make cell walls easier to deconstruct and (2) microbial research to develop multitalented biocatalysts tailor made to produce biofuels from this modified plant material in a single step.

Scientists at national laboratories, universities, and private companies that make up the BESC team have extensive experience with studying biomass recalcitrance, and they have made fundamental advances in a wide range of related sciences. The new Joint Institute for Biological Sciences systems biology research facility at ORNL serves as the central hub for coordinating research among all BESC partners (see box, BESC Partners, p. 20).

BESC's research is organized into three focus areas: (1) Biomass Formation and Modification, (2) Biomass Deconstruction and Conversion, and (3) Characterization and Modeling. Some recent highlights of BESC research are featured on p. 21. By understanding the myriad factors that collectively determine biomass recalcitrance, BESC researchers are providing foundational knowledge that will streamline processing and reduce costs for many different approaches to plant feedstock and cellulosic biofuel production.

Research Strategy

1. Biomass Formation and Modification

BESC biomass formation and modification research involves understanding the genetics and biochemistry of plant cell-wall biosynthesis and working directly with two potential bioenergy crops—switchgrass and poplar—to develop varieties that are easier to break down into fermentable sugars. Currently, little is known about how cellulose and hemicelluloses are synthesized; distributed within cell walls; and attached to each other, to lignin, or to cell-wall proteins. Computational models will help BESC researchers identify target genes and successful strategies for modifying biosynthetic pathways to generate biomass that can be readily deconstructed into sugars for biofuel

BESC BioEnergy Science Center

"For the first time, this assembles an integrated multidisciplinary team to give us the best chance to understand and overcome recalcitrance, the current bottleneck to economical, efficient bioethanol. By tapping nature, DOE BESC intends to replace the current expensive processes with a microbe that can combine multiple steps into one and can act on a plant cell wall that is designed to be deconstructed. This is an exciting moment where a dedicated team can work together to make an immediate impact." – Martin Keller

Martin Keller
BESC Director

Martin Keller, BESC director, also leads the Oak Ridge National Laboratory Biosciences Division. Before joining ORNL, he directed technology development programs for Diversa Corporation (now Verenium), a global leader in enzyme technology, and pioneered technologies enabling single-cell microbiology.

BESC Headquarters. Oak Ridge National Laboratory's new Joint Institute for Biological Sciences facility in Oak Ridge, Tennessee, houses BESC administration and ORNL-based research staff. [Photo courtesy of Oak Ridge National Laboratory] production. Target genes are turned on or off in thousands of poplar and switchgrass samples generated and studied by BESC, and then these samples are characterized to assess how these modifications affect plant cell walls. By understanding the synthesis and assembly of the polysaccharides and lignin in plant biomass, BESC researchers will develop methods for reducing cell-wall recalcitrance that can be applied to a wide range of woody and herbaceous plants.

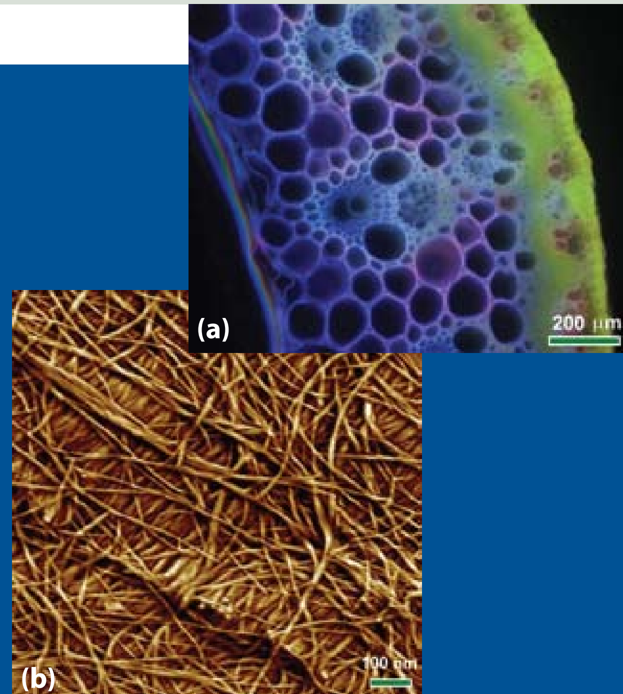
2. Biomass Deconstruction and Conversion

Two key hypotheses drive biomass deconstruction and conversion research at BESC: (1) microorganisms can be engineered to enable consolidated bioprocessing (CBP)—a game-changing, one-step, microbe-mediated strategy for directly converting plant biomass into ethanol and (2) enzymes and microbial biocatalysts can be understood and engineered to synergize with recalcitrance-reducing plant modifications to achieve better biomass deconstruction.

