DOE/SC-0127

U.S. DEPARTMENT OF ENERGY

Office of Science

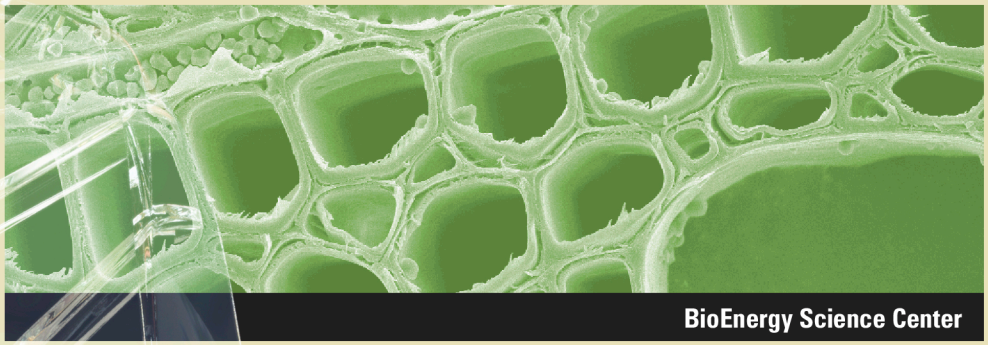
Office of Biological and Environmental Research

U.S. DEPARTMENT OF ENERGY'S

# Bioenergy Research Centers

## *An Overview of the Science*

Joint BioEnergy Institute



BioEnergy Science Center

Great Lakes Bioenergy Research Center

July 2010

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## Websites for DOE Bioenergy Research Centers

**DOE Joint BioEnergy Institute**
jbei.org

**DOE BioEnergy Science Center**
bioenergycenter.org

**DOE Great Lakes Bioenergy Research Center**
glbrc.org

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**Prepared for the**
**U.S. Department of Energy Office of Science**
**Office of Biological and Environmental Research**

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# For More Information

**DOE Bioenergy Research Centers**
genomicscience.energy.gov/centers/

**Joint BioEnergy Institute (JBEI)**
jbei.org

**BioEnergy Science Center (BESC)**
bioenergycenter.org

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

**DOE Genomic Science Program**
genomicscience.energy.gov

**DOE Mission Focus: Biofuels**
genomicscience.energy.gov/biofuels/

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

**DOE Joint Genome Institute**
jgi.doe.gov

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

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# 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."

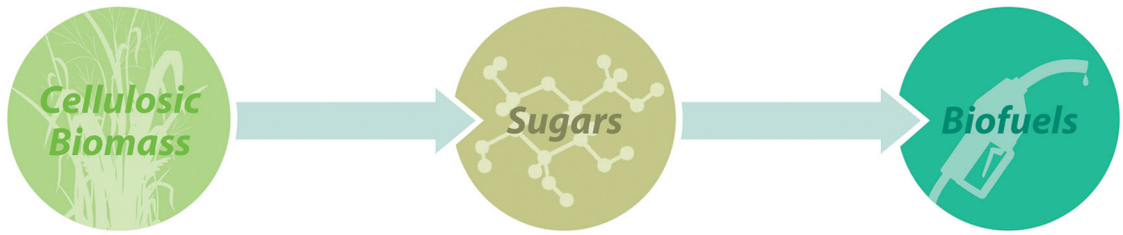
Although cellulosic ethanol production has been demonstrated on a pilot level, developing a cost-effective, commercial-scale cellulosic biofuel industry will require transformational science to significantly streamline current production processes. Woodchips, grasses, cornstalks, and other cellulosic 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 figure below, From Biomass to Biofuels).

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 30 years. The DOE Genomic Science program is advancing a new generation of research focused on achieving whole-systems understanding of 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. For more information on the Genomic Science program, see p. 26.

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 are providing the knowledge needed to develop new biobased products, methods, and tools that the emerging biofuel industry can use (see sidebar, Bridging the Gap from Fundamental Biology to Industrial Innovation for Bioenergy, p. 6). The DOE BRCs have developed automated, high-throughput analysis pipelines that will accelerate scientific discovery for biology-based biofuel research.

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 Lawrence Berkeley National Laboratory leads the DOE Joint BioEnergy Institute (JBEI) in California; DOE's Oak Ridge National Laboratory leads the BioEnergy Science Center (BESC) in Tennessee; and the University of Wisconsin–Madison leads the Great Lakes Bioenergy Research Center (GLBRC). Each center represents a multidisciplinary partnership with expertise

## From Biomass to Biofuels




**Feedstock Development**
*Develop crops with cell walls optimized for deconstruction and biofuel production.*

**Biomass Deconstruction**
*Improve enzymes and microbes that break down biomass into sugars.*

**Fuel Synthesis**
*Engineer metabolic pathways in microbes to produce diverse biofuels.*

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.

## Biofuels: Research Strategies

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

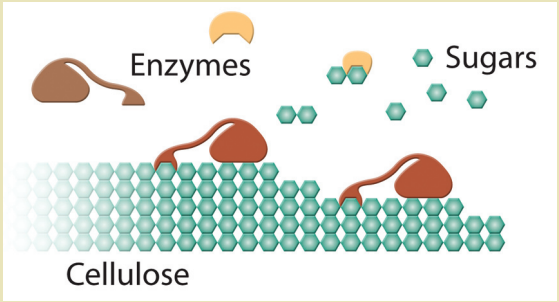
The extremely complex science needed to solve these challenges requires multiple interdisciplinary teams that approach the same problems from different directions to accelerate scientific progress (see table, DOE Bioenergy Research Center Strategies at a Glance, p. 4). The following sections explain some scientific issues related to these challenges.

## Tapping Nature's Strategies for Biomass Degradation

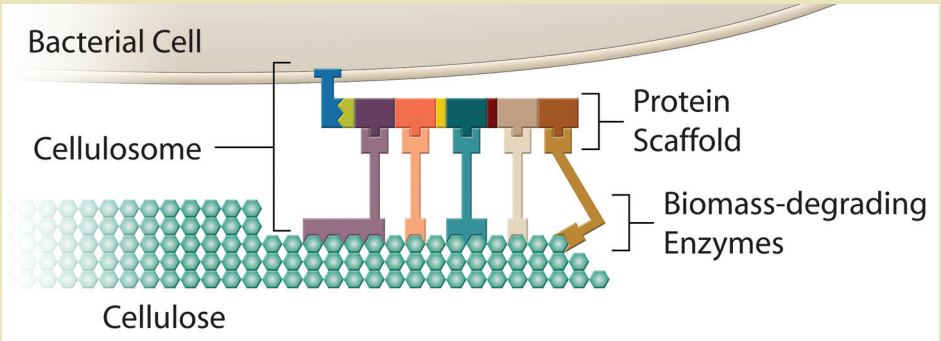
Nature has devised two types of enzyme-based strategies for breaking down plant cell walls. One strategy observed in oxygen-rich environments involves fungi and bacteria that secrete individual enzymes with complementary functions for degrading cell walls. These free enzymes work as a team to deconstruct 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



# DOE Bioenergy Research Centers

## 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. Cellulosic biomass primarily consists of cellulose and other complex cell-wall compounds, such as lignin, that strengthen and support plant structure. The main 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. Many of these genes are now being characterized by the BRCs, and our knowledge of associated biological functions continues to expand as a result of this research.

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 "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 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. 2). 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 compost piles.

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.

## 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.

### Research Strategies: Development of Next-Generation Bioenergy Crops

**Center Strategies**

- **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 manipulating the composition and cross-linking of the plant cell-wall polymers in poplar and switchgrass; simultaneously increase total biomass produced per acre.
- **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.

### Research Strategies: Discovery and Design of Enzymes and Microbes with Novel Biomass-Degrading Capabilities

**Center Strategies**

- **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).
- **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.

### Research Strategies: Development of Transformational Microbe-Mediated Strategies for Biofuel Production

**Center Strategies**

- **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- or butanol-producing capabilities to substantially reduce costs; develop a knowledgebase and pathway analysis tools to aid these manipulations; test degrading microbes on modified plants.
- **GLBRC** – Start with an ethanol-producing microbe and add lignocellulose-degrading capabilities to substantially reduce costs.

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

## 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 6-carbon sugar (glucose) that is readily converted into ethanol and other products, microbial fermentation of the 5- and 6-carbon sugar mix from hemicelluloses is less efficient and thus is a key area for improvement.

