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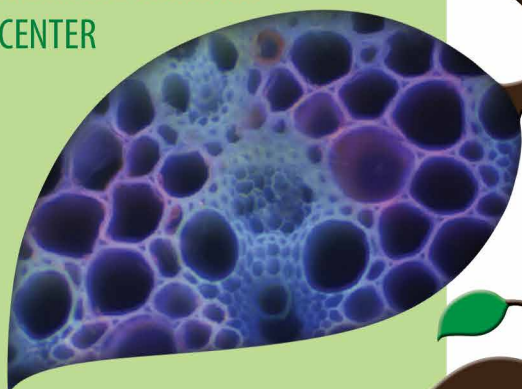
U.S. DEPARTMENT OF ENERGY

Bioenergy Research Centers

GREAT LAKES BIOENERGY RESEARCH CENTER



BIOENERGY SCIENCE
CENTER



JOINT BIOENERGY INSTITUTE

February 2014



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Websites for DOE Bioenergy Research Centers

DOE BioEnergy Science Center (BESC)

bioenergycenter.org

DOE Great Lakes Bioenergy Research Center (GLBRC)

glbrc.org

DOE Joint BioEnergy Institute (JBEI)

jbei.org

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U.S. DEPARTMENT OF ENERGY
Bioenergy Research Centers

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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 advanced biofuels (see sidebar, Advanced Biofuels, this page) from cellulosic biomass are renewable alternatives that can increase domestic production of transportation fuels, revitalize rural economies, and reduce carbon dioxide and pollutant emissions relative to petroleum-based fuels. According to U.S. Secretary of Energy Ernest Moniz, “By partnering with industry and universities, we can help make clean, renewable biofuels cost competitive with gasoline, give drivers more options at the pump, and cut harmful carbon pollution.”

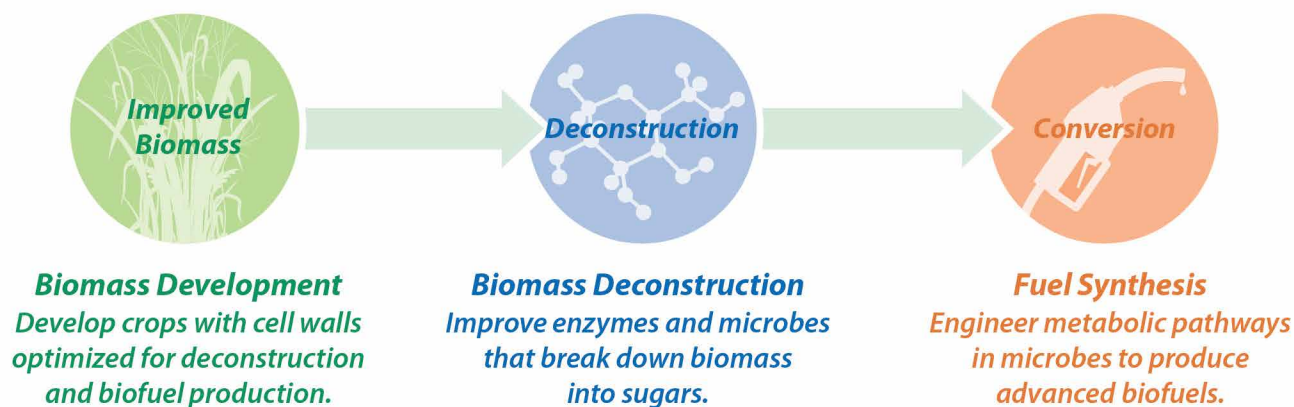
The primary source of U.S. ethanol fuel production today comes from corn grain, but the biofuels of the future will come from other biomass sources including specially designed bioenergy feedstocks. Woodchips, grasses, corn stalks, and other cellulosic biomass are widely abundant but more difficult to break down into sugars than corn grain because of the lignin present in these forms of biomass. Although cellulosic ethanol production has been demonstrated at the pilot scale, developing a cost-effective, widespread, and commercial-scale cellulosic biofuel industry will require significant improvements in current production processes. Innovation stemming from advanced biotechnology-based research is key to accelerating needed improvements in the production of cellulosic biomass, its deconstruction into sugars, and conversion to biofuels (see figure below, From Biomass to Biofuels).

Advanced Biofuels

Unlike so-called first-generation biofuels made from sugars, starches, and vegetable oils found in food crops such as corn and soybeans, next-generation or advanced biofuels are high-energy fuel compounds made from nonfood, sustainably grown feedstocks and agricultural wastes. These advanced biofuels have a similar molecular composition as today’s petrochemical-derived fuels, allowing them to be seamlessly integrated into the current fuel infrastructure as “drop-in” replacements for traditional fuels. Related conversion processes also can yield a range of other chemicals and products in addition to liquid fuels.

To focus the most advanced biotechnology-based resources on the challenges of biofuel production, the U.S. Department of Energy (DOE) established three Bioenergy Research Centers (BRCs) in September 2007, with a second 5-year phase beginning in 2012 (see sidebar, Genomic Science to Advance DOE Missions, p. 2). Each center represents an integrative, multidisciplinary partnership with expertise spanning the physical, chemical, biological, and computational sciences, including genomics, microbial and plant biology, analytical chemistry, computational biology and bioinformatics, and engineering. The BRCs are structured to facilitate knowledge sharing among multiple disciplines so that breakthroughs in one area can be capitalized on and translated to other areas of emphasis. In these integrated and collaborative environments, the BRCs pursue the necessary fundamental research to improve the processes needed for large-scale, cost-effective production of advanced biofuels from cellulosic biomass. Additionally, as each center approaches biofuel production

From Biomass to Biofuels



DOE Bioenergy Research Centers

challenges from different angles, the types of knowledge gained are multiplied, new questions opened up, and new avenues of research pursued, ultimately accelerating the pathway to improving and scaling up biofuel production processes (see sidebar, Scaling Up, p. 3).

The three centers, which were selected through a scientific peer review process, are based in geographically diverse locations—the Southeast, the Midwest, and the West Coast—with partners across the nation (see U.S. map, DOE Bioenergy Research Centers and Partners, on back cover). DOE's Oak Ridge National Laboratory leads the BioEnergy Science Center (BESC; see p. 10) in Oak Ridge, Tennessee; the University of Wisconsin–Madison leads the Great Lakes Bioenergy Research Center (GLBRC; see p. 18) in Madison in partnership with Michigan State University; and DOE's Lawrence Berkeley National Laboratory leads the Joint Bio-Energy Institute (JBEI; see p. 25) in Emeryville, California. Partners include a number of universities, private companies, nonprofit organizations, and DOE national laboratories.

Advances resulting from high-risk, high-return BRC research are providing the knowledge needed to accelerate scientific discovery and develop new biobased products, methods, and tools crucial to an emerging biofuels industry. For example, through the development of new, high-throughput technologies, the BRCs have created new plant strains optimized for bioenergy production; using advanced genomic techniques, they have discovered numerous new enzymes for deconstructing lignocellulose into sugars; and with some of the most advanced approaches to metabolic engineering, the BRCs have re-engineered microbes to produce not just ethanol, but drop-in hydrocarbon substitutes for gasoline, diesel, and potentially jet fuel. These advances from the BRCs' first 6 years are summarized in *Bioenergy Research Centers: Key Advances for Biofuels Production*, p. 4.

Center Research Strategies

The ultimate goal for the three DOE BRCs is to provide the fundamental science to underpin a cost-effective, advanced cellulosic biofuels industry. Using systems biology approaches, the BRCs are focusing on new strategies to reduce the impact of key cost-driving processes in the overall production of cellulosic biofuels from biomass. For these biofuels to be adopted on a large scale, they must represent environmentally sustainable and economically competitive alternatives to existing fuel systems. New strategies and findings emanating from the centers' fundamental research are addressing three grand challenges for cost-effective advanced biofuels production:

- Develop next-generation bioenergy crops by unraveling the biology of plant development.
- Discover and design enzymes and microbes with novel biomass-degrading capabilities.
- Develop transformational microbe-mediated strategies for advanced biofuels production.

The science needed to solve these complex challenges requires multiple, coordinated, multidisciplinary teams approaching problems from varied perspectives to accelerate scientific progress (see table, DOE Bioenergy Research Center Strategies at a Glance, p. 6). Some key scientific approaches to these challenges are described below.

1. Develop Next-Generation Bioenergy Crops by Unraveling the Biology of Plant Development

The raw material for biofuel production is cellulose, which is derived primarily from the cell walls of plants. Cellulose is a polymer of glucose that is easily broken down and converted into biofuels, hence the term cellulosic biofuels. However, the recovery of cellulose from plant biomass is an

(Center Research Strategies continued on p. 7)

Genomic Science to Advance DOE Missions

Over the past 30 years, the Department of Energy's (DOE) Office of Science has continued to play a major role in inspiring, supporting, and guiding the biotechnology revolution. Through its Genomic Science program, the Office of Biological and Environmental Research (BER) within DOE's Office of Science is advancing a new generation of research focused on achieving a whole-system predictive understanding of biology. The Bioenergy Research Centers are part of the Genomic Science program, which is bringing together scientists from diverse fields to understand the complex biology underlying solutions to DOE missions in energy production and climate change science. For more information on the Genomic Science program as well as BER and DOE's Office of Science, see pp. 33 and 34, respectively.

Scaling Up: Creating a Knowledgebase for Future Biofuel Technologies

The scale of the basic science collectively being explored by the Department of Energy's (DOE) Bioenergy Research Centers (BRCs) not only allows research teams to attack problems more efficiently, it allows them to ask deeper, more comprehensive questions. For example, across the BRCs, scientists and engineers are developing promising, innovative methods of biomass pretreatment for more efficient conversion into fuels. The strategies being pursued are distinct to each of the centers:

- The Great Lakes Bioenergy Research Center (GLBRC) is working on ammonia-based pretreatment processes such as ammonia fiber expansion (AFEX™). Using ammonia, heat, and pressure, the AFEX method blasts open cell walls, allowing enzymes easier access to the sugar polymers that make up plant cellulose. Enzymes then break the polymers apart into simple sugars.
- The Joint BioEnergy Institute (JBEI) is developing pretreatment technologies based on ionic liquids, environmentally benign organic salts often used as green chemistry substitutes for volatile organic solvents. These liquids possess a unique capacity for dissolving lignocellulosic biomass and helping hydrolyze the resulting slurry into sugars.
- One of the BioEnergy Science Center's (BESC) focus areas is looking at ways to reduce or even eliminate the need for pretreatment through consolidated bioprocessing (CBP) and

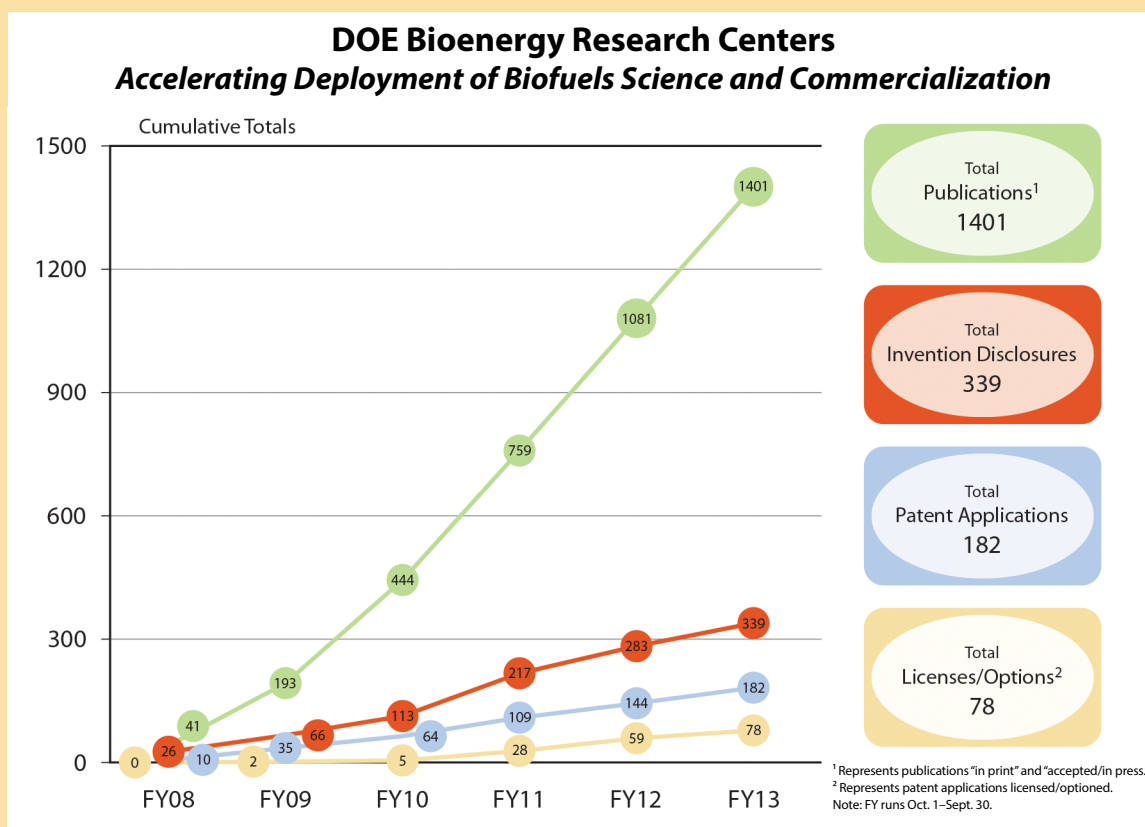
improved feedstocks. This effort involves developing multifunctional microbes to convert lignocellulosic biomass into desired products in a single step, without added enzymes.

The three BRCs also are completing a joint study of pretreatment technologies (hot water, AFEX, and ionic liquids) on shared lignocellulosic feedstocks.

These varied but complementary pretreatment approaches are yielding a battery of different techniques that can be honed and combined based on the feedstock type being used. Such versatility is important because biofuels will be a very regional industry, with feedstocks varying from region to region and even within a region, depending on weather conditions, handling, storage, and crop variety.

Another complementary area is feedstock development. While each BRC conducts research in switchgrass, BESC also targets poplar, GLBRC corn stover, and JBEI rice straw.

In short, the BRCs are producing a portfolio of diverse and complementary scientific strategies that address the challenges of biomass conversion to fuel on a scale far greater than any effort to date. This knowledgebase (see figure below) is providing new insights to help industry meet the broad challenges of reducing the cost of and meeting demand for advanced biofuels.



Bioenergy Research Centers: Key Advances for Biofuels Production

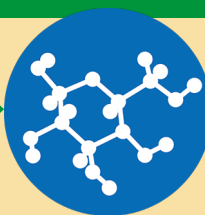


Improved Biomass

Overcome the natural resistance of plant biomass to microbial and chemical hydrolysis through the development and optimization of high-yield, sustainable feedstocks that are easily converted into biofuels. To date, the BRCs have identified more than 100 plant genes that have a beneficial impact on plant cell wall chemistry, production yield, or conversion to biofuels.

Advances and Results

- Bred and selected switchgrass crop variants with desired traits, enabling the potential doubling of switchgrass yields (Casler 2010; Zalapa et al. 2011; Zhang et al. 2011a; Zhang et al. 2011b).
- Developed efficient and rapid stable transformation protocols for switchgrass to accelerate gene functional analysis (Mann et al. 2012; Nageswara-Rao et al. 2013; Zhang et al. 2013).
- Genetically modified lignin cross-linking to improve hydrolysis, while maintaining plant strength (Eudes et al. 2013; Bartley et al. 2013; Shen et al. 2013; Vanholme et al. 2012).
- Engineered plants with modified hemicellulose deposition and increased 6-carbon sugar content, thereby facilitating processing and reducing deconstruction and conversion costs (Petersen et al. 2012; Liwanag et al. 2012).
- Genetically engineered a plant with altered deposition of lignin and increased secondary cell wall synthesis, thereby reducing recalcitrance and increasing yield of fermentable sugars (Yang et al. 2013).
- Identified a plant gene involved in synthesizing oil with 30% less viscosity than existing vegetable oils, creating the potential for producing extractable oils from nonfood perennial crops (Durrett et al. 2010).
- Characterized the expanded lignin biosynthetic pathway in *Ara-bidopsis* after identifying a new gene responsible for producing a previously unknown enzyme central to lignin synthesis (Vanholme et al. 2013).
- Identified a cell wall proteoglycan with pectin and arabinoxylan covalently attached to a protein, creating a cross-linked polysaccharide-wall protein matrix with implications for cell wall structure, function, and synthesis (Tan et al. 2013).
- Identified why lignin H subunits in β - β or β -5 linkages react poorly and tend to “cap” the lignin polymer; results provide a coherent framework for understanding the propensity of different terminal subunits in lignin to undergo growth or termination (Sangha et al. 2014).
- Identified the cellulose digestion mechanism of CelA, a hydrolytic enzyme secreted by *Caldicellulosiruptor bescii* that holds several advantages over other fungal and bacterial cellulases used in biofuels production, namely high specific activity and stability at elevated temperatures. The activity of CelA on Avicel is seven times greater than the standard exo- and endocellulase mixture (Brunecky et al. 2013).



Deconstruction

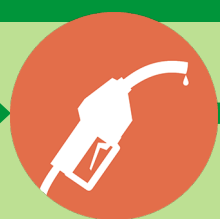
Develop more energy-efficient and cost-effective processes for deconstructing lignocellulosic biomass into its constituent sugars, while minimizing the formation of inhibitors and using as few chemicals as possible.