A model organism for CBP development is *Clostridium thermocellum*, a bacterium that rapidly degrades pure cellulose and then ferments the resulting sugars into ethanol. This microbe's strategy for combined biomass deconstruction and conversion employs cellulosomes—multifunctional enzyme complexes that specialize in degrading cellulose. BESC is studying the structures and activities of these poorly understood multienzyme complexes to design new variants that are more efficient at deconstructing cell walls. BESC researchers also are investigating microbes that tolerate near-boiling temperatures, such as species of *Anaerocellum* isolated from hot springs at Yellowstone National Park. These microbes and their enzymes could provide new biomass-degrading capabilities resistant to the heat and stresses of industrial processing.

3. Characterization and Modeling

Advancing BESC goals to develop improved plant materials and CBP methods that facilitate cost-effective conversion of biomass to fermentable sugars will require detailed knowledge of (1) the chemical and physical properties of biomass that influence recalcitrance, (2) how these properties can be altered by engineering plant biosynthetic pathways, and (3) how biomass properties change during pretreatment and how such changes affect biomass-biocatalyst interactions during deconstruction by enzymes and microorganisms.



BESC Imaging of Plant Cell Walls at Multiple Scales. BESC researchers are integrating technologies for imaging plant tissues and cell walls from millimeter to nanometer scales. **(a) Part of a cross-section of a switchgrass stem at micrometer scale.** Autofluorescent signals from compounds in the stem show less lignin and more sunlight-absorbing chlorophyll in the green outer layer on the right. Deeper blue areas toward the stem's center show more highly lignified xylem tissue used for water and nutrient transport. **(b) Atomic force micrograph showing nanometer-scale detail of the interwoven mesh of rope-like, lignocellulosic microfibril bundles in a switchgrass cell wall.** [Images courtesy of BESC researcher Shi-You Ding, National Renewable Energy Laboratory]

To examine chemical and structural changes that occur in the modified plant cell walls of switchgrass and poplar, BESC has developed a high-throughput screening pipeline that can perform compositional analysis, pretreatment, and enzyme digestibility studies on thousands of biomass samples. Selected samples from this screening pipeline assembled at the National Renewable Energy Laboratory are passed along to other partner institutions for a variety of detailed chemical, physical, and imaging analyses (see figure above, BESC Imaging of Plant Cell Walls at Multiple Scales). The resulting data are incorporated into computational models and simulations used to predict relationships between biomass structure and recalcitrance.

Modeling and simulation tools are part of a knowledgebase BESC is establishing to maintain and share data, materials, experimental processes, and scientific insights across the distributed BESC community. One component of this knowledgebase is a comprehensive set of tools for discovering biomass recalcitrance genes in plant genomes and building pathways for cell-wall synthesis. By extracting and combining results from isolated experiments, this knowledgebase serves as a biological discovery platform for integrating diverse experimental, theoretical, and computational approaches that will help define the genomic and physical basis of plant cell-wall recalcitrance.

BESC Partners

DOE's Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee (lead institution): DOE's largest science and energy laboratory, ORNL features research programs in poplar genomics, computational science, bioenergy, and plant and microbial systems biology. The ORNL Spallation Neutron Source and supercomputers at the ORNL National Leadership Computing Facility will be used to investigate and simulate the activity of enzyme complexes.

University of Georgia, Athens (UGA): UGA's Complex Carbohydrate Research Center maintains state-of-the-art capabilities in mass spectrometry, nuclear magnetic resonance spectroscopy, chemical and enzymatic synthesis, computer modeling, cell and molecular biology, and immunocytochemistry for studying the structures of complex carbohydrates and the genes and pathways controlling plant cell-wall biosynthesis.