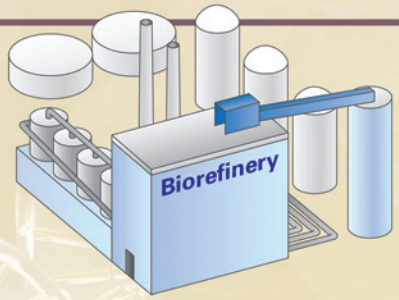
En route to the fermentation tank, biomass currently is subjected to physical, chemical, and enzymatic processing steps that can create by-products and conditions that might inhibit microbial conversion of sugars into biofuels. Ethanol and other biofuel products also inhibit microbial fermentation at 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 5- and 6-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.

# Bridging the Gap

## *from Fundamental Biology to Industrial Innovation for Bioenergy*




Although the predominant focus of the DOE Bioenergy Research Centers (BRCs) is the basic research underlying feedstock development and conversion improvements, each center has close ties to an industrial community. These ties mutually benefit the BRCs and industry. For example, companies with products such as biomass crops, enzymes, and microbial production take advantage of BRC research results. In turn, the BRCs use industrial connections to identify and refine the research goals that will be most useful for developing a vibrant biofuels sector.

By working with industrial partners and targeting key biological challenges that directly impact the complexity, cost, and energy intensity of the cellulosic biorefinery, the BRCs continue to achieve critical advances that streamline processing and improve the overall economic performance of cellulosic biofuels.

The following three examples illustrate bridges from the basic BRC research agenda to more applied uses.

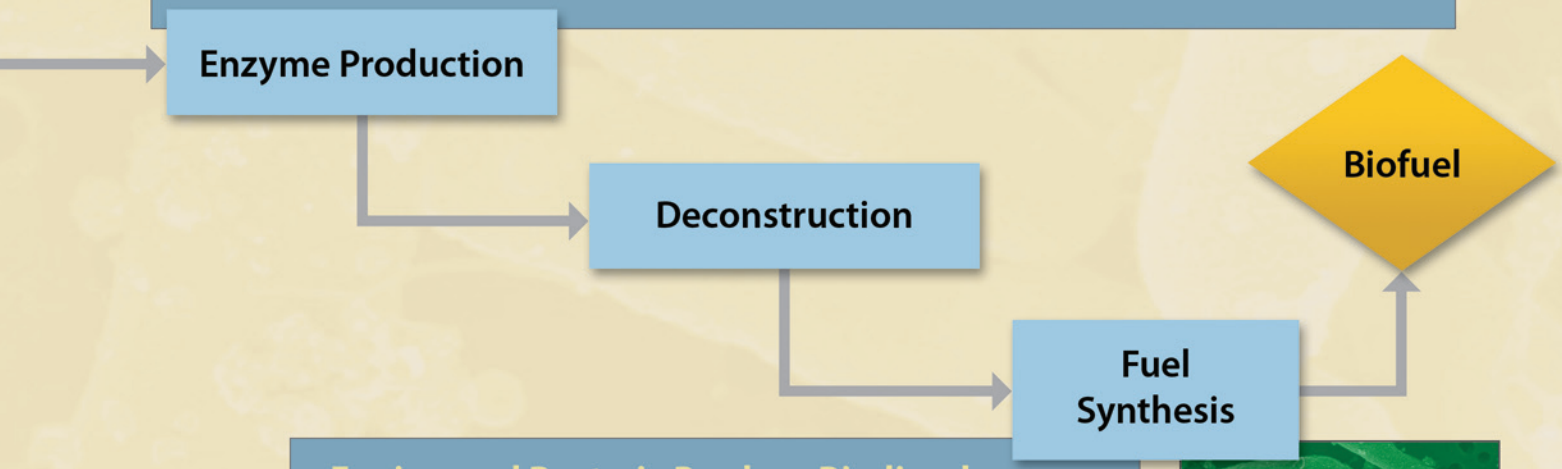
Cellulosic Biomass → Pretreatment → Detoxification and Nutrient Supplementation

### Pretreatment Strategy Eliminates Detoxification and Nutrient Supplementation Costs for Ethanol Production from Corn Stover

Post-pretreatment detoxification and commercial nutrient supplementation are estimated to account for 25% of the total processing cost of corn stover conversion to ethanol. Unlike acid pretreatment of corn stover at high temperature, which results in nutrient loss by requiring washing and detoxification, ammonia fiber expansion (AFEX) pretreatment produces fewer chemical by-products that inhibit fermentation and leaves sufficient nutrients in pretreated stover to support the robust growth of ethanol-producing yeast. Using AFEX-treated corn stover and a recombinant strain of the industrial yeast *Saccharomyces cerevisiae* that can coferment glucose and xylose, Great Lakes Bioenergy Research Center scientists have shown that detoxification and nutrient supplementation steps can be eliminated and have achieved a final ethanol concentration of 40 g/L—a benchmark for commercial cellulosic ethanol production. By reducing the costs associated with raw materials, water requirements, and capital equipment, this platform has the potential to significantly improve the economics of cellulosic ethanol. This research was reported in Lau, M. W., and B. E. Dale. 2009. "Cellulosic Ethanol Production from AFEX-Treated Corn Stover using *Saccharomyces cerevisiae* 424A(LNH-ST)," *Proceedings of the National Academy of Sciences* **106**(5), 1368–73.

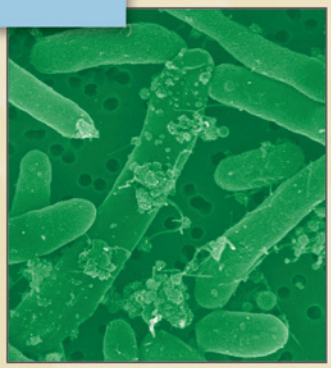
## New Yeast Reduces the Requirement for Commercial Cellulase Addition

The Boston-based biotech company Mascoma, which is an institutional partner at the BioEnergy Science Center, has achieved more than 3,000-fold improvement in the expression levels of cellulase enzymes in a yeast strain that can ferment 5- and 6-carbon sugars into ethanol. This development provides proof of concept for achieving consolidated bioprocessing—the engineering of a microorganism that can both degrade cellulosic biomass and produce a biofuel (ethanol) in a single step. Process testing at Mascoma revealed a significant 2.5-fold reduction in the amount of cellulase that must be added to convert pretreated hardwood into ethanol. The addition of cellulase was completely eliminated for the conversion of waste paper sludge into ethanol. By reducing the requirement for commercial cellulase addition, this new yeast has great potential for reducing and possibly eliminating the costs of enzyme production, one of the most expensive steps in the biomass-to-biofuel conversion process. These results were first presented by Mascoma Chief Technology Officer Mike Ladisch at the 31st Symposium on Biotechnology for Fuels and Chemicals in San Francisco in May 2009.




## Engineered Bacteria Produce Biodiesel, Fatty Alcohols, and Waxes from Sugars in Plant Biomass

Deploying the tools of synthetic biology, Joint BioEnergy Institute researchers collaborating with the San Francisco–based biotech firm LS9 have redirected the fatty acid metabolism of a strain of *Escherichia coli* bacteria to produce biodiesel and other important chemicals from plant biomass sugars. The *E. coli* strain was genetically manipulated to provide a control mechanism to tailor the structure and composition, and hence the performance, of the fatty acid–derived fuels or chemicals that it generates. These biodiesel-producing bacteria also have been engineered to produce hemicellulases—enzymes that hydrolyze hemicelluloses (complex carbohydrates that are major constituents of plant cell walls). Future engineering efforts will add cellulose-degrading enzymes, enabling the production of biodiesel and other fatty acid–derived chemicals directly from cellulose and hemicelluloses without the addition of commercial enzymes. Although this bacteria-mediated biodiesel production system has been successfully demonstrated, improvements in titer, productivity, and yield still are needed to make the transition to commercial applications. This research was reported in Steen, E. J., et al. 2010. "Microbial Production of Fatty Acid–Derived Fuels and Chemicals from Plant Biomass," *Nature* **463**, 559–63.



**Engineered Bacteria Produce Biodiesel.** Electron micrograph shows rod-shaped *Escherichia coli* secreting oil droplets containing biodiesel fuel, along with fatty acids and alcohol. [Image by Jonathan Remis, Lawrence Berkeley National Laboratory]

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. 11). 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 pp. 12–13.

## 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 (see figure, Bioenergy Crop Research at JBEI, p. 9).

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 and polymers, while improving the economics of converting cellulosic biomass into fuels.

jbei
Joint BioEnergy Institute

**Jay Keasling**
JBEI Chief Executive Officer

"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 is the JBEI Chief Executive Officer 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, Lawrence Berkeley National Laboratory]

## 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. 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. 10). Biologically produced alkanes and other oil-like hydrocarbons could replace gasoline in today's cars on a gallon-for-gallon basis.