Advances and Results

- Achieved real-time bioimaging of cell wall lignin and polysaccharides for greater understanding of relationships between biomass cell wall structure and enzyme digestibility, facilitating evaluation of pretreatments and breeding programs (Ding et al. 2012; L. Sun et al. 2013).
- Identified thermophilic cellulolytic bacteria (*Caldicellulosiruptor bescii*) that can deconstruct biomass without pretreatment (Kataeva et al. 2013; Resch et al. 2013).
- Compared various biomass pretreatment strategies including ammonia fiber expansion (AFEX™), dilute acid, and ionic liquids, informing the selection of appropriate pretreatment strategies for different processing configurations (Lau, Gunawan, and Dale 2009; Li et al. 2010).
- Provided the first genome-wide analysis of *Streptomyces* sp. ActE, an aerobic bacterium associated with wood-boring wasps, revealing key enzymes used to deconstruct cellulosic biomass (Takasuka et al. 2013).
- Developed ionic liquids for efficient pretreatment by completely solubilizing the plant cell walls of switchgrass, corn stover, and other plants, resulting in the separation of lignin and cellulose chains and significantly improving subsequent breakdown to fermentable sugars (Groff et al. 2013; N. Sun et al. 2013; Shi et al. 2013; Sen et al. 2012).
- Discovered that down regulation of a single lignin synthesis gene reduces recalcitrance with no apparent growth defects and leads to increased ethanol production and a reduced need for harsh pretreatment protocols (Fu et al. 2011).
- Identified and produced highly efficient cellulases and other hydrolytic enzymes from directed investigation of microbial communities and metagenomics-driven bioprospecting (Aylward et al. 2013; DeAngelis et al. 2010).
- Used a mixture of water and a recyclable green solvent called gamma-valerolactone (GVL) to generate high yields of solubilized sugars from biomass that were then separated from GVL into a concentrated aqueous phase compatible with subsequent upgrading into biofuels by chemical or biological processes (Luterbacher et al. 2014).



In their first 6 years of operation, the U.S. Department of Energy's three Bioenergy Research Centers (BRCs) have made numerous advances toward overcoming critical challenges to cost-effective production of renewable fuels from biomass. BRC advances listed below are key to improved biomass feedstocks, deconstruction, and conversion, as well as the cross-cutting areas of enabling technologies and sustainability. Results from BRC research are grouped under their respective challenges and goals.



Conversion

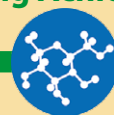
Develop improved methods of converting plant sugars into fuels, along with synthesis processes for new fuels in addition to ethanol.

Advances and Results

- Developed genetic tools for metabolic pathway engineering of thermophilic cellulolytic bacteria and demonstrated substantially increased biofuel production, providing a new paradigm for consolidated bioprocessing of fuels and other products from biomass (Cha et al. 2013; Chung et al. 2012; Li et al. 2012).
- Discovered and evolved yeast strains with increased tolerance of inhibitors to bioconversion of biomass, demonstrating improved biofuel yields (Dunlop et al. 2011; Rutherford and Mukhopadhyay 2012; Jin et al. 2013).
- Engineered *Escherichia coli* to degrade both cellulose and hemicellulose from switchgrass and to express metabolic pathways directing conversion of the resulting sugars into drop-in automotive biofuels or a jet fuel precursor terpene compound (Bokinsky et al. 2011).
- Used catalytic conversion of lignin (currently a waste product) to produce chemical feedstocks that potentially could replace petroleum-based compounds (Rahimi et al. 2013; Azarpira, Ralph, and Lu 2013; Varanasi et al. 2013).
- Identified the terpene compound bisabolane as a promising bio-synthetic fuel molecule with properties similar to D2 diesel and engineered microbial platforms for overproduction of its immediate precursor, bisabolene (Peralta-Yahya et al. 2011).
- Developed a synthetic system capable of dynamically regulating microbial metabolic pathways, enabling higher yields of fatty acid-based fuels and chemicals (Zhang, Carothers, and Keasling 2012).
- Genetically engineered a cellulose-degrading microbe, *Clostridium cellulolyticum*, to produce isobutanol directly from cellulose, the first demonstration of consolidated bioprocessing for producing next-generation biofuels (Higashide et al. 2011).



Cross-Cutting Achievements



Enabling Technologies

Develop new technologies to facilitate and accelerate BRC research, including, but not limited to, high-throughput laboratory technologies and computational and information systems, several of which have applications to biological research as a whole.

- Developed computational modeling approaches to more accurately predict the effects of genetic manipulations of metabolic flux in biofuel-producing microbes, enabling more effective metabolic engineering strategies for improved synthesis (Kim and Reed 2012).
- Developed a combination of carbohydrate arrays and nanoscale mass spectrometry to rapidly screen novel environmental microbes for cellulose-degrading enzyme activities against a wide range of plant compounds (Greving et al. 2012; Reindl et al. 2012).
- Created a high-throughput platform to handle tens of thousands of feedstock samples, both from transgenic and natural variants, to determine the chemical, structural, and genetic features that make cellulosic biomass recalcitrant (Studer et al. 2010; Decker et al. 2009; Foster, Martin, and Pauly 2010a and 2010b).
- Developed a web resource, dbCAN, for automated carbohydrate-active enzyme (CAZyme) annotation on a genome scale for any given protein dataset (Yin et al. 2012).
- Developed and optimized experimental and bioinformatics protocols for proteome measurement based on high-performance mass spectrometry to generate a high-coverage proteome atlas for *Populus*, enabling a first-time, systems-wide survey of all the changes associated with a given cellular perturbation (Abraham et al. 2013).

Sustainability and Economic Analysis

Produce biomass for biofuels with no negative impacts on food production or the environment.

- Identified marginal lands for cellulosic ethanol production from crops such as switchgrass, with climate benefits twice those of feed corn; quantified the impact of greater bioenergy feedstock demand on the agricultural landscape (Gelfand et al. 2013).
- Developed a sustainability index for various cropping systems by quantifying ecosystem changes in response to differences in land use and growth of different feedstocks—including *Miscanthus*, switchgrass, and mixed prairie grasses—to determine productivity (Zhang et al. 2010).
- Defined a science-based land-use policy that includes dedicated bioenergy crops (Egbenkewe-Mondzozo et al. 2013).
- Developed a wiki-based, publicly available technoeconomic analysis of biofuels production (Klein-Marcuschamer et al. 2010).

DOE Bioenergy Research Center Strategies at a Glance

Overcoming the complexity of the three biological grand challenges to achieve industrial-scale bioenergy production requires the coordinated pursuit of numerous research approaches to ensure timely success. Collectively, the DOE Bioenergy Research Centers (BRCs*) provide a portfolio of diverse and complementary scientific strategies that address these challenges on a scale far greater than any effort to date. Building on advances made during their first 5 years, BRC strategies being pursued during the second 5-year phase are listed briefly in the table below.

Develop Next-Generation Bioenergy Crops by Unraveling the Biology of Plant Development

Center Strategies	• BESC: Improve molecular-level understanding of biomass polysaccharide and lignin synthesis and cell wall assembly to gain in-depth mechanistic understanding of recalcitrance in two dedicated biomass crops, poplar and switchgrass; use genetic engineering and select natural feedstock variants to reduce recalcitrance and improve biomass characteristics. • GLBRC: Investigate how genes affect cell wall digestibility in model plants, cornstalks, and switchgrass; engineer new forms of lignin that facilitate biomass digestibility. • JBEI: Increase the understanding of genes and enzymes involved in the synthesis and modification of plant cell walls that impact deconstruction into sugars and fermentation into biofuels; use synthetic biology and genomics to re-engineer plant cell wall synthesis for improving deconstruction.
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Discover and Design Enzymes and Microbes with Novel Biomass-Degrading Capabilities

Center Strategies	• BESC: Experiment with microbes and microbial consortia to enable consolidated bioprocessing—a one-step, microbe-mediated process for directly converting plant biomass into biofuels; understand and engineer enzymes and microbial biocatalysts to achieve better biomass deconstruction. • GLBRC: Discover and improve natural cellulose-degrading enzymes extracted from diverse environments; improve enzymes created by the protein-production pipeline; analyze 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. • JBEI: Experiment with novel means of pretreating biomass using ionic liquids (salts that are liquid at room temperature); understand the impact of ionic liquids on downstream enzymes and microbes; and engineer enzymes that can tolerate high temperatures and ionic liquids to enable “one-pot” pretreatment and saccharification.
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Develop Transformational Microbe-Mediated Strategies for Advanced Biofuels Production

Center Strategies	• BESC: Engineer microorganisms capable of consolidated bioprocessing to synthesize next-generation biofuels. • GLBRC: Use systems biology approaches to improve biological and chemical methods for converting plant material into advanced biofuels and chemicals that can replace fossil fuels. • JBEI: Engineer new strains of <i>Escherichia coli</i> and yeast to more quickly and efficiently ferment sugars derived from cellulosic biomass into biofuels; design synthetic pathways to produce advanced biofuels that yield as much energy per volume as petroleum-based fuels and that can be shipped through existing fuel pipelines and burned in existing engines.
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* BESC: BioEnergy Science Center; GLBRC: Great Lakes Bioenergy Research Center; JBEI: Joint BioEnergy Institute

DOE Bioenergy Research Centers

(Center Research Strategies continued from p. 2)

energy-intensive (and costly) process. In addition to cellulose, plant cell walls also contain hemicellulose and other complex cell wall compounds, such as lignin, that together give plant tissues their structural strength. These other plant cell wall constituents are less amenable to breakdown and fermentation and impede cost-efficient biomass conversion to biofuels (see sidebar, Plant Cell Wall Recalcitrance, p. 8).

Many potential bioenergy 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. However, the availability of plant genome sequences has greatly accelerated bioenergy crop research, enabling scientists to more rapidly develop DNA markers to identify and isolate genes associated with beneficial traits that could improve crop yield, degradability, and sustainability. Mapping DNA markers and developing new tools for high-throughput genetic modification in plants are significantly reducing the time required to identify desired genetic variants and produce new energy crops.

The BRCs use genome-enabled approaches to gain a better fundamental understanding of plant cell wall synthesis to improve the bioenergy attributes of potential bioenergy crops. Genome-enabled studies have yielded the identity of key genes thought to be involved in cell wall synthesis and maintenance. Many of these genes are now under intense BRC investigation as potential targets for influencing cell wall composition via metabolic engineering approaches, for example. By understanding the genes and mechanisms that control cell wall synthesis, scientists are developing new energy crops with altered cellulose (or lignin) composition and modified linkages within and between cell wall components. These new bioenergy crops retain robust growth characteristics in the field but can be broken down to cellulose in a biorefinery much more cost effectively than the most prominent bioenergy crops currently in use. Other genome-enabled approaches focus on increasing the accumulation in plant tissues of starches or oils that can be converted into biofuels even more easily than cellulose. These same approaches also are being used to investigate other important cost-saving enhancements such as increasing biomass productivity per acre, increasing resistance to pests and drought, and decreasing the need for fertilizer applications and other inputs crucial to improving the sustainability of bioenergy crop production on marginal lands.

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

Nature uses both cellulases and multienzyme complexes known as cellulosomes to break down cellulosic biomass. In nature, biomass breakdown is relatively slow. For example, a fallen tree in the forest persists for quite some time before it completely degrades and disappears. BRC scientists studying the activity of cellulases and cellulosomes are optimistic that this process can be accelerated significantly by modifying known biomass-degrading enzymes and discovering new ones. Several factors govern the enzymatic deconstruction of plant polymers, including the (1) recalcitrant architecture of plant cell walls, (2) chemical and physical changes to biomass during pretreatment, and (3) structural features of the enzymes. Collectively, these factors and the high cost of commercially producing biomass-degrading enzymes contribute to the inefficiency and high cost of current enzyme-based biomass deconstruction approaches. Multiple strategies are under BRC investigation to address each of these factors, combining detailed understanding of the deconstruction process with the discovery or development of new or improved enzymatic approaches to biomass degradation.

Nature harbors a staggering diversity of microorganisms in environments such as the termite gut, cow rumen, compost piles, rainforests, hot springs, and other natural habitats where enzymatic breakdown of plant material drives these ecosystems. The BRCs have “mined” these environments for new, natural capabilities for efficient biomass breakdown. Each center continues to discover, characterize, evaluate, and develop new enzymes from these environments for biofuel production purposes.

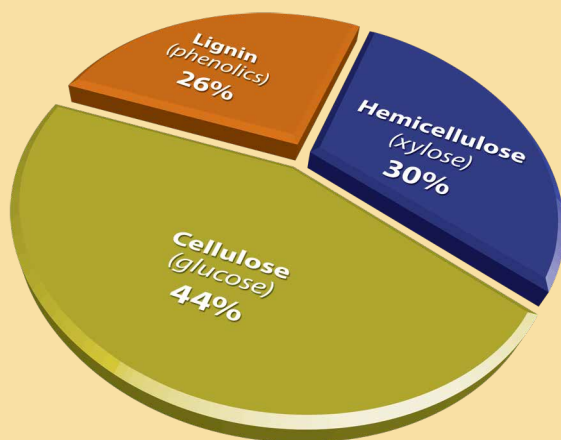
Discovering new biomass-degrading capabilities in nature is only part of the challenge. Molecular-level understanding of how enzymes degrade biomass is a prerequisite to designing improved processes. Because no single research approach can provide this understanding, each center is integrating different combinations of methodologies. These include high-throughput screens for genes, proteins, and metabolites; chemical and structural 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.

Plant Cell Wall Recalcitrance: A Key Scientific Challenge

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

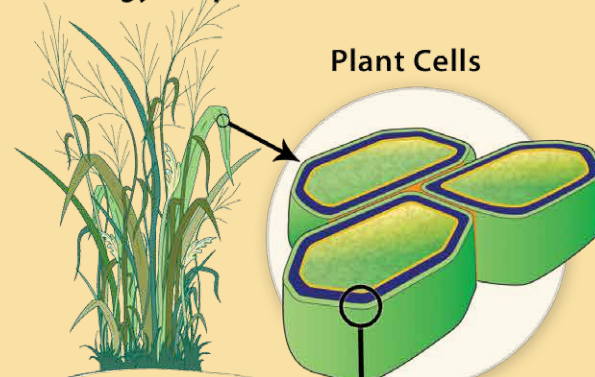
A large portion of the plant cell wall contains long chains of sugars (polysaccharides) that can be converted to biofuels. Cellulose—the most abundant biological material on Earth—consists of tightly bound sugar chains organized into strong cable-like structures (microfibrils). Like steel girders stabilizing a skyscraper’s structure, microfibrils reinforce plant cell walls. Locked away within the microfibril’s sugar chains are thousands of molecules of glucose, a type of 6-carbon sugar readily converted into biofuels by microbes. Physically accessing these sugars, however, is difficult.

Cellulose microfibrils are embedded within a matrix of other polymers (hemicellulose and lignin). Hemicellulose, a mix of branched polysaccharides made up of both 5- and 6-carbon sugars, links to a rigid noncarbohydrate polymer called lignin, which forms a protective coating to shield cellulose and hemicellulose from enzymatic attack. In addition to serving as a physical barrier to enzymes and microbes, lignin also is a source of chemical byproducts that can inhibit sugar conversion to biofuels.



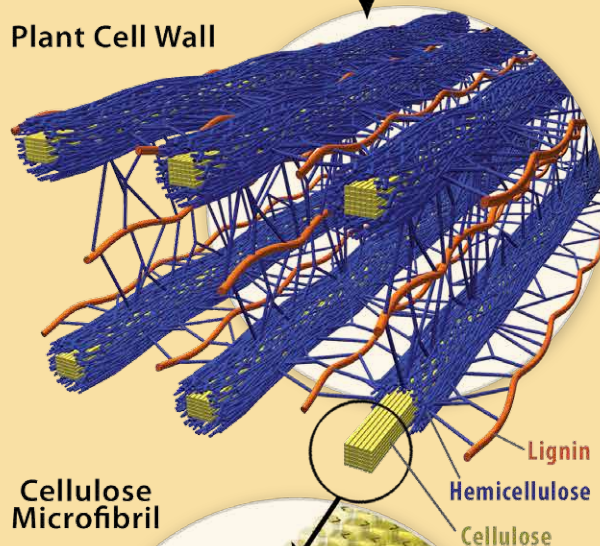
Approximate distribution of the three primary components of plant cell walls.

Bioenergy Crop

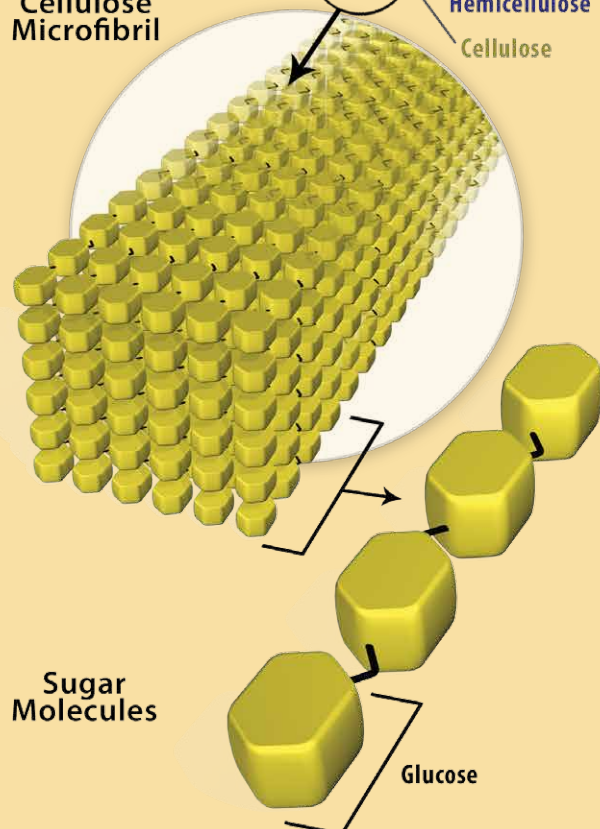


Plant Cells

Plant Cell Wall



Cellulose Microfibril



Sugar Molecules

Glucose

3. Develop Transformational Microbe-Mediated Strategies for Advanced Biofuels Production

Microorganisms can convert sugars derived from plant biomass to a range of different biofuels and bioproducts, but this activity can be altered by inhibitory and toxic compounds present in extracted biomass. These inhibitory conditions are created by the physical, chemical, and enzymatic processing steps that biomass is subjected to prior to fermentation. Biofuels themselves also can inhibit microbial fermentation at high concentrations. Costly extraction steps often must be incorporated into the treatment process to remove these inhibitory compounds. One method to reduce the overall cost of the biofuel production process is to develop robust microorganisms that can tolerate biorefinery conditions and still produce the desired biofuel. Consequently, the BRCs are using genome-enabled methods to understand stress-tolerance mechanisms in a variety of microbial strains used in biofuel production. These studies have led to the re-engineering of several cellular processes, including cross-membrane transport pathways to increase the tolerance of fermentative microbes to conditions found in a biorefinery process train and decrease the impact of biofuel product inhibition.