DOE's National Renewable Energy Laboratory (NREL), Golden, Colorado: NREL has more than 30 years of experience in biomass and biofuel research and houses premiere facilities for analyzing biomass surfaces. NREL also has a long and successful history of establishing biofuel pilot plants and partnering with industry for commercial development of technologies.

University of Tennessee, Knoxville (UT): UT conducts successful programs in bioenergy-crop genetic and field research (particularly switchgrass) and biotechnological applications of environmental microbiology.

Dartmouth College, Hanover, New Hampshire: Dartmouth's Thayer School of Engineering is a leader in the fundamental engineering of microbial cellulose utilization and consolidated bioprocessing approaches.

Georgia Institute of Technology, Atlanta: Georgia Tech's Institute for Paper Science and Technology provides BESC with expertise in biomass processing and instrumentation for high-resolution analysis of plant cell walls.

ArborGen, Summerville, South Carolina: ArborGen provides expertise in forest genetics research, tree development, and commercialization.

Verenium Corporation, Cambridge, Massachusetts: Verenium is a biofuels-focused biotechnology company and developer of specialty enzymes found in diverse natural environments and optimized for targeted applications.

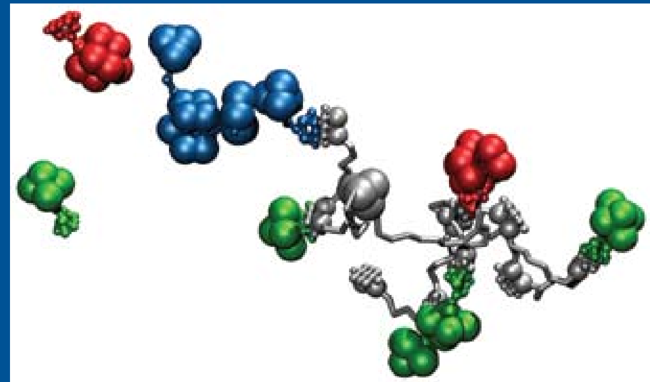
Mascoma Corporation, Boston, Massachusetts: Mascoma develops microbes and processes for economical conversion of cellulosic feedstocks into ethanol.

The Samuel Roberts Noble Foundation, Ardmore, Oklahoma: This nonprofit research foundation is devoted to improving agricultural production and advancing the development of switchgrass and other grasses through genomic research. The foundation's activities are conducted through programs in agriculture, plant biology, and forage improvement.

Ceres, Inc., Thousand Oaks, California: Ceres uses advanced plant breeding and biotechnology to develop and market low-carbon, nonfood crops for next-generation biofuels and biopower.

Individual Researchers. Specializing in biomass pretreatment, characterization of plant-associated microbes, cellulose and enzyme modeling, consolidated bioprocessing, and lignin biochemistry are researchers from the University of California, Riverside; DOE Brookhaven National Laboratory (Upton, New York); Cornell University (Ithaca, New York); Virginia Polytechnic Institute and State University (Blacksburg); University of Minnesota (St. Paul); North Carolina State University (Raleigh); and Washington State University (Pullman).

High-Throughput Biomass Characterization Pipeline. BESC researchers have developed a high-throughput pipeline to rapidly identify the chemical, structural, and genetic features that determine biomass recalcitrance. By integrating advanced, multi-sample technologies for compositional analysis, water-only or dilute-acid pretreatments, and assays for enzyme digestibility and sugar release, this pipeline screens over 100-fold more biomass samples per day than conventional methods. BESC researchers used this pipeline to investigate the diverse, natural range of digestibility for poplar trees sampled from various environments and growth conditions in the Pacific Northwest. Sugar release for the 1,100 trees sampled ranged from 0.2 to 0.7 grams of sugar per gram of biomass, and the most exciting data revealed that some trees release most of their available sugar using very mild (hot water) treatment conditions. This large-scale screening of potential feedstocks will accelerate the discovery and isolation of samples most easily converted to sugars, identify the factors that make them less recalcitrant, and provide new paths toward the development of improved biomass varieties.