## 4. Creating Technologies that Advance Biofuel Research

JBEI scientists are creating new, broadly applicable technologies to advance research that will speed biofuel development (see figure, JBEI Technology Development to Advance Biofuel Research, p. 10). 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 generating large volumes of data that are collected and catalogued in a centralized database and then analyzed using new bioinformatic tools.

**Bioenergy Crop Research at JBEI.** JBEI's director of Grass Genetics, Pam Ronald, in the *Miscanthus* plot at the University of California, Davis. [Photo courtesy of Dan Putnam, UC, Davis]

# 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 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/.

**JBEI Technology Development to Advance Biofuel Research.** JBEI's Chris Petzold and Alyssa Redding develop and apply mass spectrometry approaches to sort through the complex protein mixtures in biological cells and detect multiple target proteins in the same sample. [Photo by Dino Vournas, Sandia National Laboratories]

## Education and Outreach

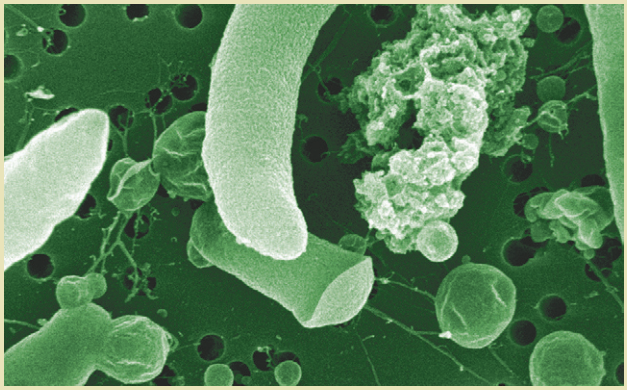
Educational efforts at JBEI build on strong undergraduate, graduate, and postdoctoral training programs, plus nationally recognized K–12 and community college science outreach programs already in place at JBEI's member institutions. In addition to starting a new student fellowship program, JBEI is collaborating 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 5-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 are assembling 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.



[Image courtesy of Manfred Auer, Lawrence Berkeley National Laboratory]

## 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 and analysis, 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.

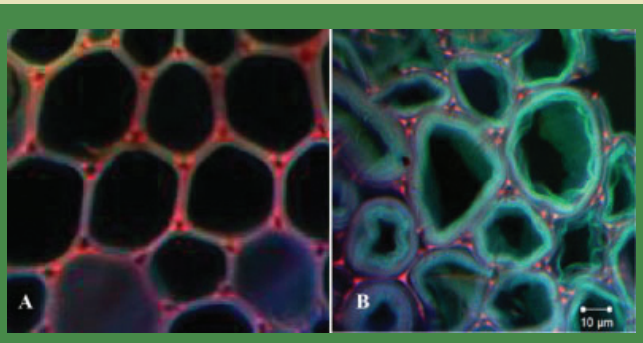
**University of California, Davis:** UC Davis oversees the California Biomass Collaborative and is home to the Northern California Nanotechnology Center, the Western Regional Center of the National Institute for Global Environmental Change, and the UC Davis Genome Center. The university provides expertise 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 one of the world's fastest supercomputers. 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.

## New Approach to Visualize Biomass Solubilization During Ionic Liquid Pretreatment

JBEI researchers have developed a technique, based on the natural autofluorescence of plant cell walls, that enables the dynamic imaging of biomass solubilization during ionic liquid pretreatment. Using this technique, researchers can accurately and quickly assess the ionic liquid's performance without the need for labor-intensive and time-consuming chemical and immunological labeling. Working with switchgrass and using the ionic liquid known as 1-n-ethyl-3-methylimidazolium acetate (EmimAc), the researchers observed a rapid swelling of secondary plant cell walls (see figure) within 10 minutes of exposure at relatively mild pretreatment temperatures (120°C). This reaction indicates a disruption of hydrogen bonding within cellulose and between cellulose and lignin. The swelling was followed by complete dissolution of biomass over 3 hours. By adding water to the solubilized biomass mixture, cellulose can be precipitated out and separated from the lignin, which remains in solution. This recovered cellulose was efficiently hydrolyzed into its sugar components by a commercial cellulase cocktail over a relatively short time interval. Currently, those ionic liquids that are most effective at dissolving plant cell-wall polymers are prohibitively expensive for use on a mass scale. Understanding how ionic liquids are able to dissolve lignocellulosic biomass could pave the way for finding new and better varieties for use in biofuel production. This research was reported in Singh, S., B. A. Simmons, and K. P. Vogel. 2009. "Visualization of Biomass Solubilization and Cellulose Regeneration During Ionic Liquid Pretreatment of Switchgrass," *Biotechnology and Bioengineering* **104**(1), 68–75.



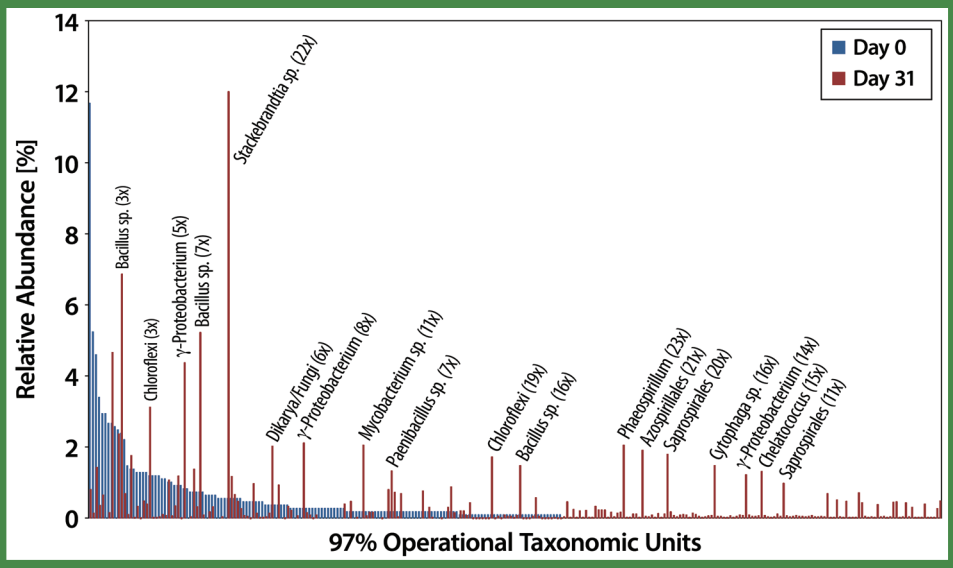

**Dissolving Cell-Wall Compounds with Ionic Liquids.** These confocal fluorescence images show switchgrass cell walls (A) before pretreatment with the EmimAc ionic liquid and (B) 10 minutes after treatment, in which the cell walls have swollen in size, a prelude to complete solubilization of cellulose, hemicellulose, and lignin. [Image courtesy of Seema Singh, Sandia National Laboratories]

## Unique Database Provides Functional and Phylogenomic Information for Rice Glycosyltransferases

JBEI researchers have made major advances in comprehensively identifying all rice glycosyltransferases (GT), an important class of enzymes involved in synthesizing polysaccharide sugars in plant cell walls. Because rice and other grasses such as switchgrass and *Miscanthus* share similar cell-wall characteristics, whole genome–scale analysis of rice has enabled the discovery of several candidate genes for more in-depth functional analysis that can help researchers understand and manipulate grass cell walls for biofuel production. This research has led to the development of JBEI's Rice GT Database, a publicly available resource for integrating and displaying diverse sets of functional genomic information for GTs (ricephylogenomics.ucdavis.edu/cellwalls/gt/). The database contains information on 793 putative gene models for rice GTs, and the loci for these genes are distributed across all 12 rice chromosomes. In addition to defining phylogenetic relationships among groups of rice GT genes based on sequence similarity, JBEI researchers also compared the number of different GT gene models identified for rice, *Arabidopsis*, and poplar (*Populus trichocarpa*). From the hundreds of possible GT genes that have been identified, scientists revealed 33 rice-diverged GTs that are highly expressed in vegetative, aboveground tissues and that serve as prime targets for mutagenesis studies and enzyme activity screens. This database was reported in Cao, P. J., et al. 2008. "Construction of a Rice Glycosyltransferase Phylogenomic Database and Identification of Rice-Diverged Glycosyltransferases," *Molecular Plant* **1**(5), 858–77.