New BRC research is also exploring alternative microbial methods to use plant biomass more efficiently. Plant cell walls contain cellulose, the prime target for biofuel production purposes, but they also contain hemicellulose and lignin. Together, these polymers impart rigidity to plant cells and serve as the structural backbone of plants, thereby comprising a significant percentage of the total terrestrial plant biomass. To use these materials more efficiently, the BRCs are investigating the recovery, breakdown, and conversion of other cell wall components such as hemicellulose. Unlike cellulose, hemicellulose is comprised of both 5- and 6-carbon sugars that are less easily broken down into fermentable sugars. BRC researchers are targeting new processes to break down hemicellulose into its component sugars and developing new microbial strains to convert these sugars to biofuel products, as well as altering the nature of the hemicellulose incorporated into cell walls. These methods target more efficient uses of bulk plant biomass used for biofuel feedstocks and help increase biofuel yields from bioenergy plants on a per weight basis.

The BRCs also are developing ways to combine unit processes into consolidated bioprocessing (CBP) to further decrease costs and increase the overall efficiency of biofuels production. The CBP strategy combines cellulose deconstruction and sugar fermentation into advanced biofuels in a single step mediated by a “multitalented” microbe or mixture of microbes (called a microbial consortium). Approaches that use a CBP microbe or microbial consortium combine knowledge of microbial stress tolerance and fermentative pathways with genome-enabled techniques to incorporate the enzymatic machinery (developed from other organisms) necessary to break down cellulose polymers into component sugars. Unprecedented understanding of microbial systems is required to incorporate and optimize expression of numerous non-native genetic elements that catalyze the conversion of plant polymers to biofuels in one step.

BRC researchers are taking CBP a step further by determining the necessary metabolic pathway modifications to develop a range of high-energy “drop-in” biofuels as substitutes for gasoline and petroleum-derived diesel fuel. These advanced biofuels are compatible with existing internal combustion engines and fuel transportation infrastructure and contain as much energy per unit volume as gasoline or diesel. Jet fuel components have even been produced via metabolic engineering of microbes. Production of these fuels draws on remarkable developments in metabolic engineering, enabling BRC researchers to “borrow” enzymatic capabilities from other plants and microbes found in nature and express these traits in a modified host microbe engineered for biofuel production. These techniques are at the very forefront of biotechnological innovation and are laying the scientific foundation for developing numerous beneficial products, in addition to biofuels, from renewable biomass.

BESC Overview

Biomass recalcitrance—the resistance of plants to deconstruction—is the primary barrier to efficiently and economically accessing fermentable sugars for advanced biofuels that will directly displace petroleum. Understanding and overcoming this recalcitrance are central research themes of the DOE BioEnergy Science Center (BESC) led by Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee. BESC's defining goal is *to enable the emergence of a sustainable cellulosic biofuels industry by leading advancements in science and science-based innovation resulting in removal of recalcitrance as an economic barrier*. Convinced that biotechnological approaches hold the most promise for achieving these breakthroughs, BESC is developing plants that are easier to deconstruct and microbes that more effectively convert lignocellulose into simple sugars. When established by DOE in 2007, BESC sought researchers from institutions across the United States to bring breadth and depth of expertise to the challenge of overcoming biomass recalcitrance (see BESC Partners, p. 14).

Transformative advances in understanding recalcitrance require detailed knowledge of the chemical and physical properties of biomass that influence its resistance to degradation. Research has been aimed at determining (1) how these properties can be altered by engineering plant biosynthetic pathways, (2) how biomass properties change during pretreatment, and (3) how such changes affect biomass-biocatalyst interactions during deconstruction by enzymes and microorganisms. Historically, the term “recalcitrance” was coined to describe an overall phenotypic trait of biomass, namely the degree of difficulty in obtaining access to sugars complexed in the plant cell wall. However, based on new knowledge about cell wall chemistry, structure, and biochemistry, BESC researchers have redefined recalcitrance as a phenomenon in terms of pathways and interactions, both in cell wall formation and bioconversion. This increasing knowledge of the scientific basis of recalcitrance underpins the overall BESC goal of eliminating it as an economic barrier to cost-effective biofuel production.

In the past 6 years, BESC has made crucial progress toward understanding, manipulating, and managing plant cell wall recalcitrance and conversion. Notably, the BESC team proved the core concept that multiple genes control cell wall recalcitrance and that manipulating these genes potentially could



“The initial vision of BESC was to understand and overcome recalcitrance in plants, since this will lead to outcomes that impact the economics of biofuel production from lignocellulosic materials. Significant advances toward that goal have positioned BESC to directly address many underlying key cost factors in the overall process. From engineering microbes that consolidate multiple processes to modifying plants that exhibit reduced recalcitrance, the BESC team has moved the boundaries of cellulosic biofuel science to reveal insights that in turn have led to these achievements. Furthermore, BESC has demonstrated how to successfully run an organization whose collaborators are highly distributed both geographically and by scientific discipline. Based on the extensive body of scientific knowledge that BESC scientists have contributed to the field, the center’s goals now are to go well beyond proof of principle and deliver functional developments to the biofuels enterprise.”



Paul Gilna
BESC Director

– Paul Gilna

Paul Gilna is BESC’s director and deputy director of Oak Ridge National Laboratory’s Biosciences Division. With a multidisciplinary background in pharmacology, bioinformatics, computational biology, and microbial genomics, Gilna has led other large collaborative bioscience projects, including ones associated with GenBank, the DOE Joint Genome Institute, and CAMERA.* He also has served as a program director at the National Science Foundation.

*Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis



BESC Headquarters. ORNL’s Joint Institute for Biological Sciences facility in Oak Ridge, Tennessee, houses BESC administration and ORNL-based research staff. [Image courtesy Oak Ridge National Laboratory]

yield perennial biofeedstocks that are easier to deconstruct. This research paves the way for improving feedstocks directly or by genetically assisted breeding. In conversion science, BESC researchers have identified and validated key genes for consolidated bioprocessing (CBP), a game-changing, one-step strategy that uses a single microbe or microbial consortium to both deconstruct biomass and ferment resulting sugars into fuels. Researchers are beginning to modify CBP target organisms to improve conversion and enhance products. In addition, they have shown the potential of thermophilic (heat-loving) microbes in biomass conversion and identified the critical deconstruction enzymes for key components of ligno-cellulosic biomass. Currently, the BESC team is demonstrating the action of improved CBP on modified plant cell walls.

Research Focus Areas

BESC is organized into three research focus areas: (1) Biomass Formation and Modification, (2) Biomass Deconstruction and Conversion, and (3) Enabling Technologies—all supported by integrating activities. Some recent highlights of BESC research are featured on p. 15, and all BESC publications are available for download from the center's website, bioenergycenter.org.

1. Biomass Formation and Modification

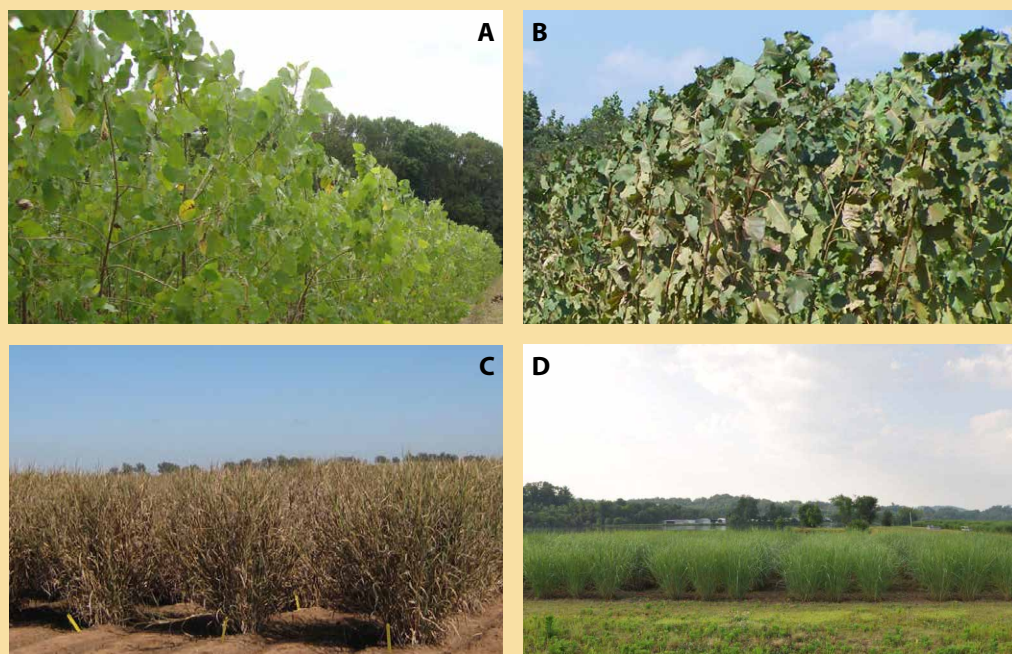
As part of its goal to reduce biomass recalcitrance, BESC conducts molecular-level investigations of cell wall assembly and polysaccharide and lignin synthesis to gain an in-depth mechanistic understanding of recalcitrance. Scientists also use targeted modification of plant cell walls and selection of natural feedstock variants to reduce recalcitrance and improve biomass characteristics. Although these methods can be applied to a wide range of woody and herbaceous plants, BESC

focuses on two potential bioenergy crops, poplar and switchgrass, with improved characteristics for converting cellulosic biomass to fermentable sugars. Researchers are moving promising plants with improved traits from greenhouses into field trials where their real-world usefulness can be evaluated (see figure, Field Testing of Improved Feedstocks, below). Successfully reducing recalcitrance will greatly facilitate the efficiency and economic viability of advanced biofuels.

2. Biomass Deconstruction and Conversion

Two key hypotheses drive Biomass Deconstruction and Conversion research at BESC: (1) microorganisms can be engineered to enable CBP and (2) enzymes and microbial biocatalysts can be understood and engineered to synergize with recalcitrance-reducing plant modifications to achieve better biomass deconstruction.

To test these hypotheses, BESC targets three microbial CBP platforms: yeast, *Clostridium thermocellum*, and *Caldicellulosiruptor* species. In yeast, a robust industrial ethanol producer, researchers have pursued genome modifications aimed at improving the expression and activity of enzymes that will digest cellulose. Already natural cellulose degraders, the other



Field Testing of Improved Feedstocks. BESC is conducting field trials of poplar and switchgrass species with enhanced biofuel-relevant characteristics. Testing includes (A) assessments of over 40 poplar constructs in South Carolina, (B) more than a thousand poplar genotypes in four common gardens in the Pacific Northwest, and genetically improved switchgrass plots in (C) Texas and (D) Tennessee. [Images courtesy BESC]

two microbes are thermophilic anaerobes whose metabolism is being manipulated by BESC to produce useful products such as ethanol or butanol.

Studies of microbial cellulose utilization at all levels—including enzyme components, enzyme systems, pure cultures, and mixed cultures—demonstrate the increased effectiveness of microbial cultures compared with free enzymes acting in the absence of cells. Enabling CBP will reduce the need for pretreatment chemicals toxic to microbes and will reduce the energy required to deconstruct and convert biomass into bio-fuels (see top figure, Optimizing Microbes for Biomass Deconstruction and Conversion, this page).

3. Enabling Technologies

BESC characterization and computational modeling efforts are focused on developing and applying chemical, immunological, physical, and imaging methods to characterize biomass. The resulting information is then used to identify relationships between biomass structure and recalcitrance.

BESC has developed a suite of new enabling technologies that support and enhance feedstock and CBP research. These technologies include high-throughput (HTP) assays for determining the recalcitrance properties of tens of thousands of feedstock samples (see bottom figure, Analyzing Biomass Recalcitrance, this page.) The information obtained from HTP pipelines for pretreatment, digestibility, and chemistry has enabled plant scientists to discover improved plant lines and single-nucleotide polymorphisms (SNPs) that link genotypic variation to cell wall chemistry in natural populations of poplar and switchgrass.

Tests that integrate enhanced-biomass grasses with cellulolytic microorganisms are showing greater effectiveness in conversion and biofuel production, supporting a BESC hypothesis that minimal to no pretreatment may be possible for some conversion regimes.

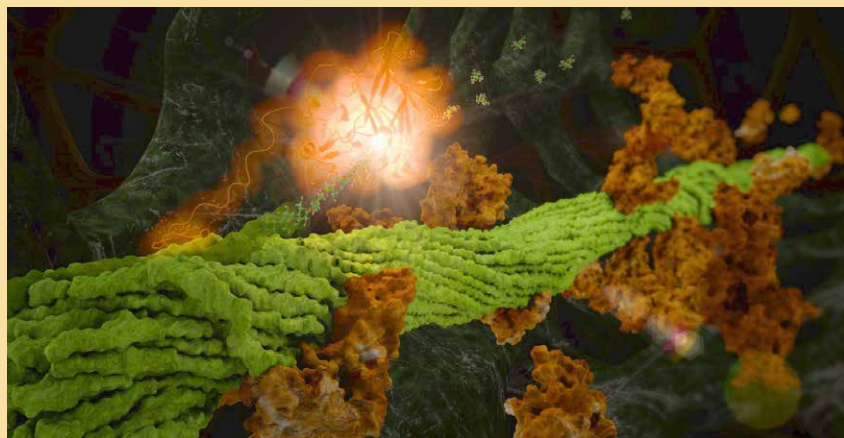
Optimizing Microbes for Biomass Deconstruction and Conversion. Kelsey Yee operates a process-controlled Applikon fermenter to evaluate how well *Caldicellulosiruptor obsidiansis* (a consolidated bioprocessing microbe) ferments simple sugars derived from poplar pretreated with dilute acid. [Image courtesy Oak Ridge National Laboratory]



Analyzing Biomass Recalcitrance. Steve Decker watches a robot dispense samples of powdered biomass into a reactor plate as part of a high-throughput recalcitrance pipeline for studying sugar release in potential biofuel feedstocks. [Image courtesy National Renewable Energy Laboratory]

Modeling and simulation tools developed by BESC researchers are being used to analyze various aspects of recalcitrance (see figure, Simulating Cell Wall Degradation, p. 13). Flux-balance analyses have provided insight into lignin synthesis, and several tools are publicly available within the BESC Knowledge-Base to analyze, for example, carbohydrate-active enzymes (CAZymes) and regulons.

These enabling technologies not only have advanced the science of biomass production more quickly, but also can be applied to numerous other areas of biological research.



Simulating Cell Wall Degradation. BESC researchers are using computational modeling to gain a molecular-level understanding of the plant cell wall and its major components, including cellulose (green) and lignin (brown). Here, the enzyme cellulase (orange) catalyzes cell wall breakdown. [Illustration courtesy Thomas Splettstoesser, www.scistyle.com, for Oak Ridge National Laboratory]

Other direct interactions with industry include partnering with Ceres, ArborGen, and GreenWood Resources, Inc., to evaluate improved bioenergy feedstocks. For example, Ceres and BESC are conducting field trials of modified switchgrass lines. Preliminary results from these trials, which are approved by the U.S. Department of Agriculture, closely follow those from greenhouse and laboratory studies. For field testing of woody feedstocks, ArborGen generates stable poplar transgenic species, including lines with altered cell wall composition. GreenWood Resources operates one of the field sites in a study examining 1,000 natural poplar variants now in its fourth year.

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. Using a "commercialization council" of technology-transfer and intellectual property (IP) management professionals from partner institutions, BESC evaluates the commercial potential of new inventions arising from BESC research and promotes and facilitates the licensing of BESC IP. A searchable BESC invention website is located under the Industry tab on the BESC home page (bioenergycenter.org), providing easy access to BESC IP available for licensing. Along with BESC researchers, industrial partners and members of the commercialization council attend and actively participate in the center's annual retreat.

Results from BESC's Biomass Deconstruction and Conversion research are being tested and applied by industrial partners Mascoma Corporation and DuPont. BESC works with Mascoma to test on a pilot scale the first CBP yeast strains able to express multiple hydrolytic enzymes while remaining robust enough to produce ethanol with little to no added commercial enzymes. Other yeast strains are studied at various levels—including pathways, enzymes, CAZymes, protein domains, SNPs, and indels—to identify functionally significant variations. BESC partners with DuPont to test improved plant lines in a bench-top simulation of the company's commercial process for lignocellulosic biofuel production.