BESC Cellulosome Modeling. Cellulosomes are large multiprotein complexes that can exceed hundreds of millions of atoms in size, making them too massive for atomistic modeling with current computational technologies. Coarser, mesoscale models that describe interactions among structural subunits of the cellulosome rather than individual atoms are used to investigate cellulosome activities. The image shows a mesoscale model of cellulosome assembly developed at BESC. The gray "scaffoldin" protein forms the structural backbone of this simple cellulosome. The red, green, and blue groups are different types of enzymatic subunits that bind to the scaffoldin and break the bonds of sugar chains in plant cell walls. [Image courtesy of BESC researchers Yannick Bomble, Michael Crowley, and Michael Himmel, National Renewable Energy Laboratory]

Advances in Microbe-Mediated Consolidated Bioprocessing (CBP). BESC researchers have completed a new study of the *Clostridium thermocellum* cellulosome under conditions representative of real-world environments using heterogeneous, natural lignocellulosic material (such as pretreated switchgrass). Other work with this potential CBP microbe has identified and experimentally verified 16 "new" cellulosome components and revealed how the relative composition and synergistic activity of different biomass-degrading enzymes in the cellulosome complex are altered by growth substrate. Other candidate CBP microbes that tolerate temperatures above 70˚C and rapidly degrade natural and pretreated biomass have been identified from Yellowstone samples and existing *Anaerocellum* strains.

New Insights from Computer Simulations Build a Foundation for Cellulosome Design. BESC scientists used computational simulations to understand cellulosome assembly (see image above) and the binding dynamics of two key cellulosome proteins, cohesions and dockerins. These simulations showed how a single substitution of one key amino acid triggers major structural changes that may enable the design of more effective cellulosomes.

Development of a Novel *In Vitro* Assay for Xylan Biosynthesis. Xylan is the most abundant hemicellulose present in poplar and switchgrass plant biomass. This key polymer in cell-wall architecture has been proposed to bind cellulose microfibrils and lignin; new technologies to modify xylan structures have the potential to decrease biomass recalcitrance. BESC researchers established *in vitro* biosynthesis assays that were used to detect several novel enzymatic activities involved in xylan synthesis in poplar and switchgrass.

New Cellulose-Degrading Enzymes Discovered by Screening Environmental DNA. BESC researchers have isolated, sequenced, and analyzed DNA from microbial communities actively decaying poplar biomass. By comparing these environmental sequences with known gene sequences, about 4,000 new carbohydrate-hydrolyzing enzymes were identified, and 5 of the most promising cellulose- and hemicellulose-degrading enzyme candidates were selected for more detailed investigations. In another approach, millions of genes isolated from the guts of plant-eating mammals and insects, wood-boring shipworms, and other environments have been expressed and screened for carbohydrate-hydrolyzing activities. Preliminary results have identified 29 unique, active enzymes, and additional activity screening is under way.

Translation of BESC Science into Commercial Applications

Translating BESC research results into the testing of applications and potential commercial deployment is an important step toward reaching DOE's bioenergy objectives. BESC has formed a "commercialization council" of technology-transfer and intellectual property (IP) management professionals from partner institutions to evaluate the commercial potential of new inventions arising from BESC research and to promote and facilitate the licensing of BESC IP. BESC inventions are posted on the center's website (bioenergycenter.org/licensing/). Some early inventions address techniques for plant and microbial genetic transformation, special microscopy methods, and innovations in biomass sample handling. To build external relationships that can promote commercialization of new technologies, BESC provides opportunities for companies to become BESC Industry Affiliates.

Education and Outreach

To prepare the next generation of bioenergy scientists, BESC provides interdisciplinary research opportunities to graduate students, postdocs, and visiting scientists. In addition to these activities in higher education, BESC is teaming with the Creative Discovery Museum in Chattanooga, Tennessee, to raise awareness of cellulosic biofuels, carbon emissions from energy use, and obstacles to a successful biofuel economy. Targeting fifth-graders, BESC education and outreach efforts make information accessible to the general public and reach students when they still are excited about science. Lessons piloted at schools in Georgia and Tennessee will be made available to schools nationwide. BESC also has begun to pilot "Science Night" programs that build on these classroom lessons and are offered to students and the general public through local schools, museums, and community centers. The BESC website features announcements about BESC outreach and educational programs, seminars and presentations describing BESC research, and other resources.