## Compost Microbes Adapted to Produce Switchgrass-Degrading Enzymes

By incubating switchgrass with a mix of microbes isolated from compost, JBEI researchers provided the selective pressure needed to grow a new microbial community enriched with enzymes that degrade cell-wall polymers specific to switchgrass. The sample was incubated in a bioreactor for 31 days under typical composting conditions. Metagenomic sequencing of the switchgrass-adapted compost (SAC) community on day 31 was carried out to investigate the sample's diverse pool of glycoside hydrolases—enzymes that break bonds between carbohydrate molecules. The sample contained a high proportion of genes encoding enzymes that attack the branches and backbone of a major hemicellulose in grass cell walls. Analysis of the small-subunit ribosomal RNA (rRNA) isolated from the microbial community revealed dramatic changes in the




**Growth on Switchgrass Changes Microbial Community Composition.** The populations of microbes present after 31 days of growth on switchgrass (indicated in red) are considerably different from those populations in the compost community (indicated in blue). This suggests a selection and enrichment of specific populations to degrade switchgrass. [Image from Allgaier et al. 2010]

community profile with more than a 20-fold increase for some bacterial populations in the SAC (see figure above). Although metagenomic DNA sequence is highly fragmented, making isolation of full genes from complex communities difficult, two full-length genes for cellulose-degrading enzymes were discovered, synthesized, expressed in *Escherichia coli*, and tested for enzyme activity. This research was reported in Allgaier, M., et al. 2010. "Targeted Discovery of Glycoside Hydrolases from a Switchgrass-Adapted Compost Community," *PLoS One* **5**(1), e8812.

## Mass Spectrometry–Based Protein Detection Technique Speeds Optimization of Biofuel Protein Levels in Metabolically Engineered Microbes

JBEI researchers have developed a mass spectrometry–based protein detection technique called multiple-reaction monitoring (MRM) for identifying microbial proteins that can convert cellulosic sugars into biofuels. With the MRM technique, researchers can detect multiple target proteins in the complex protein mixtures of native cells and rapidly change the specific proteins to be targeted, something not possible with conventional protein detection technology. When coupled to liquid chromatography, MRM analysis offers high selectivity and sensitivity. It eliminates background signal and noise even in the most complex protein mixtures by utilizing two targeted points—a peptide mass and a specific fragment mass generated by mass spectrometry. Since the entire mass range is not scanned and only combinations of peptide and fragment masses are monitored, MRM can be used to detect and quantify up to 10 different proteins in a single liquid chromatography separation. The MRM technique is a valuable tool for analyzing enzyme complexes in a variety of JBEI projects such as the synthetic protein scaffold work reported in Dueber, J. E., et al. 2009. "Synthetic Protein Scaffolds Provide Modular Control over Metabolic Flux," *Nature Biotechnology* **27**(8), 753–59.

## Key Genes for Biosynthesis of Hydrocarbon Biofuels Identified in Bacterium *Micrococcus luteus*

JBEI researchers have elucidated the genes and a proposed biochemical pathway for the production of long-chain alkenes—key chemical components of petroleum-based gasoline and diesel fuels—in the bacterium *Micrococcus luteus*. Building on insights from microbial alkene research reported 4 decades ago, JBEI researchers hypothesized that a key mechanism for long-chain alkene biosynthesis would involve decarboxylation and condensation of fatty acids. By searching the genome of the alkene-producing bacterium *M. luteus*, researchers found three candidate genes with conserved sequences associated with condensing enzymes. Expression of these genes in *E. coli* resulted in long-chain alkene production, but additional research will be needed to reveal the specific biochemical role that each of the enzymes encoded by these genes plays in alkene synthesis. A wide range of bacteria has been found to contain genes similar to those that encode *M. luteus* alkene biosynthesis enzymes, so researchers will have an opportunity to learn more about these enzymes by exploring their diversity in nature. This research was reported in Beller, H. R., E. B. Goh, and J. D. Keasling. 2010. "Genes Involved in Long-Chain Alkene Biosynthesis in *Micrococcus luteus*," *Applied and Environmental Microbiology* **76**(4), 1212–23.

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 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. 17).

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 pp. 18–19. 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. Computational models are being developed to help BESC researchers identify target genes and successful strategies for modifying biosynthetic pathways to generate cell walls that can be readily deconstructed into sugars for biofuel 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

BESC
BioEnergy Science Center

**Paul Gilna**
BESC Director

"The BioEnergy Science Center is focused specifically on the challenge of overcoming biomass recalcitrance. If we can manage to understand and solve this challenge, we will not only improve our ability to generate cellulosic ethanol, but we also will open doors to a vast array of renewable and sustainable product pipelines that can serve many different missions. To address these important issues, we have assembled a team of world leaders in their fields from 20 different institutions and have built a culture of integration and collaboration that has significantly accelerated our rate of scientific discovery and application."
– Paul Gilna

Paul Gilna, BESC director, also is deputy director of the Biosciences Division at Oak Ridge National Laboratory. An expert in computational biology, Gilna previously served as executive director of CAMERA (Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis), a project providing researchers with bioinformatic tools and services.

**BESC Headquarters.** Oak Ridge National Laboratory's 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]

to assess how these modifications affect plant cell walls. These modified plants are growing in greenhouses and at several sites to help differentiate genetic versus environmental influences on plant trait development (see figure, Bioenergy Crop Research at BESC, this page). By understanding the synthesis and assembly of the polysaccharides and lignin in plant biomass, BESC researchers are reducing cell-wall recalcitrance using methods that can be applied to a wide range of woody and herbaceous plants.

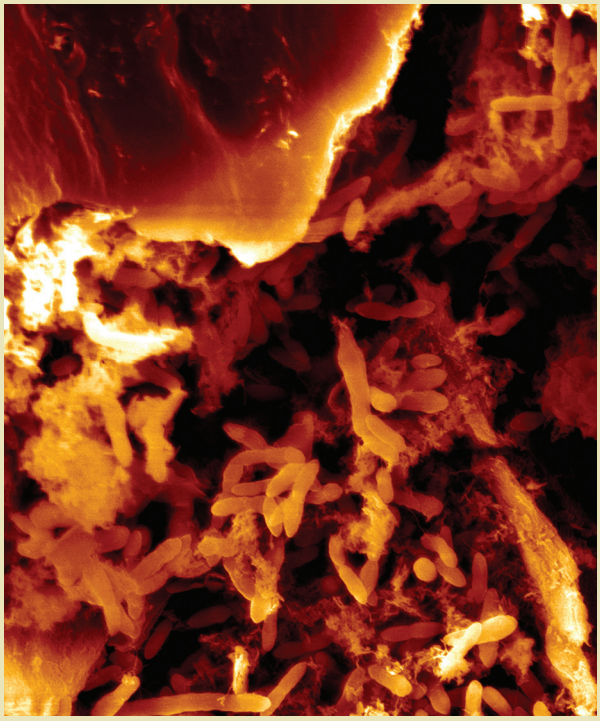
## 2. Biomass Deconstruction and Conversion

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.

Model organisms for CBP development include species of *Clostridia* bacteria that rapidly degrade pure cellulose and then ferment the resulting sugars into ethanol (see figure, BESC Research on Biomass-Deconstructing Microbes, this page). These microbes deconstruct biomass using cellulosomes—multifunctional enzyme complexes that specialize in degrading the mix of complex carbohydrates in cell walls. 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 can rapidly degrade biomass at near-boiling temperatures, such as species of *Caldicellulosiruptor* isolated from hot springs at Yellowstone National Park. By developing genetic tools for these difficult-to-manipulate microbes, BESC is working to engineer novel microbes that add biofuel production capabilities to their native cellulose-degrading abilities. These microbes and their enzymes could provide new biomass-degrading capabilities resistant to the heat and stresses of industrial processing.

**Bioenergy Crop Research at BESC.** Transformed *Populus* shoots grow in a greenhouse. These plants are altered in targeted cell-wall pathway genes. [Image courtesy of Oak Ridge National Laboratory]



**BESC Research on Biomass-Deconstructing Microbes.** Isolated from decaying grass compost, *Clostridium cellulolyticum* degrades cellulosic biomass using multienzyme complexes called cellulosomes. This scanning electron micrograph shows *C. cellulolyticum* cells growing on switchgrass biomass. [Photo by Thomas Hass and Shi-You Ding, National Renewable Energy Laboratory]

## 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.

To examine chemical and structural changes that occur in the modified plant cell walls of switchgrass and poplar, BESC analyzes thousands of native and modified biomass samples in a high-throughput screening pipeline that can perform compositional analysis, pretreatment, and enzyme digestibility studies. This screen already has revealed that a few native poplar samples can release most of their sugars using just a mild (hot water) pretreatment. Promising candidates selected from this screening pipeline are passed along to other partner institutions for a variety of detailed chemical, physical, and imaging analyses. 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 bases of plant cell-wall recalcitrance. A public version of the knowledgebase is at besckb.ornl.gov.