In addition to these activities, BESC recently initiated a webinar series to inform interested industry professionals of BESC science. In the first webinar, BESC Director Paul Gilna provided an overview of the center. The series continued with webinars by BESC researchers Steve Brown and Neal Stewart highlighting, respectively, BESC microbial and "omics" capabilities and switchgrass field trials at the University of Tennessee.

Education and Outreach

To prepare the next generation of bioenergy scientists, BESC provides interdisciplinary research opportunities to graduate students, postdocs, and visiting scientists. Hands-on "Farming for Fuel" lessons educate students in the fourth–sixth grades about a bio-based fuel economy. Working with the Creative Discovery Museum in Chattanooga, Tennessee, BESC has reached 80,000 (~20,000 in year 6 alone) students, teachers, and parents nationwide. A free iPad app featuring a Biofuel Road Trip Challenge is available for download under the education category on iTunes. Materials and lessons are available at bioenergycenter.org/besc/education.cfm.

BESC Partners*

DOE's Oak Ridge National Laboratory (ORNL, 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. Additional resources such as supercomputers at the ORNL National Leadership Computing Facility are being used to investigate and simulate biomass reactions.

University of Georgia (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): NREL has more than 30 years of experience in biomass and biofuel research and houses premier 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 (UT): UT conducts successful programs in bioenergy-crop genetic and field research (particularly switchgrass) and biotechnological applications of environmental microbiology.

Dartmouth College: 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: 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: ArborGen provides expertise in forest genetics research, tree development, and commercialization.

Mascoma Corporation: Mascoma develops microbes and processes for economic conversion of cellulosic feedstocks into ethanol.

The Samuel Roberts Noble Foundation: 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: Ceres uses advanced plant breeding and biotechnology to develop and market nonfood crops with low-carbon footprints for next-generation biofuels and biopower.

DuPont: A leader in next-generation biofuels and bioproducts, DuPont has a conversion process under commercialization for cellulosic ethanol from corn stover. DuPont will test improved BESC feedstocks using its technology and, as warranted by bench performance, progress into process-development unit evaluations.

GreenWood Resources, Inc.: GreenWood develops and manages sustainable environmentally certified tree farms and is a world leader in the hybridization of fast-growing, high-yield poplar trees.

North Carolina State University (NC State): NC State is a leader in discovering and studying novel enzymes from thermophilic anaerobes to break down biomass.

University of California–Riverside (UC Riverside): Individual researchers at UC Riverside specialize in biomass pretreatment, characterization of plant-associated microbes, lignin biochemistry, and other related areas.

University of California–Los Angeles (UCLA): UCLA explores advanced biofuels in the context of other BESC deconstruction activities, including preliminary efforts on consolidated bioprocessing microbes for advanced biofuels and metabolic engineering to develop nonethanol products.

University of North Texas (UNT): Research at UNT focuses on using metabolic engineering to produce plant-derived chemicals that could be used, for example, to create biorenewable products and improve the quality of forage crops.

Cornell University: Individual researchers at Cornell focus on cellulose and enzyme modeling, lignin biochemistry, and the characterization of plant-associated microbes.

West Virginia University (WVU): As part of an ongoing association study of *Populus* supported by the National Science Foundation, WVU researchers have been developing analytical and technical tools that will be directly applicable in the association mapping component of BESC.

*See back cover for map showing partner locations.

BESC Research Highlights

Nanoscale Imaging Offers Insights for Improving Biomass Pretreatment and Processing

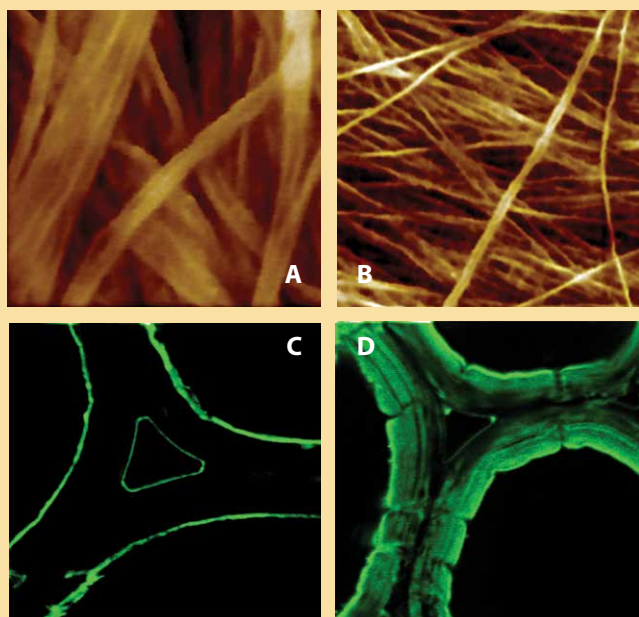
BESC researchers are building and applying imaging technologies and platforms to characterize the structure of plant biomass at the molecular level and assess how it is affected by chemical pretreatment. A BESC-developed integrated microscopy system combining atomic force microscopy, stimulated Raman scattering microscopy, and single-molecule spectroscopy was used for subnanometer imaging and quantitative chemical mapping of pretreatment and enzyme digestion in real time. With this new technology, researchers were able to localize the enzymatic sites of action without compromising the cell wall's structural integrity. The study revealed that biomass reactivity is determined by the nanoscale architecture of plant cell walls. Lignin, a major component of this architecture, physically impedes the accessibility of chemical and enzymatic catalysts to substrates. Results suggest that biomass pretreatment ideally should focus on eliminating lignin while leaving intact the structural polysaccharides within cell walls. Such pretreatment would result in a structure that allows easy access by enzymes and rapid digestion of polysaccharides, leading to more efficient and cost-effective biofuel production processes (Ding et al. 2012).

BESC scientists also have devised new imaging methods to study biomass-biocatalyst interactions. ORNL researchers at BESC received an R&D 100 Award for developing a mode-synthesizing atomic force microscope that allows molecular-level spectroscopic measurements of plant tissues at 50 nm resolution (Tetard, Passian, and Thundat 2010). Other advanced imaging methods have been applied to analyze, for example, surface biomass using time-of-flight secondary ion mass spectrometry/matrix-assisted laser desorption and ionization mass spectrometry (Jung et al. 2012), as well as cell wall lignin and cellulose using a coherent Raman scattering technique. In addition, BESC researchers have used total internal reflection fluorescence microscopy to image how enzymes interact with plant polysaccharides and have obtained nanometer-scale images of hydrated plant cells, which identified key parameters in reducing the recalcitrance of transgenic plants (Liu, Ding, and Himmel 2012).

Genes Beyond Those Involved in Lignin Synthesis Have Important Effects on Cell Wall Recalcitrance

Previously, the majority of genes responsible for plant cell wall recalcitrance were thought to be located in the metabolic pathways responsible for lignin synthesis. BESC's early work showed that recalcitrance, and thus biofuel yields, could be improved by manipulating genes in these pathways. However, in recent years, BESC researchers have demonstrated that additional cell wall biosynthesis genes outside the lignin pathway have significant effects on recalcitrance. These include:

- MYB4, a regulatory transcription factor that represses multiple biosynthetic genes in switchgrass (Shen et al. 2013).
- UDP-glucose pyrophosphorylase, a cellulose biosynthesis gene (Yang and Bar-Peled 2010).
- FPGS (folylpolyglutamate synthetase), a co-factor (Srivastava et al. 2011).
- Certain pectin synthesis genes (a surprising finding, since pectin makes up only a small fraction of mature secondary cell walls) (Atmodjo, Hao, and Mohnen 2013).
- The primary nucleotide-sugar precursors in xylan biosynthesis (Bar-Peled, Urbanowicz, and O'Neill 2012; Nag et al. 2012).
- Cell wall arabinogalactan proteins (Tan et al. 2013).



Imaging Enzyme Systems in Action. Atomic force microscopy reveals the different nanoscale architecture of cellulose microfibrils in (A) the plant primary cell wall and (B) secondary wall. Confocal laser scanning microscopy allowed BESC researchers to examine accessibility of the cell wall to two different enzyme systems: (C) bacterial cellulosomes and (D) fungal cellulases. [Images from Ding, S. -Y., et al. 2012. "How Does Plant Cell Wall Nanoscale Architecture Correlate with Enzymatic Digestibility?" *Science* 338(6110), 1055–60. Reprinted with permission from AAAS.]

BESC Research Highlights

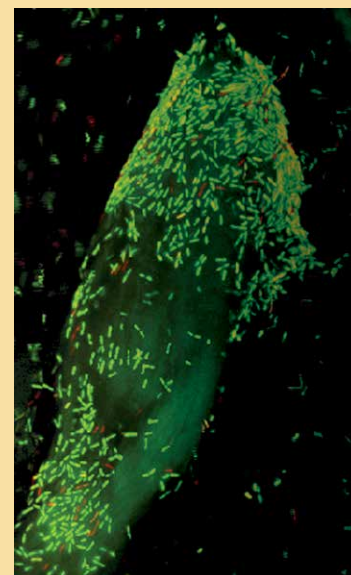
Genetic Engineering Boosts Thermophiles' Ability to Convert Biomass to Fuels

Because higher temperatures facilitate the deconstruction of lignin and release of simple sugars within plant biomass, thermophilic bacteria are promising candidates for biofuel production systems. The thermophiles *Clostridium thermocellum* and *Caldicellulosiruptor bescii*, in particular, hold great potential for consolidated bioprocessing (CBP), a strategy that uses a single microbe or culture to both deconstruct biomass and ferment resulting sugars into fuels. To take full advantage of this potential, BESC researchers are using new genetic tools to create CBP production strains of these bacteria with high yield and conversion. In *C. thermocellum*, researchers conducted various manipulations of the microbe's carbon, electron, and ethanol tolerance pathways. The most successful strategy to date improved ethanol yield to nearly a third of a gram of fuel per gram of carbohydrate (Deng et al. 2013). This yield represents more than a threefold increase over the wild type strain and is a significant step toward a theoretical goal of half a gram of fuel per gram of carbohydrate.

BESC scientists also have developed the first system allowing stable introduction of foreign DNA elements into *C. bescii*, which produces primarily lactate, acetate, and hydrogen as fermentation products. This breakthrough is based on identification of a *Caldicellulosiruptor* “immune system” that normally protects the bacterium from viral infection, destroying outside DNA before it can be integrated into the host genome. The BESC team developed a set of targeted nucleic acid modifications that protects DNA from the host immune system, allowing introduction of new genes and regulatory elements into the organism. This technology will enable metabolic engineering of *C. bescii* for direct conversion of lignocellulose to biofuels such as ethanol and butanol (Chung et al. 2012).

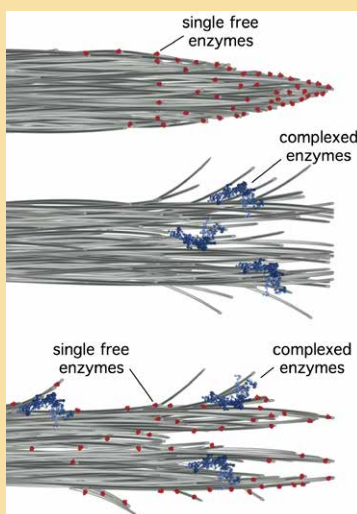
In related research, BESC demonstrated the first targeted gene deletion for this microbe—the gene encoding lactate dehydrogenase (*ldh*). The deletion was constructed within a nonreplicating plasmid (a small DNA molecule physically separate from chromosomal DNA) and then introduced into the *C. bescii* chromosome. Because the plasmid contains a gene for which there is both positive and negative selection, researchers were able to select first for recombination of the deleted *ldh* gene and then for loss of the plasmid sequences. Because this method allows for clean genetic insertions and deletions that leave no residual genetic material, it can be used repeatedly for metabolic engineering. The *C. bescii* strain containing the *ldh* gene deletion exhibited the expected changes in metabolism, namely the inability to produce lactate, leading to increased acetate and hydrogen production. This demonstration of a gene replacement strategy paves the way for further genetic manipulation of *C. bescii* to produce desired biofuel fermentation

products directly from plant biomass. Future goals target increased yield and titer as well as improved understanding of cellulolytic fermentation, enzymes, and regulation (Cha et al. 2013).



Leveraging Thermophiles for Biofuels. Members of the *Caldicellulosiruptor* genus—such as *C. bescii* and *C. obsidiansis* (shown here growing on crystalline cellulose)—hold great potential for lowering the cost of biofuel production processes. [Image reprinted with permission from *Journal of Proteome Research*, Jan. 1, 2012, 11(1). Copyright 2012 American Chemical Society.]

Understanding How Different Enzyme Systems Deconstruct Biomass. Illustration of the mechanisms by which free enzymes (top) and cellulosomes (middle) differ in their action on cellulose microfibril bundles and act synergistically to degrade cellulose (bottom). [Image reprinted with permission from the Royal Society of Chemistry from Resch, M. G., et al. 2013. “Fungal Cellulases and Complexed Cellulosomal Enzymes Exhibit Synergistic Mechanisms in Cellulose Deconstruction,” *Energy & Environmental Science* 6(6), 1858–67.]



Better Understanding of Microbial Deconstruction Mechanisms Paves Way for Optimizing Biomass Degradation

In addition to their natural ability to break down lignocellulose, *C. thermocellum* and *C. bescii* have the surprising capacity to extensively deconstruct biomass (especially grasses) after minimal or no chemical pretreatment, a typically harsh and expensive step in biofuel production. To understand the mechanisms underlying these abilities, BESC researchers compared

BESC Research Highlights

two enzyme systems for degrading biomass: “cell-free” fungal cellulases and cellulosomes—large multiprotein complexes that protrude from a bacterium’s surface, attaching to and digesting plant cell walls. The BESC team demonstrated that solubilization of the cell wall is far more effective when mediated by cellulolytic microbes (e.g., *C. thermocellum* and *C. bescii*) than by cell-free enzymes. Provisional patents were filed on the discoveries that CelA from *C. bescii* is the most active enzyme reported to date on Avicel cellulose and that *C. thermocellum* cellulosomes will enhance the activity of fungal hydrolytic enzyme cocktails. Improved understanding of the mechanistic differences between these two enzyme systems highlights new opportunities for combining them to enhance cellulose degradation and sugar release in biofuel production processes (Resch et al. 2013).

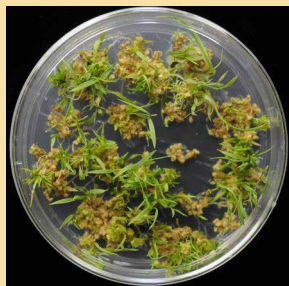
BESC researchers also have shown that *C. bescii* can simultaneously solubilize lignin, cellulose, and hemicellulose from switchgrass at high temperatures (78°C) without chemical pretreatment. Until now, no anaerobic microbe had been known to degrade lignin. Although *C. bescii* did not consume lignin, it removed it, thereby improving access to the plant’s energy-rich sugars. The discovery of a single microbe that can efficiently grow on plant biomass, degrade it, and biosynthesize fermentation products offers the potential for streamlining biofuel production (Kataeva et al. 2013).

New Genetic Tools and Database Help Advance Switchgrass Analysis

When BESC was formed, methodologies for the genetic transformation of switchgrass (*Panicum virgatum* L.) were relatively inefficient. Over the last 6 years, BESC has created a rapid, stable transformation protocol for switchgrass that has now been standardized across the center. This protocol has improved the transformation efficiency from 20% to >90% and decreased the turnaround

time for generation of new transformants from months to weeks (Wang 2013, unpublished; Nageswara-Rao et al. 2013).

To support gene discovery and analysis in switchgrass, BESC researchers also developed an integrated transcript sequence database and a gene expression atlas containing quantitative transcript data for most genes in all the major switchgrass organs. Researchers assembled full-length or partial mRNA sequences for a majority of the plant’s functional genes and developed a web server (switchgrassgenomics.noble.org) for hosting all switchgrass datasets. This site serves as a robust tool for functional genomics and breeding in switchgrass, providing annotations that will help users formulate hypotheses about gene function. Because the server also handles RNA-seq data, it can act as a “one-stop” shop for switchgrass transcriptomics (Zhang et al. 2013).



Improving Tools for Studying Switchgrass. BESC-developed genetic transformation protocols and databases for switchgrass are helping accelerate research of this bioenergy feedstock. [Images courtesy University of Georgia]

Glycome Profiling Reveals New Structural Clues about Role of Lignin in Cell Wall Recalcitrance

A new study that uses glycome profiling to monitor biomass changes in structure and sugar extractability during pretreatment suggests that lignin content *per se* does not affect recalcitrance. Instead, it is the integration of lignin and polysaccharides within cell walls—and their associations with one another—that appear to play a larger role. To better understand how the pretreatment process reduces plant cell wall recalcitrance, researchers adapted glycome profiling protocols to develop a medium-throughput technique to analyze poplar samples from BESC and stover samples from the Great Lakes Bioenergy Research Center. Glycome profiling uses polysaccharide antibodies to identify changes in plant cell wall polysaccharide epitopes. Results from this study demonstrated the sequence of structural changes that occur in plant cell walls during pretreatment-induced deconstruction, namely, the initial disruption of lignin-polysaccharide interactions in concert with a loss of pectins and arabinogalactans; this is followed by significant removal of xylans and xyloglucans (Pattathil et al. 2012).

GLBRC Overview

The DOE Great Lakes Bioenergy Research Center (GLBRC) is led by the University of Wisconsin–Madison, in close partnership with Michigan State University. 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.

The center supports nearly 400 researchers, students, and staff spanning a wide array of disciplines, from microbiology and plant biology to engineering and economics (see GLBRC Partners, p. 21). The innovations born of these unique collaborations provide the basic scientific foundation for the sustainable, large-scale production of advanced cellulosic biofuel technologies that will help meet the nation’s growing energy needs.