The Genomic Science Program (formerly Genomics:GTL and Genomes to Life) is run by the DOE Office of Biological and Environmental Research (BER). This program aims to develop a predictive understanding of biological systems relevant to energy production and other DOE missions in environmental remediation and climate change mitigation (see illustration, Systems Biology for DOE Missions, this page).

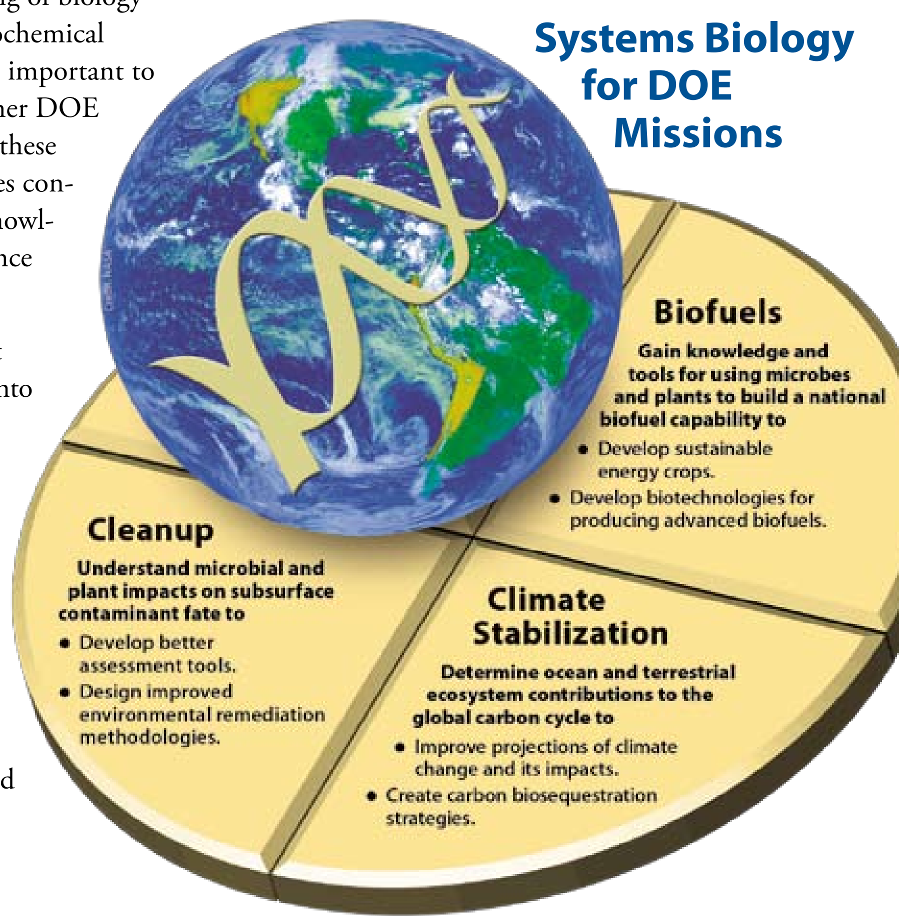
Genomic Science Program research is conducted at national laboratories and universities and includes single-investigator projects, multi-institutional collaborations, and fundamental research centers.

The DNA sequence of an organism's complete genome is the starting point to understanding any biological system. Scientists from the three DOE Bioenergy Research Centers and other projects of the Genomic Science Program are working with the DOE Joint Genome Institute to sequence the genomes of energy-related plants, as well as microbes and fungi that degrade biomass or impact plant productivity. Building on this foundation of genomic information, the whole-systems understanding of biology will enable scientists to redesign proteins, biochemical pathways, and even entire plants or microbes important to solving bioenergy challenges and meeting other DOE needs. Even though the specific functions of these systems vary, common fundamental principles control the behavior of all biological systems. Knowledge of these underlying principles will advance biological solutions to DOE missions.