## 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. 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. Several BESC research partners and affiliates have pilot facilities for testing BESC improvements. The University of Tennessee–Genera Energy and Dupont Danisco Cellulosic Ethanol have established a demonstration plant for turning corn cobs and switchgrass into ethanol in Vonore, Tennessee. Mascoma, Verenium, Ceres, and ArborGen also have pilot and field sites.

## 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 to thousands of students at schools in Georgia and Tennessee are available to schools nationwide. BESC also began "Science Night" programs that build on these classroom lessons and are offered to students and their families. The National Geographic Jason Project featured BESC science in a program developed for high school students. The BESC website features announcements about BESC outreach and educational programs, seminars and presentations describing BESC research, and other resources.

## BESC Partners

**DOE's Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee (lead institution):** As 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.

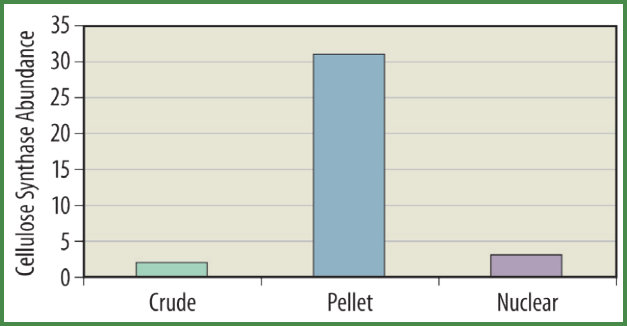
**Individual Researchers:** Specializing in biomass pretreatment, characterization of plant-associated microbes, cellulose and enzyme modeling, consolidated bioprocessing, and lignin biochemistry are researchers from the University of California, Riverside; DOE's Brookhaven National Laboratory (Upton,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

## Key Targets from a Complex Family of Lignin Biosynthesis Genes Identified in Switchgrass

Although lignin content and composition have been manipulated in several plant species by targeting the monolignol biosynthesis pathway, little is known about the genes and enzymes associated with this pathway in switchgrass. Cinnamoyl CoA reductase (CCR) catalyzes the first step in this pathway dedicated to monolignol synthesis. However, switchgrass contains numerous copies of CCR-like genes, complicating the selection of the best gene targets for altering lignin to reduce cell-wall recalcitrance. By analyzing the RNA of expressed CCR genes, BESC researchers show that one of the expressed genes (PvCCR1) encodes an enzyme actively involved in lignification and thus is a prime target for down-regulation to improve the degradability and sugar yield from switchgrass. Ongoing research is investigating how reducing the expression of the PvCCR1 gene impacts lignin composition and plant structure. This research was reported in Escamilla-Treviño, L. L., et al. 2009. "Switchgrass (*Panicum virgatum)* Possesses a Divergent Family of Cinnamoyl CoA Reductases with Distinct Biochemical Properties," *New Phytologist* **185**, 143–55.

## Thousands of Proteins from Developing Xylem Cells in Poplar Are Identified

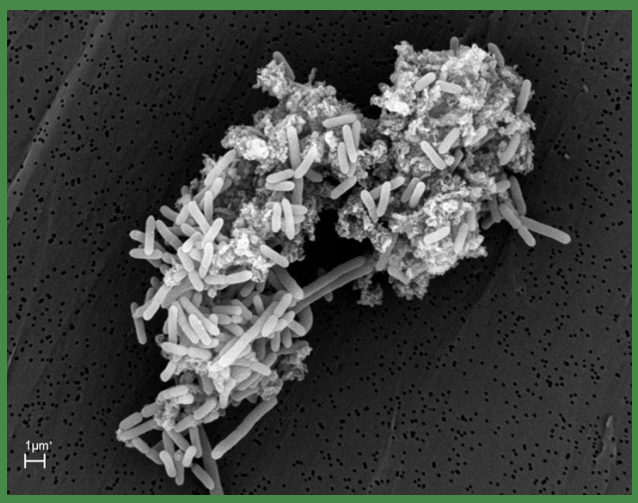
Woody biomass in trees primarily consists of the secondary cell walls of dead xylem tissue, so developing xylem cells are useful models for investigating secondary cell-wall formation. To provide subcellular context for identified protein functions and to enhance the detection of low-abundance proteins, subcellular fractionation techniques were used to obtain crude (soluble protein), pellet (insoluble protein), and nuclear protein fractions for analysis. Applying an automated approach known as MudPIT (Multidimensional Protein Identification Technology), BESC researchers successfully isolated and identified 6,000 different proteins from developing xylem cells in the stems of poplar plants. Results from this project greatly expanded the number of proteins that had been identified in previous poplar proteome studies. The protein products of several cell-wall synthesis genes (e.g., cellulose synthase, sucrose synthase, and polygalacturonase) were found to be associated with cellular membranes (see figure), and numerous new candidate genes for cell-wall synthesis were discovered—many are promising targets for further functional genomic analysis. Measuring differences in the whole proteomes of different poplar variations will increase understanding of the fundamental properties that underlie the recalcitrance of woody biomass to degradation. This research was reported in Kalluri, U. C., et al. 2009. "Shotgun Proteome Profile of *Populus* Developing Xylem," *Proteomics* **9**, 4871–80.




**Amount of Cellulose Synthase Proteins in Different Subcellular Fractions.** The pellet fraction of extracted xylem proteins is enriched in membrane proteins. Significantly higher amounts of a key membrane protein, cellulose synthase, are associated with the pellet fraction relative to the other fractions. [Image from Kalluri et al. 2009]

## Heat-Tolerant Bacteria Efficiently Degrade Non-Pretreated Biomass

Presenting the possibility of eliminating the pretreatment step from cellulosic biofuel production, a hot springs bacterium known as *Caldicellulosiruptor bescii* has shown that it can efficiently degrade crystalline cellulose, xylan (a hemicellulose), and various types of non-pretreated biomass including hardwoods such as poplar, high-lignin grasses such as switchgrass, and low-lignin grasses such as Bermuda grass (see top figure, p. 19). With an optimal growth temperature of 75°C, *C. bescii* was able to break down 65% of switchgrass biomass without pretreatment. This bacterium is the most heat-tolerant biomass degrader known (withstanding temperatures up to 90°C), and it primarily produces hydrogen as an end product when grown on plant biomass. BESC researchers have discovered another hot springs bacterium (*Caldicellulosiruptor obsidiansis*), isolated from Yellowstone National Park, that thrives at 78°C and can ferment all the simple sugars in cell-wall polysaccharides into diverse products including ethanol. Combining the functional capabilities of *C. bescii* and *C. obsidiansis* theoretically could yield organisms that both deconstruct and ferment plant biomass at temperatures above the boiling point of ethanol (78.4°C). Producing ethanol in the vapor phase could greatly reduce the inhibitory effects of ethanol on cell growth. *C. bescii* (formerly called *Anaerocellum thermophilum* DSM 6725) findings are from Yang, S. J., et al. 2009. "Efficient Degradation of Lignocellulosic Plant Biomass, Without Pretreatment, by the Thermophilic Anaerobe '*Anaerocellum thermophilum*' DSM

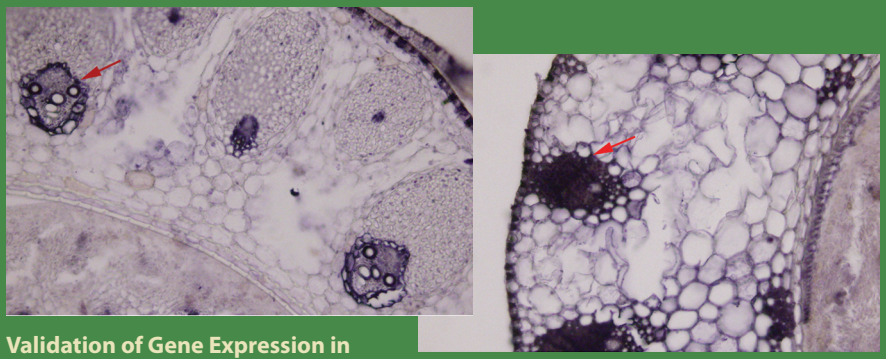



***Caldicellulosiruptor bescii* on Birchwood Xylan.** [Image courtesy of Mike Adams, University of Georgia]

6725," *Applied and Environmental Microbiology* **75**(14), 4762–69. The discovery of *C. obsidiansis* was reported in Hamilton-Brehm, S. D., et al. 2010. "*Caldicellulosiruptor obsidiansis* sp. nov., an Anaerobic, Extremely Thermophilic, Cellulolytic Bacterium Isolated from Obsidian Pool, Yellowstone National Park," *Applied and Environmental Microbiology* **76**(4), 1014–20.