Taking full advantage of the diverse expertise of its research team, GLBRC leadership is focusing on two key knowledge gaps: sustainable production of crops with desirable biofuel traits and efficient conversion of biomass into fuels and chemicals. The economic and environmental sustainability of cellulosic technologies depends greatly on how biofuel crops are produced and whether they compete with food production for land use. Optimal sustainable biofuel crops will have different traits than those of crops used solely for food. Alternatives to cellulosic ethanol—referred to as advanced biofuels—also hold promise for the automotive, diesel, and aviation sectors. The GLBRC research portfolio is strategically positioned to increase emphasis on production of advanced biofuels and to modify deconstruction and conversion technologies to maximize the efficiency of lignocellulosic biomass processing.

As the center has matured, it has increased its focus on areas that the biofuels industry categorizes as obstacles to the sustainable, efficient, and cost-effective production of energy-dense fuels and chemicals from lignocellulosic biomass. One such obstacle is lignin, and GLBRC researchers have made important advances that will reduce the energy needed to use this promising source of fuels and chemicals. By applying unique alkaline pretreatment strategies, the GLBRC hopes to harness a reliable lignin stream that will open up tremendous possibilities for coproducts and materials that can add value to the biofuels pipeline.



“GLBRC researchers, in partnership with the state of Wisconsin, the state of Michigan, and affiliated industries, have made substantial progress toward developing the next generation of advanced biofuels. We are committed to building on these scientific breakthroughs and accelerating our efforts to develop sustainable biofuel strategies, from growing plants for use as energy feedstocks to exploring novel ways to convert the non-edible components of plants into fuels for the automotive, diesel, and aviation sectors.”



Tim Donohue
GLBRC Director

– Tim Donohue

Tim Donohue is GLBRC’s 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.



Located on the University of Wisconsin–Madison campus, the Wisconsin Energy Institute opened in April 2013. [Image courtesy GLBRC]

From an organismal perspective, researchers are closely examining the stressors that keep microbes from doing their jobs effectively. Going forward, the GLBRC will continue using genomic tools to discover insights that will enable microbes to handle those stressors. From a broader perspective, GLBRC sustainability researchers have been concerned since the center's inception about how to introduce bioenergy crops into the landscape without causing unintended environmental, economic, or social consequences. One possible and promising solution is the use of marginal lands. For more details on these and other GLBRC studies, see Research Highlights, p. 22.

Research Focus Areas

With a focus on sustainable biofuel production as an organizing principle, the GLBRC science portfolio is built upon four integrated research areas: Sustainability, Plants, Deconstruction, and Conversion (see figure, GLBRC Research, below). Research support activities within the center enable faster, more collaborative research and engage scientists in training future bioenergy leaders.

1. Sustainability

For the bioenergy economy to have a positive impact on the United States, complex issues in agricultural, industrial, and behavioral systems must be addressed. To determine the best practices for biofuel production, GLBRC researchers study issues such as minimizing energy and chemical inputs for bioenergy crop production; reducing greenhouse gas emissions

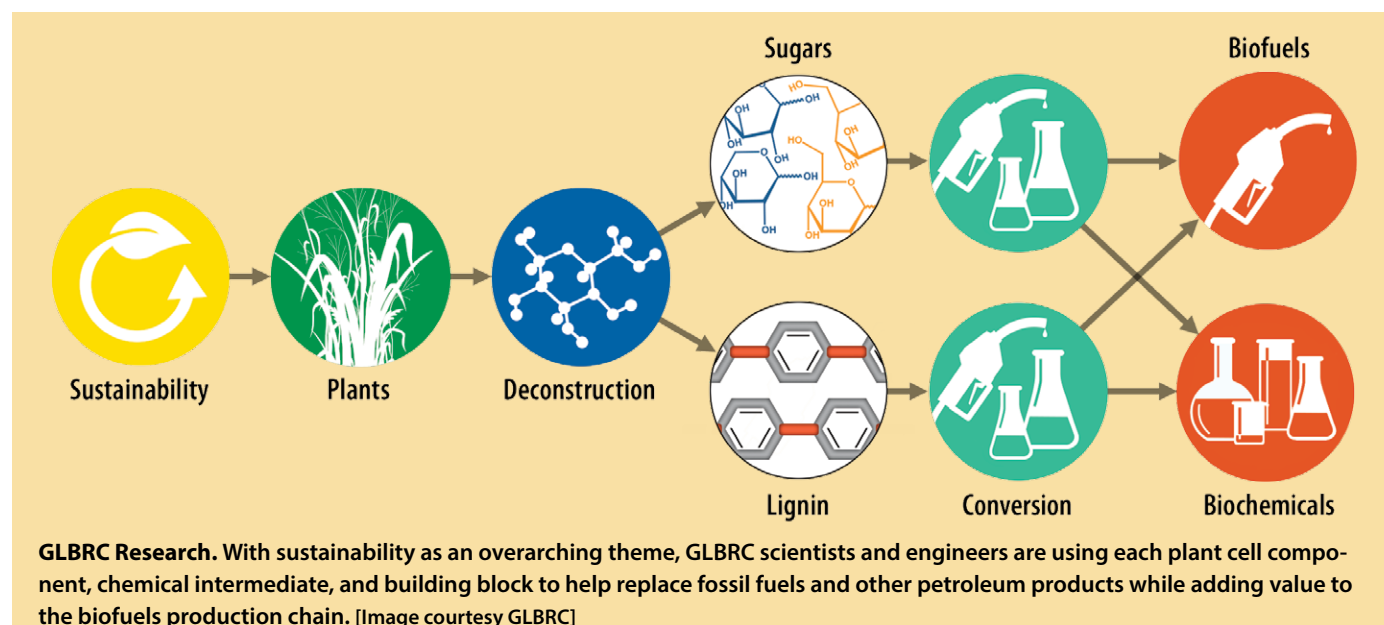
from the entire biofuel production life cycle; and understanding the environmental impacts of removing leftover stalks, stems, and leaves from food crops. GLBRC scientists also study the social and financial incentives needed to promote the adoption of more environmentally beneficial practices.

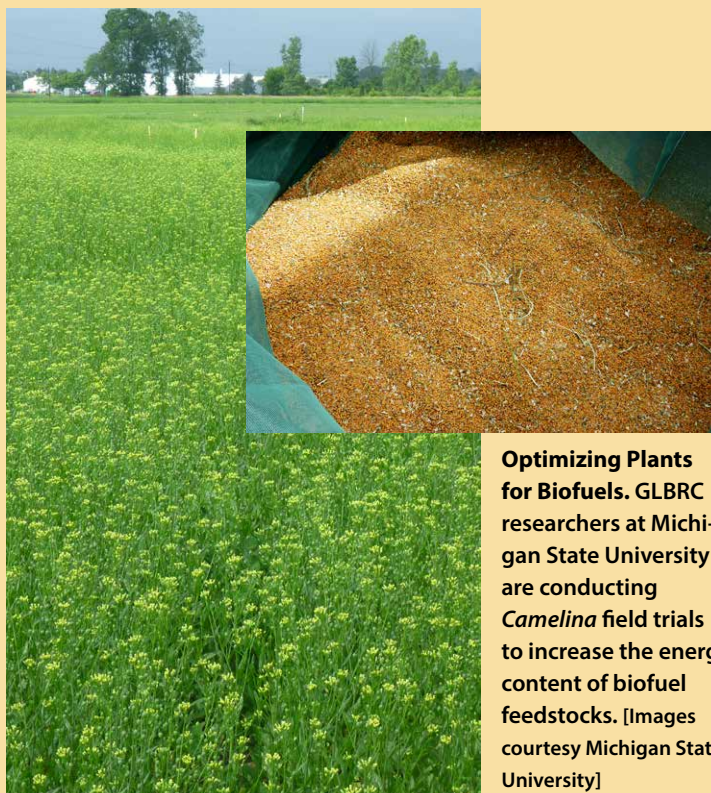
2. Plants

With the goal of improving the traits and sustainable production of bioenergy crops, GLBRC researchers are investigating how genes affect cell wall digestibility in model plants, cornstalks, and switchgrass (see figure, Optimizing Plants for Biofuels, p. 20). A key breakthrough in bioenergy feedstock development has been the engineering of new forms of lignin that promote biomass digestibility. Modified lignin molecules can significantly decrease the time and energy required for biomass processing by making recalcitrant plant tissues easier to break down and convert to fuels. Additionally, GLBRC researchers are breeding plants that produce more hemicelluloses and oils that can be converted into biofuels. They are increasing the energy density of grasses and other nontraditional oil crops by manipulating the metabolic and genetic circuits that control accumulation of oils and other easily digestible, energy-rich compounds in plant tissues.

3. Deconstruction

Located at the intersection of America's agricultural heartland and its northern forest, the GLBRC has access to a rich diversity of raw biomass for study. GLBRC Deconstruction researchers

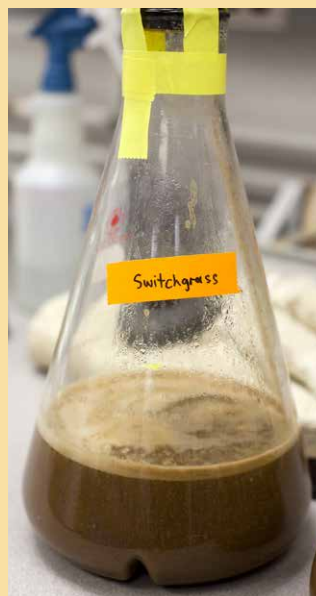




Optimizing Plants for Biofuels. GLBRC researchers at Michigan State University are conducting *Camelina* field trials to increase the energy content of biofuel feedstocks. [Images courtesy Michigan State University]

Improving Biomass Conversion. Hydrolysate is a sludgy mix of partially digested plant material resulting from the enzymatic hydrolysis of biomass. During fermentation, microbes encounter sugars in hydrolysate plus a variety of other compounds, some of which may slow

their ability to create fuel. GLBRC researchers are studying ways to make this process more efficient. [Image courtesy GLBRC]



are discovering and improving natural cellulose-degrading enzymes extracted from diverse environments. Improved enzymes created by the GLBRC protein-production pipeline are used in analyzing 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. Deconstruction researchers also are exploring ways to add value to these processes by developing pretreatment technologies that can yield additional compounds and coproducts useful for energy applications.

4. Conversion

The need for increased quantity, diversity, and efficiency of biomass-derived energy drives GLBRC Conversion research. Along with continuing to develop cellulosic ethanol processes, the center is moving forward with a focus on improving biological and chemical methods for converting plant material into advanced biofuels and chemicals that can replace fossil fuels (see figure, Improving Biomass Conversion, above).

Research Support

Crossing all research areas, GLBRC's Core Facilities area provides cutting-edge technologies that facilitate the innovative discoveries and creative solutions needed to advance bioenergy research. These facilities provide specialized high-throughput screening for plant cell wall digestibility and chemical composition, plant transformation, proteomics, and metabolomics. This research support area provides core plant transformation facilities for *Arabidopsis*, *Brachypodium*, and maize. It also operates the hydrolysate production chain that uses quality-controlled methodologies to generate biomass hydrolysates for fermentation experiments.

The Informatics and Information Technology (IIT) team develops and delivers optimal computational solutions and ensures their effective adoption in support of the GLBRC's overall mission. IIT focuses on the delivery and management of a laboratory information management system and the creation of tools and methodologies for collaboration and knowledge management among GLBRC staff. The team also works closely with center scientists to support experimental design, data analysis, and interpretation, enabling practical and conceptual breakthroughs in biofuels production.

GLBRC Partners*

University of Wisconsin–Madison (lead institution): The GLBRC’s lead partner provides world-renowned expertise in genome-enabled analysis of plant and microbial pathways, networks, and systems; computational analysis of bioenergy proteins, organisms, and ecosystems; and discovery, production, and improvement of bioenergy enzymes.

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

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

Lucigen Corporation: Lucigen provides valuable expertise in bioprospecting for new biomass deconstruction enzymes.

*See back cover for map showing partner locations.

DOE’s Pacific Northwest National Laboratory (PNNL): PNNL enables the GLBRC to analyze the entire life cycle of bioenergy practices.

University of British Columbia (UBC): Work at UBC contributes additional lignin expertise to the GLBRC’s Plants area, including plant cell wall lignification and cross-linking to modify structure and improve biomass processing.

University of New Hampshire (UNH): GLBRC Sustainability researchers at UNH investigate how bioenergy crops influence the composition and enzymatic activity of soil organic matter.

University of Toledo: Within the GLBRC’s Sustainability area, experts in environmental studies work to understand the biogeochemical cycling of bioenergy crop production.

Texas A&M University: As part of the Plants area, Texas A&M researchers study sorghum as a source of stem sugars for energy production.

Education and Outreach

The staff and partners of the GLBRC Education and Outreach team inform a variety of audiences about biofuels research, energy concerns, and sustainability issues affecting our planet. The team’s goal is to broaden the understanding of current bioenergy issues for the general public, as well as students and educators at K–12, undergraduate, and graduate levels. In the GLBRC’s suite of bioenergy education materials for teachers and students, a strong emphasis is placed on using critical thinking, quantitative reasoning, and systems-based logic. Because bioenergy research and development are important contemporary issues, Education and Outreach members present GLBRC research to diverse audiences at numerous events and programs in an accessible and interesting way.

Industry Partnerships

The GLBRC uses a fundamental, systems-driven, and genome-informed basic science approach within a project management environment and thus operates primarily in the early research and development arena. Once technology is developed, the center works closely with industry partners—through technology transfer mechanisms or collaboratively—to achieve commercial implementation.

GLBRC intellectual property is protected by the Wisconsin Alumni Research Foundation (WARF), which was established in 1925 as the world’s first university-based technology transfer office. WARF also supports GLBRC innovation by conducting intellectual property surveys to guide new research and by licensing discoveries to companies for commercial uses that benefit society. With assistance from WARF, GLBRC researchers will begin scaling up a process that uses a recyclable organic solvent called gamma-valerolactone to generate high yields of sugars from cellulosic biomass. The sugars produced from this process can then be chemically or biologically upgraded into advanced biofuels (see *Using Green Solvents to Produce Sugars from Biomass*, p. 24).

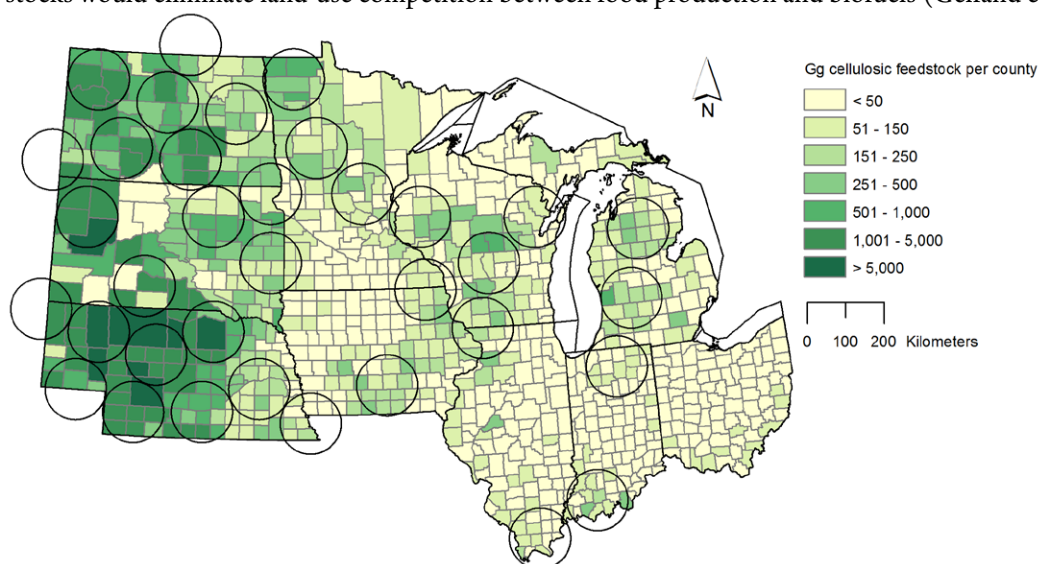
The first patent and license on GLBRC technologies were both issued in 2012, with many more expected in the next 5 years. The center has submitted 66 patent applications, 82 invention disclosures, and 23 licenses since its inception in 2007.

As one of the GLBRC’s primary industry partners, the Michigan Biotechnology Institute (MBI), a subsidiary of the Michigan State University Foundation, has worked with research area leaders to lower the commercial risk of potential biofuel technologies. In addition, the private company Hyrax Energy, Inc., was founded in 2011 based on GLBRC research. As the first company to emerge from GLBRC research, Hyrax also secured the first license on a GLBRC technology.

GLBRC Research Highlights

Examining the Capacity of Marginal Lands to Support Biorefineries

An issue concerning cellulosic biofuel systems is the use of land for bioenergy feedstocks versus food crops. Marginal lands offer a potential solution because they are unsuitable for growing food crops but can support grasses and other plants with bioenergy potential. Using 20 years' worth of long-term ecological research data from 10 midwestern states, GLBRC Sustainability researchers found that mixed-species plants from marginal lands produce as much biomass as traditional feedstocks such as corn grain. Moreover, these plants reaped twice the climate benefits of corn by sequestering more carbon in the soil and reducing fossil fuel consumption. Out of nearly 27 million acres of estimated marginal land, researchers found 35 locations in the 10 states that could each support a biorefinery with a capacity of at least 24 million gallons of ethanol per year. Including these sites, researchers identified enough marginal land in the study area to produce 5.5 billion gallons of ethanol per year, amounting to 25% of the mandated 2022 target for cellulosic biofuels. The results suggest that using marginal lands for bioenergy feedstocks would eliminate land-use competition between food production and biofuels (Gelfand et al. 2013).