Several developments have converged in recent years to suggest that systems biology research into microbes and plants may be able to overcome critical roadblocks to large-scale production of fuels from plant biomass. The ability to rapidly sequence the DNA of any organism is a critical but modest part of these new capabilities. Others include:

- Development of high-throughput techniques and commercially available reagents for protein production and characterization to test thousands of natural and engineered protein variations.
- Emergence of a range of new instrumentation for observing proteins and other cell constituents to determine, for example, how cell walls are constructed.
- Availability of technologies for high-resolution imaging—spanning spatial scales from molecular to cellular to microbial community—that can be used to help understand, for example, why rates of cellulose degradation vary.
- Major advances in the integraton of computational modeling, algorithm and software development, and model-driven laboratory experimentation to enable solutions in bioenergy and systems biology.

In addition to these activities in the Genomic Science Program, BER is supporting related genomic bioenergy collaborative research with other governmental agencies. A research program jointly sponsored by BER and the U.S. Department of Agriculture (USDA) Cooperative State Research, Education, and Extension Service supports genome-based approaches to accelerate plant-breeding programs and improve potential bioenergy crops.



DOE Biological and Environmental Research

The BER programs within the DOE Office of Science advance world-class research to understand complex biological and environmental systems and provide scientific user facilities to support DOE missions in scientific discovery and innovation, energy security, and environmental responsibility. BER's interdisciplinary research programs engage scientists from national laboratories, universities, and the private sector in exploring the frontiers of genome-enabled biology; discovering the physical, chemical, and biological drivers of climate change; and seeking the geochemical, hydrological, and biological determinants of environmental sustainability. BER programs are managed within two divisions: the Biological Systems Science Division and the Climate and Environmental Sciences Division.

BER's Biological Systems Science Division (BSSD) seeks to achieve a predictive understanding of biological systems with potential use in bioenergy, carbon cycling and biosequestration, and biogeochemistry. BSSD research activities include using genomics and systems biology to understand plant and microbial systems, supporting DOE Bioenergy Research Centers to provide transformational breakthroughs in cellulosic biofuels, and developing real-time, high-resolution technologies (assisted by integration with computational modeling) for analyzing dynamic biological processes.

The Climate and Environmental Sciences Division (CESD) aims to achieve a predictive understanding of climate change, ecosystem response to climate change, and contaminant fate and transport in the subsurface. Projects supported by CESD programs are resolving the greatest uncertainties in climate change, improving the world's most powerful climate models, providing the science to inform environmental remediation strategies, and working to understand carbon cycling in terrestrial vegetation and soils.

DOE Office of Science

The Office of Science manages fundamental research programs in basic energy sciences, high energy physics, fusion, biological and environmental sciences, and computational science. In addition to being the federal government's largest single funder of material and chemical sciences, the Office of Science supports unique and vital parts of U.S. research in climate change, geophysics, genomics, life sciences, and science education. About a third of Office of Science research funding supports projects at more than 300 colleges and universities nationwide.

The Office of Science also manages 10 world-class national laboratories with unmatched capabilities for solving complex interdisciplinary scientific problems and oversees the construction and operation of some of the nation's most advanced scientific user facilities, located at national laboratories and universities. These include particle and nuclear physics accelerators, synchrotron light sources, neutron scattering facilities, and supercomputers and high-speed computer networks. In the 2008 fiscal year, these facilities were used by more than 22,000 researchers from universities, national laboratories, private industry, and other federal science agencies.

For More Information

DOE Bioenergy Research Centers
genomicsgtl.energy.gov/centers/

Great Lakes Bioenergy Research Center (GLBRC)
glbrc.org

Joint BioEnergy Institute (JBEI)
jbei.org

BioEnergy Science Center (BESC)
bioenergycenter.org

DOE Genomic Science Program
genomicsgtl.energy.gov

DOE Mission Focus: Biofuels
genomicsgtl.energy.gov/biofuels/

DOE–USDA Plant Feedstock Genomics for Bioenergy
genomicsgtl.energy.gov/research/DOEUSDA/