## Researchers Target Expressed Genes in Vascular Tissues of Switchgrass

Using a laser-based technique for microdissecting plant tissues, BESC researchers have targeted and analyzed DNA that is actively expressed in switchgrass vascular tissues where secondary cell walls are synthesized and reinforced with lignin. A total of 2,766 unique genes were identified from 5,734 expressed DNA segments (known as expressed sequence tags or ESTs). A significant number of these expressed sequences are novel with no significant hits to existing EST data. A small subset of the identified genes was targeted with labeled probes to visualize the expression of these genes in live plant tissue (see figure below), and researchers found that several genes have much higher expression in the vascular bundles. The gene list generated from this study provides an important genomic resource for narrowing the range of molecular targets that could play key roles in modifying the lignin content of switchgrass and other related bioenergy crops. This research was reported in Srivastava, A. C., et al. 2010. "Collection and Analysis of Expressed Sequence Tags Derived from Laser Capture Microdissected Switchgrass (*Panicum virgatum* L. Alamo) Vascular Tissues," *BioEnergy Research*, DOI: 10.1007/s12155-010-9080-8.



**Validation of Gene Expression in Vascular Tissues.** To confirm the expression of identified genes in vascular tissues, probes that specifically bind the mRNA of a small number of the identifed genes were applied to switchgrass tissue. The darker regions indicate the vascular bundles where the probes bound the abundant mRNA from the active expression of targeted genes in these regions. [Image courtesy of Elison Blancaflor, The Samuel Roberts Noble Foundation]

## New Strategy Enhances Microbial Resistance to Inhibitory Pretreatment Chemicals

The chemical and physical processes for pretreating biomass help unravel the complex matrix of cell-wall components and enhance enzyme accessibility to these materials, but pretreatments also generate chemicals such as acetate that inhibit sugar fermentation to biofuels. Using a combination of adaptation, genetic engineering, and systems biology tools, BESC researchers have developed acetate-resistant strains of two industrial ethanol producers (the bacterium *Zymomonas mobilis* and the yeast *Saccharomyces cerevisiae*) by changing the expression of genes encoding transport proteins that move substances across the cell membrane. These proteins (called antiporters) transport proton and sodium ions and form gradients that are adversely impacted by the presence of acetate. By resequencing a *Z. mobilis* strain that had been adapted to withstand high acetate concentrations, BESC researchers discovered specific mutations in antiporter genes that enable acetate resistance. The specific antiporter mutations were validated using genetically engineered *Z. mobilis* and yeast showing the broad impact of these changes. This research is reported in Yang, S., et al. 2010. "Paradigm for Industrial Strain Improvement Identifies Sodium Acetate Tolerance Loci in *Zymomonas mobilis* and *Saccharomyces cerevisiae*," *Proceedings of the National Academy of Sciences* **107**(23), 10395-400.

# DOE Great Lakes Bioenergy Research Center

glbrc.org

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. 23). 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) Improved Plants, (2) Improved Processing, (3) Improved Catalysts, and (4) Sustainable Biofuels 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 (see figure, Automated Microbial Colony Picker at GLBRC, p. 21), 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 pp. 24–25.

## Research Strategy

### 1. Improved Plants

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 to increase the energy density of grasses and other nontraditional oil

"We are proud of the pieces of the scientific puzzle we have assembled. The Great Lakes Bioenergy Research Center has brought together 400 hand-picked scientists and staff and established core facilities for analyzing bioenergy microbes and plants in the lab or the field. The productivity of this team has resulted in more than 100 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]

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.

## 2. Improved 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.

## 3. Improved Catalysts

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, 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.

## 4. Sustainable Biofuels 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

**Automated Microbial Colony Picker at GLBRC.** GLBRC screening efforts begin with the automated selection of microbial colonies in multiwell plates—selecting up to 7,000 colonies per hour. The robot then dispenses growth medium into the destination plates and seals the plates for analysis. [Photo by Wolfgang Hoffmann, University of Wisconsin–Madison]

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.

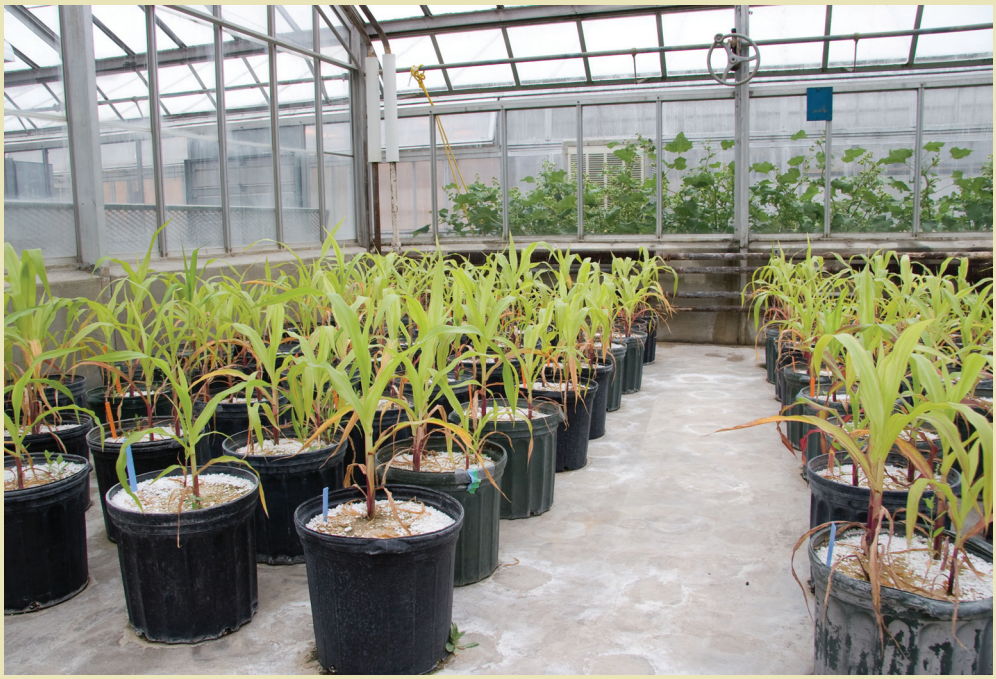
To determine the best practices for biofuel production, GLBRC researchers are analyzing the impacts of issues such as minimizing energy and chemical inputs for bioenergy crop production and reducing greenhouse gas emissions from the entire biofuel production life cycle (see figure, GLBRC Research on Bioenergy Crop Sustainability, this page). 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 for 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 laboratories 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/.

## 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. The GLBRC is positioned to make key discoveries and major advances that will lead to breakthrough technologies for eventual large-scale conversion of biomass into biofuels. Once a technology is developed, the center works closely with industry partners through technology-transfer mechanisms or collaboratively to achieve commercial implementation. More information about collaborating with the GLBRC is available at glbrc.org/industry/.



**GLBRC Research on Bioenergy Crop Sustainability.** To improve the sustainability of crops and agricultural residues used for energy production, GLBRC researchers are studying the symbiotic associations of crop roots with arbuscular mycorrhizal (AM) fungi. Interactions with AM fungi benefit host plants by improving the uptake of nutrients, especially phosphorus, nitrogen, and potassium from the soil. Establishing these symbiotic associations in crops grown under suboptimal conditions has the potential to increase biomass production while limiting use of fertilizers and pesticides. [Photo courtesy of the Great Lakes Bioenergy Research Center]

## 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.

**Cornell University, Ithaca, New York:** Individual researchers at Cornell provide expertise in the design and creation of microorganisms with new or improved protein machinery, specifically extracellular secretion of cellulases by engineered bacteria.

**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.

**University of Minnesota, St. Paul**: Individual researchers at the University of Minnesota provide expertise on complex global environmental systems and their interactions with human societies, focusing on the global land resource base for bioenergy feedstocks.

**University of Missouri, Columbia:** Individual researchers at the University of Missouri provide expertise in agricultural economics, specifically the impacts of Energy Independence and Security Act mandates on U.S. crop and livestock production.