Assessing the Use of Marginal Lands for Biofuels. Potential biomass collection areas for cellulosic biorefineries within 10 midwestern states. [Image reprinted by permission from Macmillan Publishers Ltd: Gelfand, I., et al. 2013. "Sustainable Bioenergy Production from Marginal Lands in the US Midwest," *Nature* 493(7433), 514–17 © 2013.]

Maximizing Switchgrass Productivity

GLBRC Plants researchers are developing resources and tools to improve switchgrass varieties by breeding this potential biofuel feedstock for climates similar to the midwestern and northern United States. In particular, researchers are applying genomic selection to plant breeding and developing a hybrid production system to maximize switchgrass productivity. Combining genomic selection, late-flowering winter-hardy genotypes, and hybrid deployment has the potential to double switchgrass yields by 2020 (Casler 2010).

Characterizing Switchgrass Diversity

In collaboration with the DOE Joint Genome Institute (JGI), BioEnergy Science Center (BESC), Joint BioEnergy Institute (JBEI), and others, GLBRC Plants researchers are enhancing resources that can characterize the natural diversity in switchgrass, including its reference genome sequence. This sequencing will characterize the genetic variation of structured populations and diverse genotypes of broad interest to the switchgrass community and will facilitate high-density genotyping necessary for switchgrass genomic selection, gene discovery, and evolutionary studies. These enhanced genetic resources will accelerate the breeding of switchgrass for use as a biomass crop (Zhang et al. 2011a).



Enhancing Bioenergy Feedstocks. GLBRC technician Nick Baker and researcher Rajan Sekhon harvest young switchgrass tissue for RNA and DNA analysis. [Image courtesy GLBRC]

GLBRC Research Highlights

Biomass-Deconstructing Enzymes from Wasp Communities

Biomass deconstruction is a major step in the cellulosic biofuels pipeline, and GLBRC scientists have looked to natural environments for solutions to breaking down cellulose. For example, Deconstruction researchers have isolated a new aerobic microbe called *Streptomyces* sp. ActE from a community of wood-boring wasps. ActE may aid development of specialized approaches to deconstructing woody materials because of an apparent evolutionary advantage enabling it to efficiently break down cellulose. Researchers used genome-wide transcriptomic and proteomic analyses to identify the suite of enzymes used to deconstruct crystalline cellulose and other pure polysaccharides that ActE secretes when grown on plant biomass. The mixture of enzymes obtained has biomass-degrading activity comparable to a cellulolytic enzyme cocktail from the fungus *Trichoderma reesei*. This example of high cellulolytic capacity in an aerobic bacterium is novel to biofuels research, and identification of these new enzymes greatly expands the repertoire available for biomass deconstruction (Takasuka et al. 2013).



GLBRC Pretreatment Technology. AFEXTM-treated corn stover with water before enzymatic hydrolysis, a process that breaks down cellulose polymers into simple sugars using cellulase enzymes. [Image courtesy GLBRC]

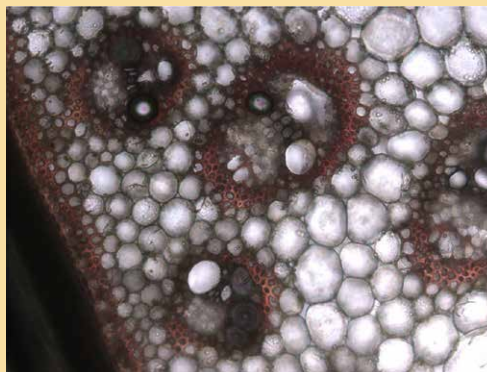
Adding Value to Biomass Processing with Lignin

GLBRC researchers are using highly selective catalysts to oxidize lignin molecules for conversion into valuable chemical feedstocks that potentially could replace petroleum-based compounds. Exemplifying the center's emphasis on transdisciplinary collaboration, the new method may facilitate lignin's use as a value-added chemical, rather than a waste product, of biofuel processing (Rahimi et al. 2013).

Accelerating Ethanol Production with Novel Yeast Strains

GLBRC Deconstruction and Conversion researchers have improved the efficiency of cellulosic biomass enzymatic hydrolysis and fermentation, decreasing the total time for ethanol production from over a week to two days. Using ammonia fiber expansion (AFEXTM), a biomass pretreatment technology, Deconstruction researchers developed the simultaneous saccharification and cofermentation process (SSCF) to combine enzymatic hydrolysis and fermentation of corn stover into a single reactor. Conversion researchers subsequently performed phenotypic screening and engineering of yeast to develop a strain with increased tolerance to the heat and degradation products present in SSCF reactions, as well as the ability to metabolize xylose. The collaboration resulted in a novel yeast strain able to thrive in the unique SSCF environment and produce ethanol concentrations comparable to a well-developed industrial benchmark strain (Jin et al. 2013).

Exploring New Uses for Lignin. As this cross-section of a corn stem reveals, lignin (stained red) accounts for a significant portion of the plant tissue.



GLBRC researchers are studying lignin as a source of useful chemicals rather than just a waste product of biofuel production. [Image courtesy Illinois State University]

GLBRC Research Highlights

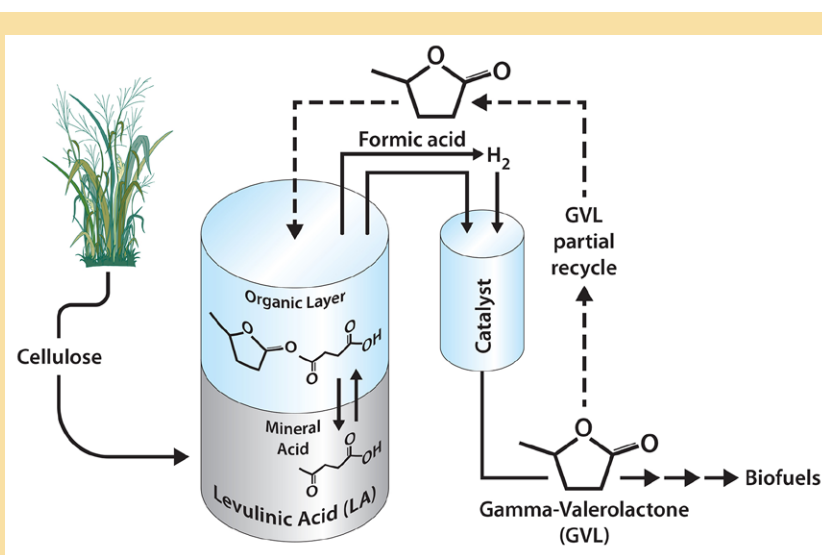
Understanding and Manipulating Lignin Biosynthesis

GLBRC Plants researchers played a key role in an international research project aimed at re-evaluating the lignin biosynthetic pathway. After researchers at Ghent University in Belgium identified a new gene that produces a previously unknown enzyme (caffeoyl shikimate esterase, or CSE) involved in lignin production, GLBRC researchers used nuclear magnetic resonance technology to analyze *Arabidopsis* mutants with knocked-out CSE genes. They found that turning off CSE production results in 36% less lignin per gram in *Arabidopsis* stem tissue, and that the remaining lignin has a significantly altered—and possibly more digestible—composition. Researchers plan to apply these findings to cellulosic bioenergy feedstocks such as poplar to reduce the amount of lignin and increase its digestibility, potentially lowering the cost and energy inputs required to convert cellulosic biomass to ethanol (Vanholme et al. 2013).

Using Green Solvents to Produce Sugars from Biomass

GLBRC Conversion researchers recently succeeded in obtaining valuable linear alpha olefins (LAOs) from an organic solvent called gamma-valerolactone (GVL) in a highly selective catalytic reaction. This process could provide an efficient and renewable option for manufacturers of plastics, detergents, and other traditionally petroleum-based materials (Wang et al. 2013).

Conversion researchers also used a mixture of GVL and water to recover solubilized sugars from biomass. GVL, which is itself obtained from cellulose, is a green solvent and thus can be recycled. High yields of solubilized sugars have been generated from this process, and an initial economic assessment of the technology has indicated that it could produce ethanol at a cost savings of roughly 10% when compared with current state-of-the-art technologies (Luterbacher et al. 2014). With support from the Wisconsin Alumni Research Foundation (WARF), the team will begin scaling up this process later this year. Under the WARF Accelerator Program, GLBRC researchers will construct a high-efficiency biomass reactor that will use GVL to produce concentrated streams of high-value sugars and intact lignin solids. Carbohydrates and lignin from the reactor will be delivered to scientific collaborators, where the fermentation potential of recovered sugars can be tested to create opportunities for integration (Wettstein et al. 2012).



Sustainable Catalytic Production of Gamma-Valerolactone (GVL). GLBRC researchers have used GVL, a recyclable solvent, to extract high yields of sugars from solubilized cellulose. This cellulose conversion approach eliminates the need to separate the final product from the solvent because the GVL product is the solvent. [Image courtesy GLBRC]

Metabolic Modeling of Microbes

GLBRC Conversion researchers are working to identify microbial strains that can thrive within the highly stressful fermentation environment. Mathematical modeling techniques have provided advances in this area by enabling unique insight into metabolic pathways. In 2012, researchers reported an improved method for predicting metabolic flux changes in key model microbes in response to environmental variation. The method, called RELATCH (RELATIVE CHange), uses gene expression data to accurately predict genome-scale flux distribution and corresponding enzyme contribution. RELATCH's ability to predict metabolic responses in *Escherichia coli*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* dramatically outperforms existing methods. These findings demonstrate the applicability of RELATCH for engineering microbial strains with improved biofuel production (Kim and Reed 2012).

JBEI Overview

The DOE Joint BioEnergy Institute (JBEI) is a partnership led by Lawrence Berkeley National Laboratory (LBNL) that leverages the scientific expertise, resources, and support of four national laboratories and three academic institutions (see JBEI Partners, p. 29). This multi-institutional research team is using the latest techniques in molecular biology, chemical and genetic engineering, and computational and robotic technologies to develop advanced biofuels. JBEI consolidates all biomass-to-biofuels research areas within three scientific divisions and one technologies division at its research center in Emeryville, California. Since 2007, JBEI has been addressing key roadblocks to converting lignocellulosic biomass into advanced fuels through an integrated, multidisciplinary approach.

In the Feedstocks Division, researchers are engineering non-food plants for optimal sugar yields and reduced resistance to breakdown. They have combined two highly targeted bioengineering strategies to redesign the cell walls of plants, resulting in a 20% increase in cell wall sugar content and a 2.5-fold increase in sugar yield. Meanwhile, researchers in the Deconstruction Division have demonstrated that certain ionic liquids—a novel class of biomass solvents—effectively pretreat a wide range of biomass types, facilitating enzymatic conversion to sugars. In a first-of-its-kind effort, they demonstrated a “one-pot,” wash-free process that combines biomass breakdown and sugar extraction into a single vessel. This process eliminates the excessive use of water and waste disposal currently associated with washing biomass after ionic liquid pretreatment. Refinement of this one-pot method could significantly simplify and lower the cost of biofuel production processes. In the Fuels Synthesis Division, JBEI’s pioneering work in synthetic biology has produced engineered microbes that transform the complex sugars derived from lignocellulosic biomass into biofuels that can directly replace petroleum-based gasoline, diesel, and jet fuel. These advanced biofuels do not require modification of today’s engines or fuel infrastructures and can be incorporated with no loss of performance. JBEI has produced a portfolio of intellectual property in feedstocks, biomass deconstruction, fuels synthesis, and enabling technologies to help advance the emerging biofuels industry. For more details on JBEI contributions to bioenergy research and development, see Research Highlights, p. 30.



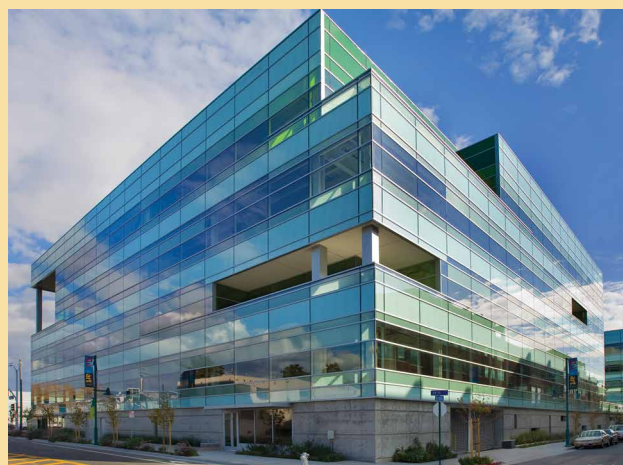
“At JBEI, we are inspired by the vision of moving the nation toward a sustainable, clean energy future. The threat of a warming planet and the need to diversify our energy sources while supplying transportation fuels for a growing population drive our research. JBEI was designed to be nimble and flexible enough to focus and refocus our research quickly and effectively. Our entrepreneurial culture acts as a bioscience incubator and has produced a portfolio of intellectual property in feedstocks, biomass deconstruction, fuel synthesis, bio-based products, and enabling technologies that will help fuel the economy and grow the emerging biofuels industry. Our scientists and staff envision a future when cellulosic biofuels provide transformative advantages for our nation.”



Jay Keasling
JBEI Chief Executive Officer

– Jay Keasling

Jay Keasling is the chief executive officer of JBEI, the associate laboratory director for Biological Sciences at Lawrence Berkeley National Laboratory, and the Hubbard Howe Jr. Distinguished Professor of Biochemical Engineering at the University of California–Berkeley. He is one of the world’s foremost authorities on synthetic biology.



JBEI Research Facility. JBEI researchers are located at a single site in Emeryville, California. [Image courtesy Lawrence Berkeley National Laboratory]

Research Focus Areas

JBEI brings the sunlight-to-biofuels pipeline under one roof in four interdependent research divisions that focus on (1) designing and developing new bioenergy crops, (2) enhancing biomass deconstruction, (3) developing routes to new biofuels through synthetic biology, and (4) creating technologies that advance biofuels research.

1. Designing and Developing New Bioenergy Crops

Researchers in JBEI's Feedstocks Division are improving the understanding of genes and enzymes involved in the synthesis and modification of plant cell walls using well-characterized genomes and genetic-engineering tools 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 crops such as switchgrass and poplar. Genetic insights from rice (a model for grasses and other monocots) and *Arabidopsis* (a model for trees and other dicots) will accelerate the breeding of new energy feedstocks (see figure, Developing Better Plants for Biofuels, below). This

knowledge is used in developing specialized fuel crops that are optimized for deconstruction into sugars and fermentation into biofuels and can thrive with little fertilization or irrigation on land not suitable for growing food crops.

In addition, scientists are investigating metabolic pathways involved in the biosynthesis of lignin, a tough structural material that shields a plant's energy-rich sugars from enzymatic attack. This unique basic research program could help transform lignin into a valuable source of chemicals and polymers while improving the economics of converting cellulosic biomass into fuels.

2. Enhancing Biomass Deconstruction

Unlike the simple starch-based sugars in corn and other grains, the complex polysaccharide sugars in nonedible plant cell walls are locked within lignin. The process of liberating fermentable sugars that can then be converted into fuels is called "deconstruction" and normally requires two steps: pretreatment and enzymatic hydrolysis. The Deconstruction Division is using ionic liquids (molten salts that are liquid at room temperature) as a novel means of pretreating biomass. Free of the inhibitors associated with other pretreatment methods, ionic

liquids help liberate high yields of fermentable sugars that can be converted to biofuels and biochemicals. Moreover, ionic liquid pretreatment is the only known technology that can efficiently process a wide range of single and mixed feedstocks, including agricultural and forest residues, grasses, and woody perennials. Researchers are investigating the effects of ionic liquids on cellulosic biomass and the recovery of sugars and lignin through selected liquid-liquid extraction and filtration (see figure, Improving Access to Energy-Rich Sugars, p. 27). Using advanced imaging and spectroscopy, coupled with computer simulations, scientists are obtaining a detailed, cellular-level understanding of the ionic liquid pretreatment process and are using this information to develop scalable and cost-competitive engineering solutions.

Another key aim of the Deconstruction Division is developing cost-effective enzyme mixtures that will tolerate potential biorefinery conditions, such as extremes of temperature



Developing Better Plants for Biofuels. April Liwanag, a research assistant in JBEI's Feedstocks Division, checks on seedlings of *Arabidopsis*, one of two model plants researchers are studying in efforts to design improved bioenergy crops. [Image courtesy JBEI]



Improving Access to Energy-Rich Sugars. Ning Sun is part of a team of researchers in JBEI's Deconstruction Division exploring the use of ionic liquids to pretreat biomass. [Image courtesy JBEI]

and pH or the presence of ionic liquids. JBEI scientists also are exploring a range of ecosystems, from rainforest floors to composts, to discover and isolate new microbial enzymes that can efficiently degrade cell wall polysaccharides and lignin. Studies of fungal biotechnology are under way to generate a genetic toolbox for more efficient protein production in fungi to expedite the discovery of high-performance industrial-strength enzyme mixtures that operate effectively at high biomass loading.