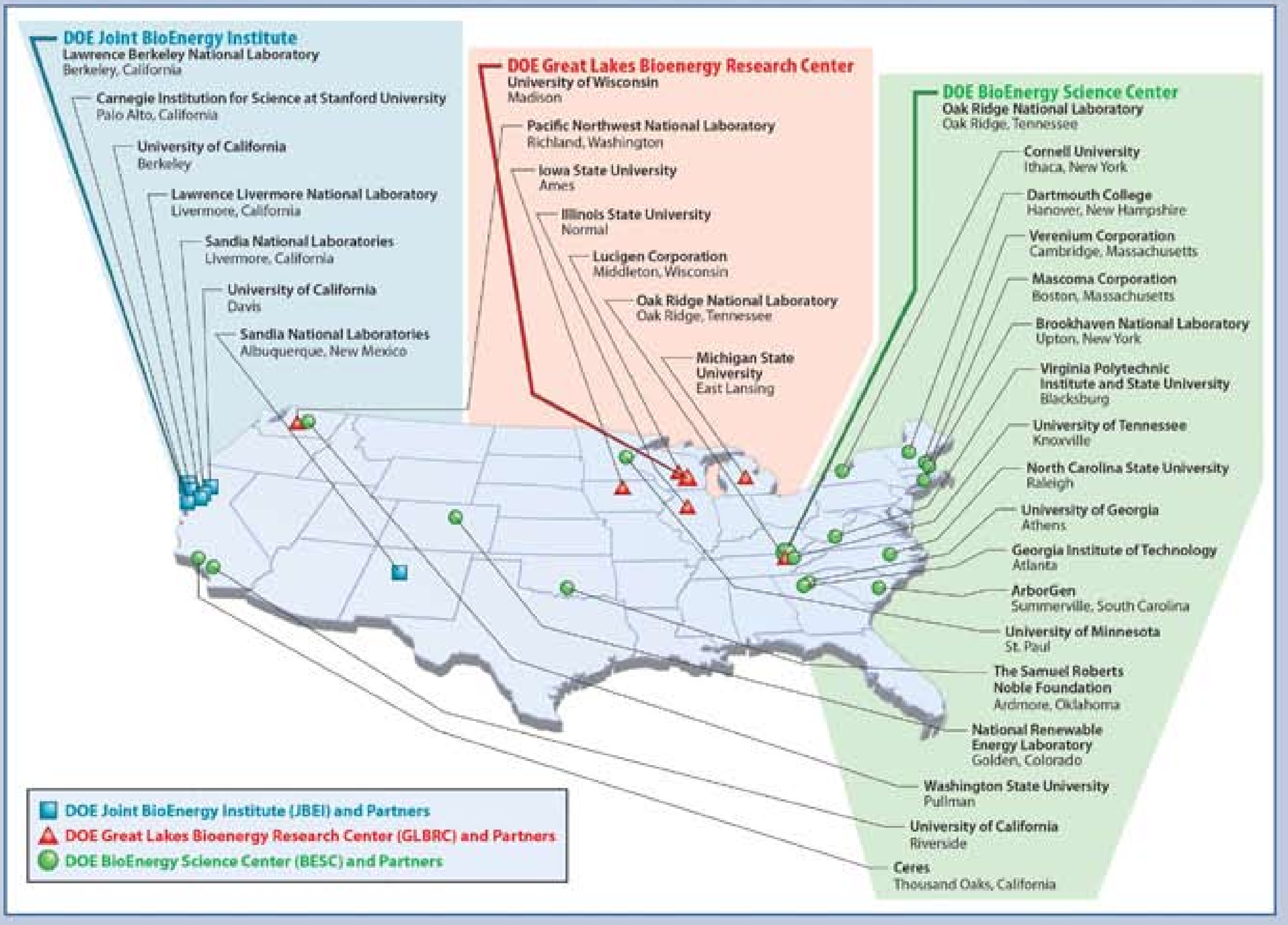
DOE Joint Genome Institute
jgi.doe.gov

Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda
genomicsgtl.energy.gov/biofuels/b2bworkshop.shtml

DOE Office of Biological and Environmental Research
science.doe.gov/ober/

DOE Office of Science
science.doe.gov

TRANSFORMATIONAL BIOLOGY FOR ENERGY BREAKTHROUGHS



DOE Bioenergy Research Centers and Partners
genomicsgtl.energy.gov/centers/

DOE Great Lakes Bioenergy Research Center (GLBRC)
glbrc.org

DOE Joint BioEnergy Institute (JBEI)
jbei.org

DOE BioEnergy Science Center (BESC)
bioenergycenter.org

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