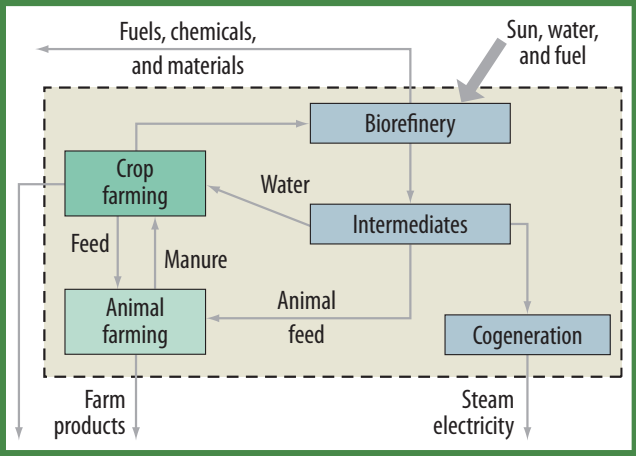
**University of Toledo, Ohio:** Individual researchers at the University of Toledo provide expertise on biophysical constraints and resource use limitations in regards to maximizing biofuel production.

## Alterations in Poplar Lignin Could Enhance Pretreatment Efficiency

Alterations in lignin content or structure in plant cell walls can have a profound effect on chemical or enzymatic degradability and the efficiency by which certain pretreatment methods remove lignin from polysaccharides. GLBRC researchers found that overexpression of a particular gene [ferulate 5-hydroxylase (F5H)] in the lignin biosynthetic pathway of a hybrid poplar created lignin with a structure and composition that can enhance lignin removal from cellulose, while still maintaining normal growth and development. When compared to wild-type poplar, the up-regulated F5H poplar has a much simpler lignin structure that is less branched and more homogeneous in its subunit composition, which makes the lignin easier to separate from cellulose during pretreatment. This and other poplar transgenic materials under investigation by GLBRC researchers have cell walls that release more sugar than wild-type poplar over a range of pretreatment methods. Ongoing work is examining the effect of ammonia fiber expansion pretreatment on these transgenic poplars. Details on the lignin structure of F5H up-regulated poplar were reported in Stewart, J. J., et al. 2009. "The Effects on Lignin Structure of Overexpression of Ferulate 5-Hydroxylase in Hybrid Poplar," *Plant Physiology* **150**(2), 621–35.

## New Modeling Tool Combines Environmental and Economic Analysis of the Biorefinery in Agricultural Landscapes

GLBRC researchers have provided a direct simulation of different biorefinery configurations in realistic agricultural landscapes for diverse locations throughout the United States. Since no full-scale commercial examples of a cellulosic biorefinery yet exist, forecasting the risks and tradeoffs of the complete biofuel production chain requires the use of modeling tools. Developed at GLBRC, the Biorefinery and Farm Integration Tool (BFIT) enables a combined modeling approach, including both crop and animal production, for analyzing potential economic profitability as well as environmental impacts (see figure). Focusing on ethanol production from the two largest anticipated sources of cellulosic biomass—corn stover and switchgrass—BFIT simulated the farm-biorefinery interactions for nine different agricultural regions using county-specific data for soil, weather, and farm practice patterns. In all cases, cellulosic biofuel production was integrated into existing farmlands. Results from the simulated scenarios include projections for land area requirements, annual farm income, nitrogen loss, greenhouse gas emissions, total project investment, and minimum ethanol selling price. Based on these projections, GLBRC researchers show that introducing the cellulosic biorefinery and associated markets could improve farm economics and reduce emissions without additional clearing of lands for biofuels. BFIT research results are reported in Sendich, E. D., and B. E. Dale. 2009. "Environmental and Economic Analysis of the Fully Integrated Biorefinery," *GCB Bioenergy* **1**, 331–45.




**Integrated Biorefinery Concept.** This figure shows the integrated system components analyzed by the Biorefinery and Farm Integration Tool. [Image from Sendich and Dale 2009]

## Study Provides Insights on Maximizing Energy-Rich Lipid Content in Leaves

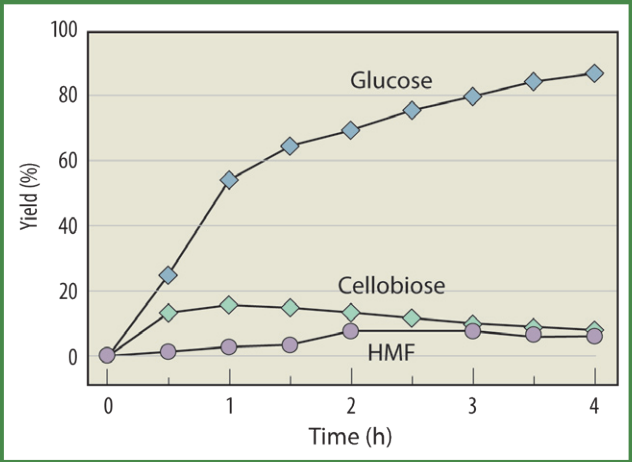
Energy-rich lipids—with two times more energy than carbohydrates or proteins—are life's primary molecules for energy storage. Preventing the breakdown of lipids as leaves age during the process of senescence is estimated to increase the energy content of leaves by about 20%. GLBRC researchers systematically studied the age-dependent changes in the fatty acids of *Arabidopsis*, *Brachypodium distachyon* (a model grass), and switchgrass leaves during natural plant senescence. Researchers found that surface lipids were more stable during senescence than membrane lipids, thus a potential strategy for increasing the energy content of biofuel crops might be to enhance surface lipid production. This research was reported in Yang, Z., and J. B. Ohlrogge. 2009. "Turnover of Fatty Acids During Natural Senescence of *Arabidopsis*, *Brachypodium*, and Switchgrass and in *Arabidopsis* β-Oxidation Mutants," *Plant Physiology* **150**, 1981–89.

## Sequencing Characterizes Bacterial Rhizosphere Communities of Biofuel Crops on Marginal Lands

Using a new high-capacity sequencing technology, GLBRC researchers characterized the structure of bacterial communities living in the rhizosphere (microscopic zone surrounding roots) of corn, soybean, canola, sunflower, and switchgrass. Samples were taken from agricultural sites and adjacent native forest in four locations with different soil types in Michigan. Three of the locations were marginal lands unsuitable for conventional agriculture, and a fourth site served as an experimental control to evaluate crop yield and quality on nonmarginal land. Although bacterial communities from biofuel crops and forest were clearly differentiated, the communities grouped mainly by location rather than by crop species, and soil environment and land management were key factors influencing community structure. Although more limited in plant diversity, greater bacterial diversity was observed in the biofuel crop samples than in the forest samples. Species of Acidobacteria were the most abundant community members in the rhizospheres of all plants, yet no strains have been isolated for cultivation and characterization in the laboratory. This research was reported in Jesus, E. C., et al. 2010. "Bacterial Communities in the Rhizosphere of Biofuel Crops Grown on Marginal Lands as Evaluated by 16S rRNA Gene Pyrosequences," *Bioenergy Research* **3**, 20–27.

## Chemical Process Produces Simple, Fermentable Sugars from Raw Biomass

A GLBRC research team has developed a promising new chemical method to liberate the sugar molecules trapped inside inedible plant biomass, a key step in the creation of cellulosic biofuels. The new chemical process combines ionic liquids and dilute acid to degrade cellulosic biomass without the use of cellulases. In this approach, ionic liquids make cell-wall polysaccharides accessible to chemical reactions by decrystallizing lignocellulosic biomass and dissolving cellulose. Then, dilute hydrochloric acid at 105°C is used to hydrolyze cellulose and hemicellulose into individual sugar subunits. Applying this process to pure cellulose resulted in nearly 90% yield of glucose (see figure), and applying it to raw corn stover achieved sugar yields of 70% to 80%. By adding the right balance of water to the mixture, the researchers reduced the formation of unwanted by-products and demonstrated significant improvement in fermentable sugar yields from ionic liquid treatment of lignocellulose with yields comparable to those of enzymatic hydrolysis. Ion-exclusion chromatography was used to separate sugars from the reaction mixture and recover the ionic liquids for reuse. Sugars recovered from the hydrolyzed stover were readily converted to ethanol by *Escherichia coli* and the yeast *Pichia stipitis*. This research was reported in Binder, J. B., and R. T. Raines. 2010. "Fermentable Sugars by Chemical Hydrolysis of Biomass," *Proceedings of the National Academy of Sciences* **107**, 4516–21.