3. Developing Routes to New Biofuels Through Synthetic Biology

JBEI researchers are applying synthetic biology techniques and mathematical models of metabolism and gene regulation to engineer microorganisms to efficiently convert sugars released from deconstructed biomass into the energy-rich molecules of advanced biofuels such as alkanes. Scientists in the Fuels Synthesis Division are engineering new strains of *Escherichia coli*, the gut bacterium, and yeast, the common single-cell organism used in baking bread and making beer, to more quickly and efficiently ferment the sugars derived from cellulosic biomass into biofuels (see sidebar, *Fueling the Future Using Synthetic Biology Tools*, p. 28). Their goal is to produce advanced biofuels that (1) yield as much energy per volume as petroleum-based fuels, (2) can be shipped through existing fuel pipelines, and (3) can burn in existing engines. Biologically produced alkanes and other oil-like hydrocarbons could replace gasoline, diesel, and jet fuel on a gallon-for-gallon basis. Work also is being done

to develop experimental wetware, software, and laboratory automation devices that facilitate, accelerate, and standardize the engineering of microbes that produce fuels from cellulosic biomass.

4. Creating Technologies that Advance Biofuels Research

JBEI scientists are devising new, broadly applicable technologies to accelerate research that will lead to renewable biofuels. Among these are techniques for characterizing genes and proteins in both natural and engineered plants and microorganisms. Innovations include chip-based systems for identifying new enzymes with cellulose- and lignin-degrading activities. Researchers also have developed high-throughput methods using microfluidics and array platforms that can screen hundreds of enzymatic reactions simultaneously to help identify the best enzymes for biomass deconstruction. High-resolution imaging visualizes in greater detail plant cell walls and their components and also helps characterize the effects of pretreatment protocols on plant biomass. These and other enabling technologies are generating large volumes of data collected in a centralized database for computational analysis.

Industry Engagement

Accelerating the transfer of JBEI inventions to private industry is a critical aspect of the institute's mission. An innovative culture has resulted in collaborations with industry and a wealth of intellectual property available for licensing. Companies sponsoring research have access to world-class scientific expertise, capabilities, and infrastructure to speed time to market. JBEI researchers also stay current on industry challenges and opportunities by interacting with members of the Industry Advisory Committee who represent the entire spectrum of the biofuels industry including energy, agribusiness, and biotechnology. JBEI research is informed by real-world needs and standards through ongoing talks with representatives from a broad range of companies. To facilitate technology transfer, JBEI's director of commercialization is authorized to implement licensing agreements for all of JBEI's institutional partners. As of spring 2013, JBEI has partnered with 29 companies under a variety of arrangements and has spun off three startup companies. In addition, the rate of JBEI invention disclosures is trending at 2.5 times the rate of the top four U.S. universities, while the rate of patent licenses is two times greater (Association of University Technology Managers FY 2011–12 survey of academic institutions, www.autm.net).

Fueling the Future Using Synthetic Biology Tools

Many of JBEI's numerous successes during its first 6 years of operation stem from pioneering work in synthetic biology. Sometimes considered a new field, the concept of synthetic biology can be traced back to when humans first began trying to improve the usefulness, efficiency, or performance of things in the natural world around them. The goal has always been to change existing materials and resources or create new ones that do what raw resources cannot.

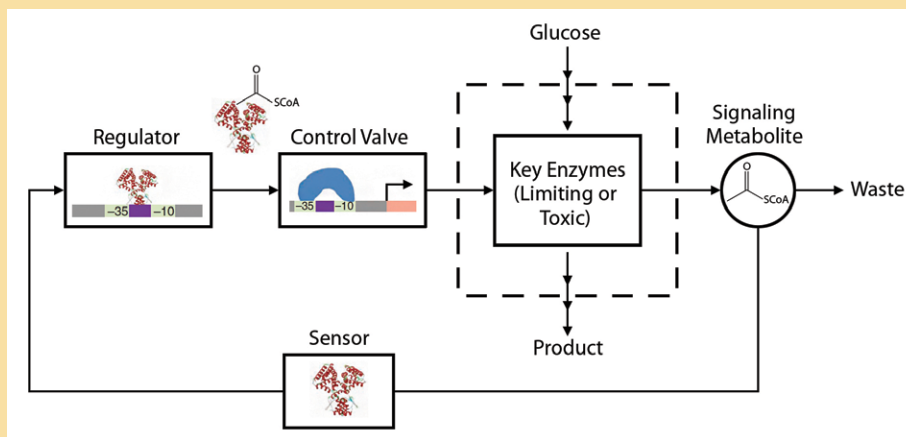
Today, synthetic biologists are designing and building biological systems for specific purposes, including biofuels. Since 1992, Jay Keasling, JBEI's chief executive officer, has been redesigning microbes to operate as miniature chemical reactors that transform sugars into valuable products.

"It's a lot like brewing beer from yeast and hops. But rather than alcohol being the end product, you program your microbe to produce fuels," Keasling says.

"Our goal at JBEI has been to put as much chemistry as we can into microbes. We graft genes from plants into the microbes. Once inside, the genes produce enzymes that do the chemistry to transform sugars into fuel," he says. "Enzymes can do in one step what might take many steps using synthetic organic chemistry."

Prominent achievements include engineering the first strains of *Escherichia coli* that can digest switchgrass and synthesize its sugars into gasoline, diesel, or jet fuel without any help from enzyme additives. Drawing on advances in molecular, cell, and systems biology, the growing synthetic biology toolset provides critical new insights into the natural world.

Synthetic biology techniques also have been used to engineer plants that produce more cellulose; less hemicellulose; and less-recalcitrant lignin, a trait enabling easier extraction of sugar-containing cellulose and hemicellulose. Together, these advances will increase sugar yields from energy crops, thereby reducing the cost of fuels derived from them. The promise of synthetic biology is the ability to turn any plant, including nonfood sources such as forest debris, weeds, even paper waste, into energy.



Leveraging Synthetic Biology for Bioenergy. As part of JBEI's synthetic biology research, scientists have developed a dynamic metabolite regulatory system that could help boost production of advanced biofuels and other valuable chemical products. [Image courtesy Lawrence Berkeley National Laboratory. See Farmer and Liao 2000; Zhang, Carothers, and Keasling 2012.]

To expedite commercialization of its research breakthroughs, JBEI conducts feasibility and scale-up tests using the facilities at LBNL's Advanced Biofuels Process Demonstration Unit (ABPDU). Located in Emeryville, ABPDU provides industry-scale test beds for discoveries made in the laboratory, including JBEI's work on ionic liquid pretreatment and microbial synthesis of bisabolene, a terpene-based precursor to an advanced biofuel that potentially could replace diesel.

In a study assessing the optimization and scaling potential of ionic liquid pretreatment, JBEI and ABPDU scientists and engineers determined that this technology can be effectively scaled to larger biorefinery operations (Chenlin et al. 2013). Using the study as a baseline, researchers will develop and apply improved biomass deconstruction processes to diverse feedstocks as a means to realize a commercially viable pretreatment method based on ionic liquids. In addition, unit operations

and process parameters obtained in the study will be essential for analyzing the technoeconomic aspects of this technology. The JBEI-ABPDU collaboration is the first of its kind in the research community, successfully identifying key opportunities and challenges associated with ionic liquid pretreatment and subsequent enzymatic saccharification beyond the bench scale.

JBEI also is working with ABPDU to scale up microbial production of bisabolene, a member of the terpene class of chemicals found in plants and used in fragrances and flavorings. When hydrogenated, bisabolene becomes bisabolane, a compound with fuel properties comparable to commercial diesel and also considered a promising jet fuel. Using the tools of synthetic biology, JBEI scientists engineered microbes that produce bisabolene from a simple carbon source. To increase production, JBEI collaborates with ABPDU, which has a sequential scale-up capacity from 1 L to 300 L. The team is working with

JBEI Partners*

DOE's Lawrence Berkeley National Laboratory (LBNL, lead institution): Established in 1931, LBNL is a founding partner of the DOE Joint Genome Institute and is home to the Advanced Light Source, the Molecular Foundry, the National Center for Electron Microscopy, and the National Energy Research Scientific Computing Center. LBNL's scientific expertise has been recognized with 13 Nobel Prizes. The University of California manages the laboratory for DOE's Office of Science.

DOE's Sandia National Laboratories (SNL): SNL has major R&D responsibilities in national security, energy and environmental technologies, and economic competitiveness. It provides expertise in systems engineering and integration function, microfluidics, computation, robotics, materials development, and manufacturing technologies. SNL is operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin, for DOE's National Nuclear Security Administration.

DOE's Pacific Northwest National Laboratory (PNNL): PNNL conducts interdisciplinary research addressing many of America's most pressing issues in energy, the environment, and national security through advances in basic and applied science. PNNL is contributing staff expertise in fungal biotechnology to JBEI's deconstruction research. Ohio-based Battelle has been managing PNNL for DOE since the laboratory's inception in 1965.

DOE's Lawrence Livermore National Laboratory (LLNL): Founded in 1952 to provide solutions to important national security challenges, LLNL is host to the Center for Accelerator Mass Spectrometry and is also one of the DOE Joint Genome Institute's founding partners. The laboratory provides expertise in genomics, bioinformatics, experimental protein

production, advanced measurement technologies, and high-performance scientific computing. LLNL is managed by Lawrence Livermore National Security, LLC, for DOE's National Nuclear Security Administration.

University of California–Berkeley: UC Berkeley is the nation's top-ranked public university. Founded in 1868, the university provides a broad range of research support in JBEI focus areas, including molecular and cellular biology, molecular genetics, proteomics, and environmental sciences. UC Berkeley counts among its current faculty 7 Nobel laureates, 130 members of the National Academy of Sciences, 94 members of the National Academy of Engineering, and 13 recipients of the National Medal of Science.

University of California–Davis: UC Davis oversees the California Biomass Collaborative, Northern California Nanotechnology Center, Western Regional Center of the National Institute for Global Environmental Change, and the UC Davis Genome Center. The university supports JBEI's research mission by providing expertise in plant and environmental sciences, plant genetics, plant physiology, evolutionary biology, and environmental science.

Carnegie Institution for Science: The Carnegie Institute for Science is a private, nonprofit institution on the campus of Stanford University. As leaders in plant biology, Carnegie researchers established and maintain The Arabidopsis Information Resource (TAIR), a database of genetic and molecular biology data for *Arabidopsis thaliana*, a model higher plant. TAIR provides findings in plant photosynthesis, bioinformatics, and the growth and development processes that enable plants to survive disease and environmental stresses.

*See back cover for map showing partner locations.

an industrially favored engineered yeast strain, aiming to generate 1 gallon of the biofuel for jet fuel specification.

Education and Outreach

JBEI is working to keep our nation at the vanguard of scientific discovery by developing future generations of scientists. Preparing a multidisciplinary workforce of researchers is key to growing the cellulosic biofuels industry.

Year-round, JBEI scientists and staff provide educational opportunities (e.g., site tours, workshops, presentations, and educational programs) for high school to graduate students. Opportunities to explore careers in science and engineering are offered through direct mentoring and internships for undergraduate and graduate students. Each summer, JBEI partners

with the Synthetic Biology Engineering Research Center (SynBERC) to manage an intensive internship program for high-potential, low-income high school students from communities underrepresented in science. Guided by JBEI scientists, students in the Introductory College-Level Experience in Microbiology (iCLEM) program participate in hands-on biofuels research. During the program's first 5 years, nearly 100% of iCLEM interns have enrolled in college, and 63% have elected to major in science or engineering.

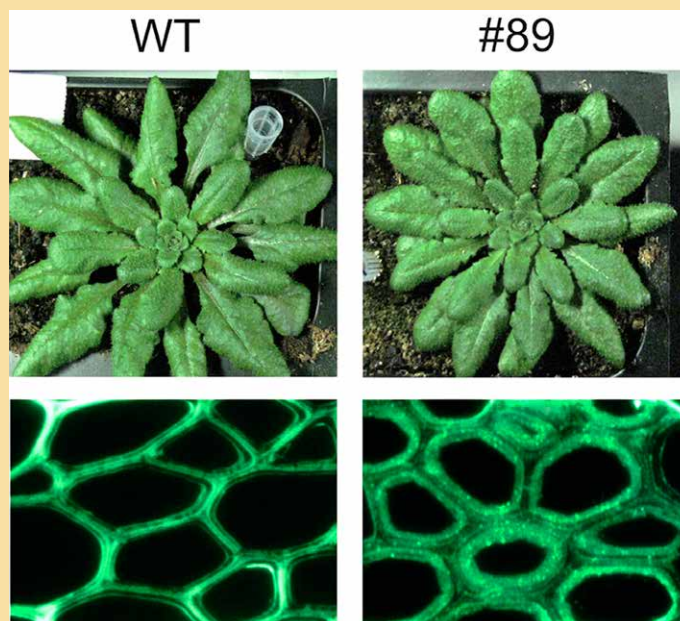
Through ongoing, in-house professional development, JBEI offers weekly seminars and workshops to enhance research performance, collaboration, and results. Industry leaders are invited to give entrepreneurial training as part of guest lectures focused on enabling young scientists and engineers to develop biofuels-related business plans and advance their careers.

JBEI Research Highlights

Engineering Plant Cell Walls to Boost Sugar Yields for Biofuels

Lignocellulosic biomass is composed mostly of plant secondary cell walls that contain, among other polymers, complex chains of sugars called polysaccharides that hold great promise as biofuel sources. These sugars, however, are embedded in lignin, a tough material that helps reinforce cell wall structure and maintain its integrity. Because lignin is primarily responsible for the resistance of biomass to enzymatic hydrolysis or breakdown, considerable research has been directed at decreasing the amount of lignin in the cell wall. Previous efforts have demonstrated that large reductions in lignin content cause growth defects and often correlate with vessel collapse, adversely affecting water and nutrient transport carried out by the plant vascular system.

JBEI researchers have developed a new approach to decrease lignin content while preventing vessel collapse and have introduced a novel strategy to boost transcription factor expression in specific tissues. Synthetic biology tools were used to rewire the secondary cell network in *Arabidopsis* by changing promoter-coding sequence associations. The result was a reduction in lignin and an increase in polysaccharide depositions in the plant's fiber cells. The promoter of a key lignin biosynthesis gene, *C4H*, was replaced by the vessel-specific promoter of transcription factor *VND6*. This replacement rewired lignin biosynthesis specifically for vessel formation while disconnecting *C4H* expression from the fiber regulatory network. Secondly, the promoter of the *IRX8* gene, encoding secondary cell wall glycosyltransferase, was used to express a new copy of the fiber transcription factor *NST1*. When the *IRX8* promoter is induced by *NST1*, it also creates an artificial positive feedback loop (APFL). The combination of strategies—lignin rewiring with APFL insertion—enhances polysaccharide deposition in stems with low lignin content, resulting in higher sugar yields after enzymatic hydrolysis. If commercialized, such plants would increase biofuel yield per acre and enhance profitability (Yang et al. 2013).



Engineering Enhanced Plants. JBEI researchers genetically engineered *Arabidopsis* plants (#89) that yielded as much biomass as wild types (WT) but with increased polysaccharide deposition in the fibers of their cell walls. [Images courtesy JBEI]

Consolidated Pretreatment and Saccharification Using Ionic Liquids

Biomass pretreatment using certain ionic liquids, such as 1-ethyl-3-methylimidazolium acetate ($[C_2mim][OAc]$), can be highly effective at reducing the recalcitrance of lignocellulosic biomass to enzymatic degradation. However, current commercial enzyme cocktails—derived from filamentous fungi and developed for dilute acid pretreatment—are inhibited by the most effective ionic liquids, whose removal from pretreated biomass requires excessive amounts of water for efficient enzyme performance. The costs associated with ionic liquid recycling and waste disposal pose significant economic and engineering challenges for the commercial scale-up of pretreatment technologies based on ionic liquids.

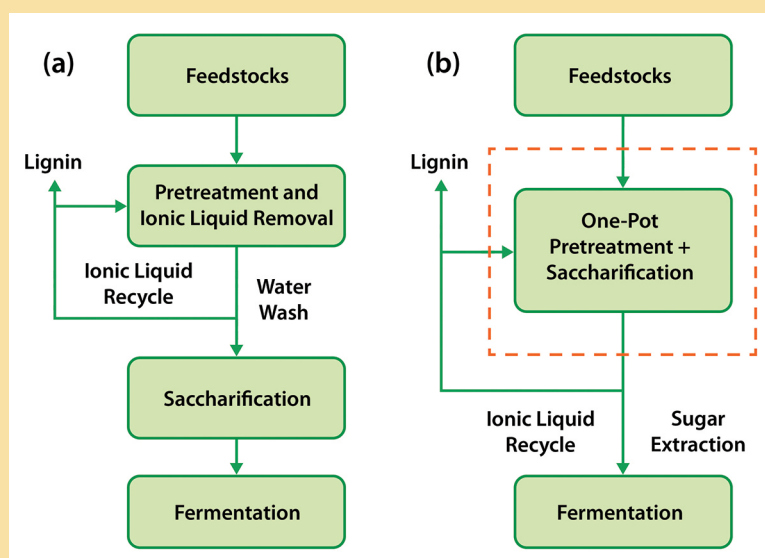
In a first-of-its-kind effort, JBEI researchers have demonstrated a wash-free process that combines, in a single vessel, ionic liquid pretreatment and saccharification (the



One-Pot Process. The right combination of enzyme cocktail and ionic liquid pretreatment can be used to extract fermentable sugars from switchgrass in a single, wash-free step. [Image courtesy Lawrence Berkeley National Laboratory]

JBEI Research Highlights

breakdown of plant polysaccharides into simple sugars). After treating switchgrass with $[C_2mim][OAc]$ and diluting with water to a final ionic liquid concentration of 10% to 20%, the pretreatment slurry was directly hydrolyzed using a thermostable, ionic liquid-tolerant enzyme cocktail previously developed at JBEI. Within three days, this “one-pot” process liberated 81.2% of the available glucose and 87.4% xylose (monomers and oligomers) at 70°C, with an enzyme loading of 5.75 mg/g of biomass at 10% $[C_2mim][OAc]$. Glucose and xylose were selectively separated by liquid-liquid extraction with over 90% efficiency, thus eliminating extensive water washing as a unit operation. This process could drastically simplify the downstream recovery of sugar and lignin and the recycling of ionic liquids, paving the way for an affordable and scalable approach to producing fermentable sugars from lignocellulosic biomass using ionic liquids (Park et al. 2012).