**Chemical Hydrolysis of Cellulose Achieves High Glucose Yields.** Applying this new chemical approach to the hydrolysis of pure cellulose results in glucose yields approaching 90% in just a few hours. Yields of unwanted by-products such as HMF (5-hydroxymethylfurfural, an inhibitor of microbial fermentation) and cellobiose (a molecule consisting of two linked glucose subunits) were minimal. [Image from Binder and Raines 2010]

# DOE Genomic Science Program
## *From Genome Sequences to Understanding*

genomicscience.energy.gov

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 box, Genomic Science Program Goal and Objectives, below). 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 integration 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) National Institute of Food and Agriculture supports genome-based approaches to accelerate plant-breeding programs and improve potential bioenergy crops.

## Genomic Science Program Goal and Objectives

**Genome Sequence** → **System-Wide Biological Investigations** → **Predictive Understanding**

**Goal:** Achieve a predictive, system-level understanding of plants, microbes, and biological communities, via integration of fundamental science and technology development, to enable biological solutions to DOE mission challenges in energy, environment, and climate.

- **Objective 1:** Determine the genomic properties, molecular and regulatory mechanisms, and resulting functional potential of microbes, plants, and biological communities central to DOE missions.
- **Objective 2:** Develop the experimental capabilities and enabling technologies needed to achieve a genome-based, dynamic system-level understanding of organism and community function.
- **Objective 3:** Develop the knowledgebase, computational infrastructure, and modeling capabilities to advance the understanding, prediction, and manipulation of complex biological systems.

## 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 important roles in sustainable biofuels, climate science and carbon cycling, and subsurface biogeochemistry (see figure). 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.

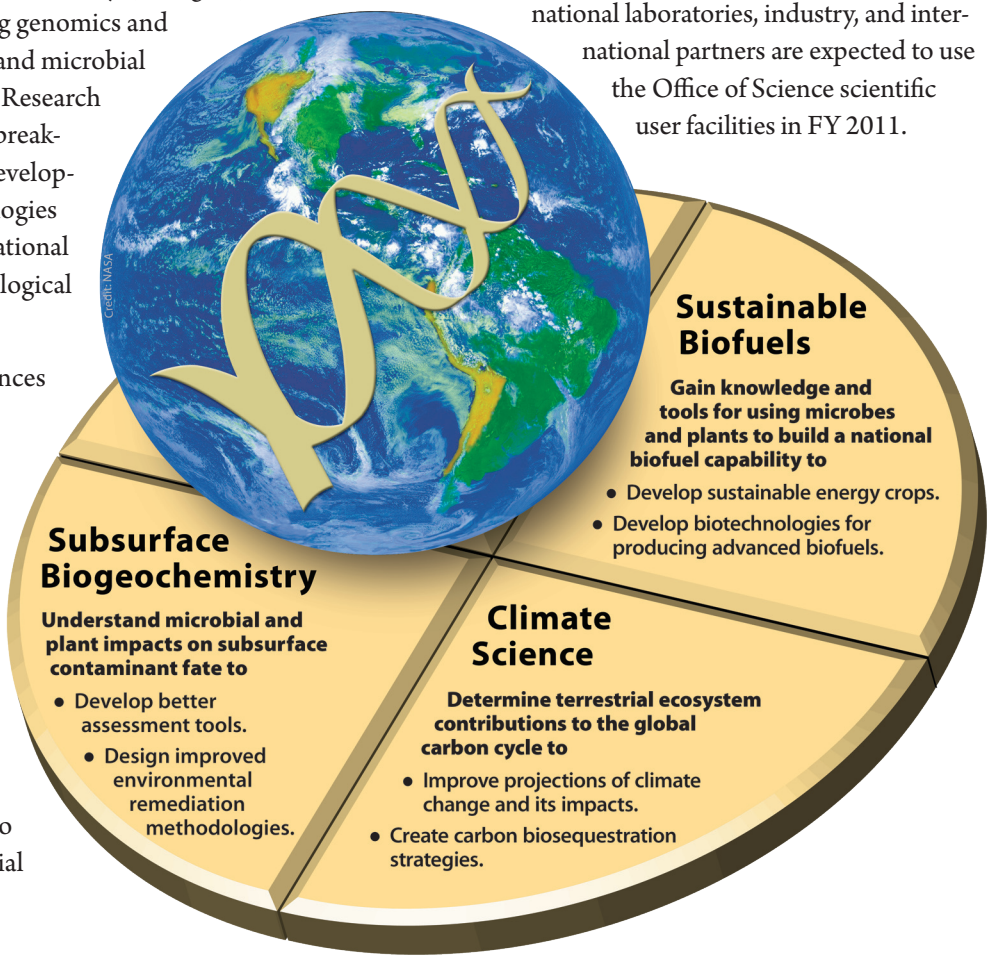
The Climate and Environmental Sciences Division (CESD) aims to achieve a predictive understanding of climate change, ecosystem response to climate change, and contaminant fate and transport in the subsurface. Projects supported by CESD programs are resolving the greatest uncertainties in climate change, 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.

Nearly 26,000 researchers from universities, national laboratories, industry, and international partners are expected to use the Office of Science scientific user facilities in FY 2011.



# TRANSFORMATIONAL BIOLOGY FOR ENERGY BREAKTHROUGHS

## DOE Joint BioEnergy Institute

**Lawrence Berkeley National Laboratory**
Berkeley, California

**Carnegie Institution for Science at Stanford University**
Palo Alto, California

**Lawrence Livermore National Laboratory**
Livermore, California

**Sandia National Laboratories**
Albuquerque, New Mexico

**Sandia National Laboratories**
Livermore, California

**University of California**
Berkeley

**University of California**
Davis

## DOE Great Lakes Bioenergy Research Center

**University of Wisconsin**
Madison

**Cornell University**
Ithaca, New York

**Illinois State University**
Normal

**Iowa State University**
Ames

**Lucigen Corporation**
Middleton, Wisconsin

**Michigan State University**
East Lansing

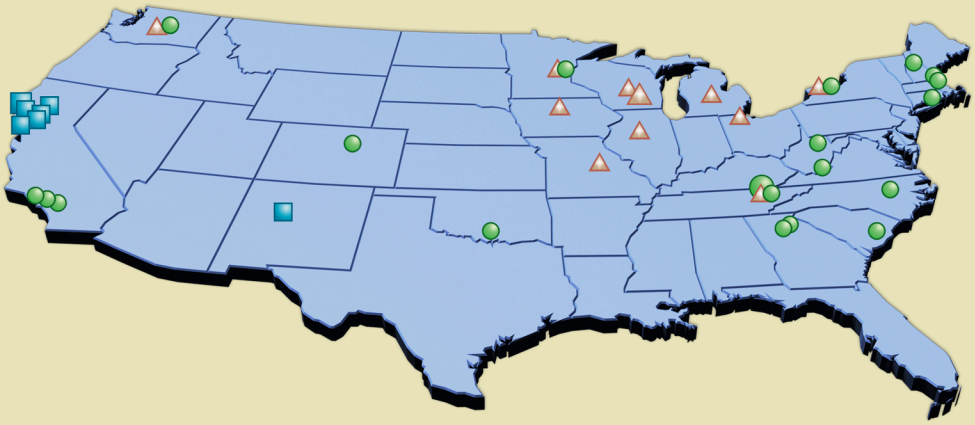
**Oak Ridge National Laboratory**
Oak Ridge, Tennessee

**Pacific Northwest National Laboratory**
Richland, Washington

**University of Minnesota**
St. Paul

**University of Missouri**
Columbia

**University of Toledo**
Toledo, Ohio



## DOE BioEnergy Science Center

**Oak Ridge National Laboratory**
Oak Ridge, Tennessee

**ArborGen**
Summerville, South Carolina

**Brookhaven National Laboratory**
Upton, New York

**Ceres**
Thousand Oaks, California

**Cornell University**
Ithaca, New York

**Dartmouth College**
Hanover, New Hampshire

**Georgia Institute of Technology**
Atlanta

**Mascoma Corporation**
Boston, Massachusetts

**National Renewable Energy Laboratory**
Golden, Colorado

**North Carolina State University**
Raleigh

**The Samuel Roberts Noble Foundation**
Ardmore, Oklahoma

**University of California**
Los Angeles

**University of California**
Riverside

**University of Georgia**
Athens

**University of Minnesota**
St. Paul

**University of Tennessee**
Knoxville

**Verenium Corporation**
Cambridge, Massachusetts

**Virginia Polytechnic Institute and State University**
Blacksburg

**Washington State University**
Pullman

**West Virginia University**
Morgantown

## DOE Bioenergy Research Centers and Partners

genomicscience.energy.gov/centers/

### DOE Joint BioEnergy Institute (JBEI)

jbei.org

### DOE BioEnergy Science Center (BESC)

bioenergycenter.org

### DOE Great Lakes Bioenergy Research Center (GLBRC)

glbrc.org

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