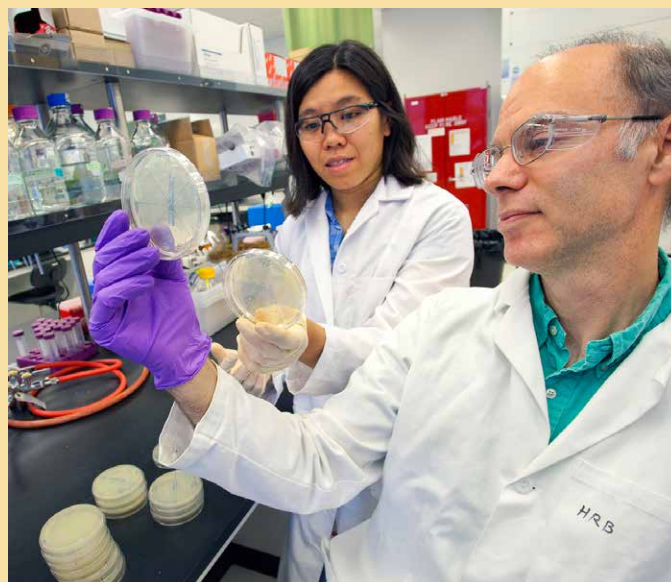


Streamlining Biofuel Production. Conventional pretreatment and saccharification of biofuel feedstocks occur in (a) separate steps entailing water washing and waste disposal that JBEI's (b) one-pot system eliminates. [Image courtesy JBEI]

Synthetic Biology Techniques Boost Fatty Acid–Based Biofuels

JBEI synthetic biologists have engineered *Escherichia coli* bacteria to generate significant quantities of methyl ketone compounds from glucose. These compounds have high cetane numbers (a diesel fuel rating comparable to the octane number for gasoline), making them strong candidates to replace petroleum-based diesel. Researchers successfully increased the methyl ketone titer production of *E. coli* more than 4,000-fold with a relatively small number of genetic modifications. These findings add to the list of naturally occurring chemical compounds that could serve as biofuels, providing more flexibility and options for the biofuels industry (Goh et al. 2012).

Synthetic biology methods also were used by researchers in the Fuels Synthesis Division to devise a new technique—called a dynamic sensor-regulator system (DSRS)—that can detect metabolic changes in microbes during the production of fatty acid-based fuels or chemicals. Because DSRS also enables researchers to control the expression of genes affecting this production, the team was able to demonstrate a threefold increase in microbial synthesis of biodiesel from glucose. DSRS is the first example of a synthetic system that can dynamically regulate a metabolic pathway for improving production of fatty acid-based fuels and chemicals while microbes are in a bioreactor (Zhang, Carothers, and Keasling 2012).



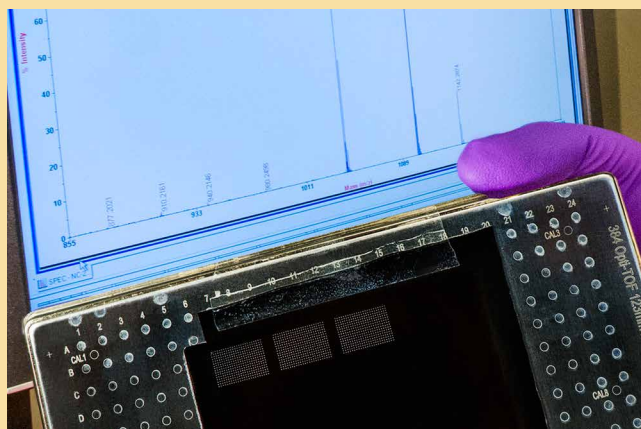
Identifying New Biofuel Candidates. JBEI researchers Harry Beller (foreground) and Ee-Been Goh have engineered *Escherichia coli* bacteria to synthesize large quantities of methyl ketone compounds that could be used as biofuels. [Image courtesy Lawrence Berkeley National Laboratory]

JBEI Research Highlights

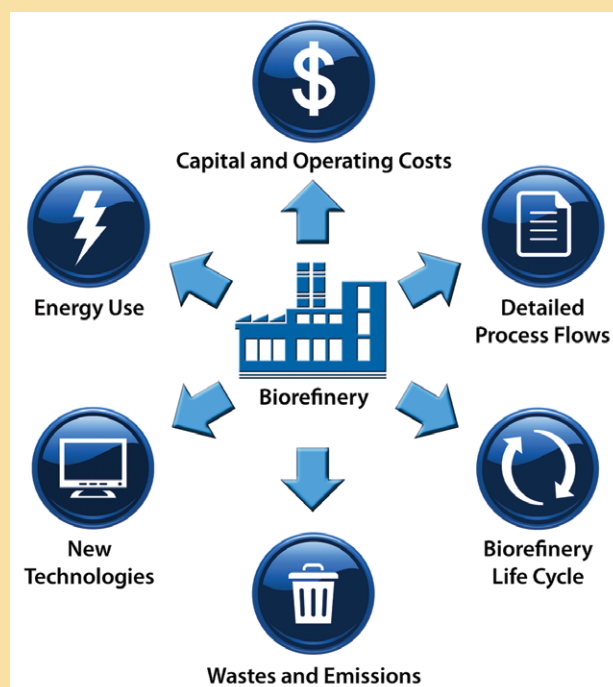
New Technology Enables Ultrafast Screening for Best Biofuel Enzymes

High-throughput nanostructure-initiator mass spectrometry (HT-NIMS), codeveloped at JBEI, is a high-speed chemical-screening technology that researchers can use to discover the function of large numbers of biologically active molecules. With speeds 100 times faster than conventional probes, HT-NIMS can rapidly screen tens of thousands of enzymatic biomass deconstruction reactions that could be used to turn grass into biofuels.

A workhorse of biotechnology, mass spectrometry (MS) offers unparalleled accuracy, but its potential as a screening tool has been limited by its slow throughput. HT-NIMS dramatically speeds up the process. Tiny sample volumes are deposited in rows and columns on a slide of silicon, creating a microarray of as many as 10,000 discrete sites. Each site is probed with a laser and analyzed in a split second. In the time it takes conventional MS to characterize one sample, HT-NIMS can cost-effectively profile hundreds. JBEI is using the technique, which was recognized with a 2013 R&D 100 Award, to screen for enzymes that can be used to modify lignocellulose for producing advanced biofuels that could replace gasoline on a gallon-for-gallon basis (Greving et al. 2012; Reindl et al. 2012).



High-Speed, Large-Scale Enzyme Screening. Codeveloped by JBEI researchers, high-throughput nanostructure-initiator mass spectrometry (HT-NIMS) can be used to quickly and precisely determine the molecular composition of thousands of samples arrayed on a small slide of silicon. JBEI researchers are using the technology to screen for enzymes useful in biomass deconstruction. [Image courtesy Lawrence Berkeley National Laboratory]



Accelerating Commercialization of Research Advances. JBEI's wiki-based technoeconomic model simulates critical factors in the biorefinery process, enabling scientists to evaluate the real-world potential of research developments and focus on the most promising strategies. [Image courtesy JBEI]

Model Assesses Commercialization Potential of JBEI Research

To evaluate the potential economic impact of JBEI technological advances, researchers have developed a technoeconomic model as a principal tool for measuring progress. This publicly available model (econ.jbei.org) can be used to simulate the performance and cost-competitiveness of engineered feedstocks, new biomass pretreatments, enzyme mixtures, enzyme loading, and type of biofuel produced. Another model representing the corn stover-to-ethanol process (i.e., dilute acid pretreatment, saccharification, and yeast fermentation) serves as the technical benchmark against which JBEI research developments can be measured. The primary metric for comparing the different biofuel technologies is the minimum ethanol selling price (MESP)—or the price at which a biorefinery can be economically viable. JBEI research advances are incorporated into the technoeconomic model, which calculates the resultant minimum biofuel selling price. This price is then compared with the benchmark MESP for the corn stover process, thereby providing an important measure of progress as it relates to potential commercialization of the technology evaluated.



DOE Genomic Science Program

Advancing Scientific Discovery Through Genomics and Systems Biology

genomicscience.energy.gov

The U.S. Department of Energy's (DOE) Genomic Science program, managed by the Office of Biological and Environmental Research (BER) within DOE's Office of Science, is driven by a grand challenge in biology: understanding biological systems so well that a predictive understanding of biological processes can be obtained (see box, Genomic Science Program Goal and Objectives, below). By revealing the genetic blueprint and fundamental principles that control plant and microbial systems, the Genomic Science program is providing the foundational knowledge underlying biological solutions to DOE missions in energy, environment, and climate. These objectives include advancing development of sustainable bioenergy systems and predicting impacts of changing environmental conditions on carbon cycling and other biogeochemical processes.

The DNA code—the genome—is the starting point to understanding any biological system because it contains the information and operating capabilities that determine structure and function. Exploring biological systems across all scales in a comprehensive and integrated way is essential to understanding how these systems operate in nature or in more applied contexts related to new technology endpoints for DOE missions. As a leader in systems biology research, the Genomic Science program builds on a foundation of sequenced genomes and metagenomes to identify the common fundamental principles that drive living systems. These principles guide the translation of genomic code into functional molecules underlying biological system behavior.

Building on this foundation of genomic information, a 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.

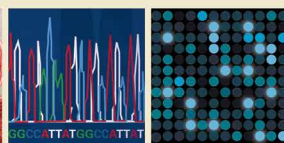
Addressing extremely complex scientific questions spanning all scales of biology, research supported by DOE's Genomic Science program requires the collective expertise of scientists from many disciplines and the coordinated application of a wide range of technologies and experimental approaches. Scientists 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. Working in concert with the DOE Systems Biology Knowledgebase (KBbase), the Genomic Science program is integrating the immense amounts of diverse information generated by its researchers to enable a more comprehensive understanding of how biological systems work. This enhanced understanding will allow more accurate predictions of system behaviors, leading to development of biologically inspired solutions to some of the most pressing energy and environmental challenges. The Genomic Science program also jointly sponsors genome-based approaches to accelerate plant breeding programs and improve potential bioenergy crops with the U.S. Department of Agriculture National Institute of Food and Agriculture.

Genomic Science Program Goal and Objectives

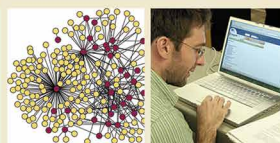
Genome Sequence



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.

Office of Science and Office of Biological and Environmental Research

The U.S. Department of Energy (DOE) Office of Science manages fundamental research programs in basic energy sciences, high-energy physics, fusion, biological and environmental sciences, and computational science, supporting unique and vital parts of U.S. research in climate change, geophysics, genomics, and life sciences. 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, supercomputers and high-speed computer networks, genome and protein sequencing facilities, and advanced resources in imaging and analysis for biological and environmental systems.

Office of Biological and Environmental Research (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 leveraging diverse scientific insights by coupling theory, observations, experiments, models, and simulations.

The programs are managed within two divisions: the Biological Systems Science Division (BSSD) and Climate and Environmental Sciences Division (CESD).

BSSD seeks to understand how genomic information is translated to functional capabilities, enabling more confident redesign of microbes and plants for sustainable biofuels production, improved carbon storage, and a better understanding of biological transformation of materials such as nutrients and contaminants in the environment. BSSD research activities support the DOE Bioenergy Research Centers (BioEnergy Science Center, Great Lakes Bioenergy Research Center, and Joint BioEnergy Institute) to provide transformational breakthroughs for cellulosic ethanol and advanced biofuels from cellulosic biomass. The division also supports development of real-time, high-resolution technologies (assisted by integration with computer modeling) for analyzing dynamic biological processes.

CESD advances understanding of the roles of Earth's biogeochemical systems (the atmosphere, land, oceans, sea ice, and subsurface) in determining climate to facilitate prediction of climate decades or centuries into the future, information needed to plan for future energy and resource needs. CESD programs are improving the world's most powerful climate models through research on atmospheric processes; terrestrial surface and subsurface ecosystems; climate change and environmental monitoring; and analysis of impacts and interdependencies of climate change with energy production and use.

For More Information

DOE Bioenergy Research Centers

genomicscience.energy.gov/centers/

BioEnergy Science Center (BESC)

bioenergycenter.org

Great Lakes Bioenergy Research Center (GLBRC)

glbrc.org

Joint BioEnergy Institute (JBEI)

jbei.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

DOE Systems Biology Knowledgebase (KBase)

kbase.us

Breaking the Biological Barriers to Cellulosic Ethanol

genomicscience.energy.gov/biofuels/b2bworkshop.shtml

DOE Office of Biological and Environmental Research

science.energy.gov/ber/

DOE Office of Science

science.energy.gov

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*See individual BRC websites for updated and more comprehensive publication lists.

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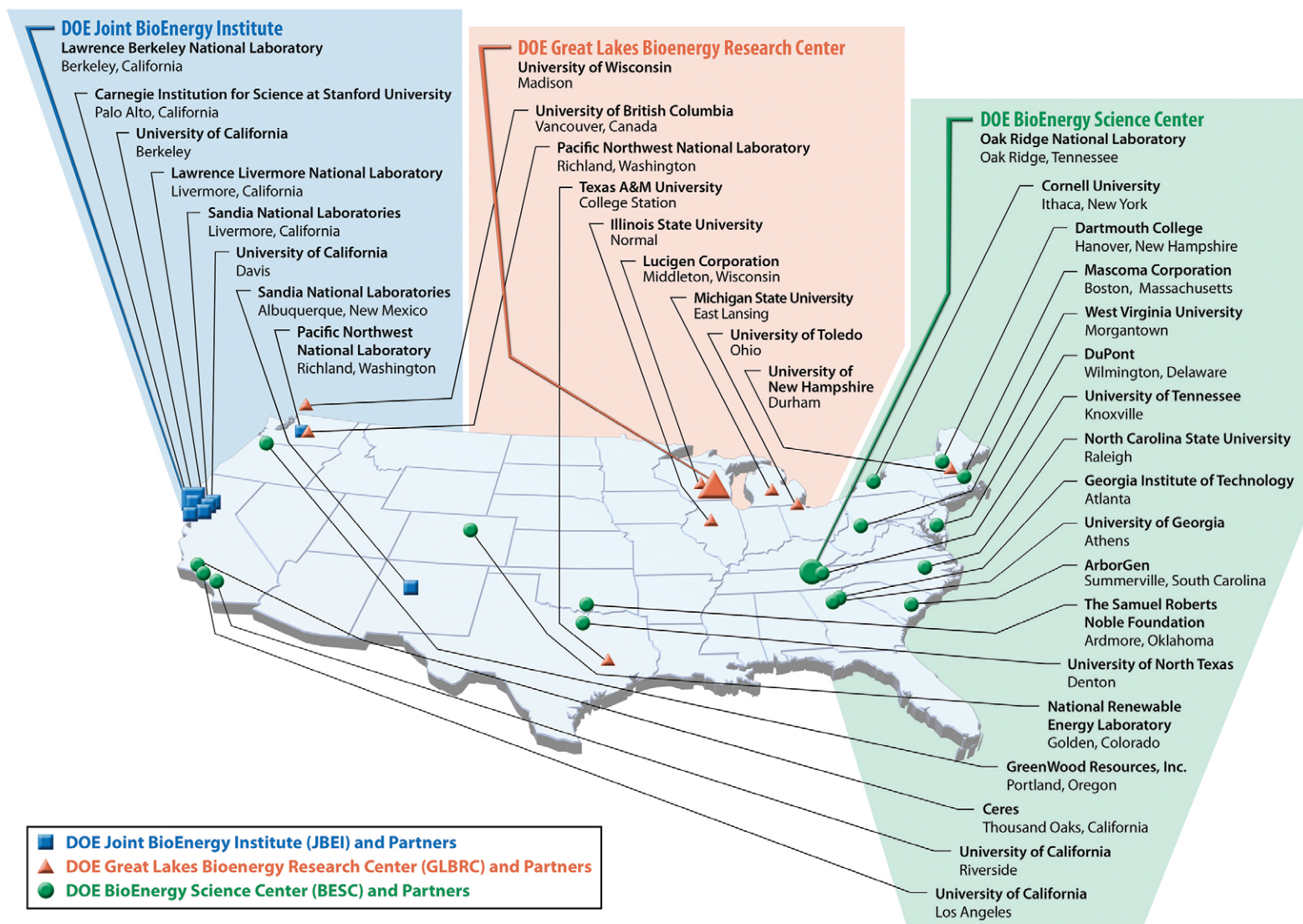
Acronyms

ABPDU	Advanced Biofuels Process Demonstration Unit	LLNL	Lawrence Livermore National Laboratory
AFEX™	ammonia fiber expansion	MBI	Michigan Biotechnology Institute
APFL	artificial positive feedback loop	MESP	minimum ethanol selling price
BER	Office of Biological and Environmental Research	MS	mass spectrometry
BESC	BioEnergy Science Center	MSU	Michigan State University
BRC	Bioenergy Research Center	NC State	North Carolina State University
BSSD	Biological Systems Science Division	NREL	National Renewable Energy Laboratory
CAZyme	carbohydrate-active enzyme	ORNL	Oak Ridge National Laboratory
CBP	consolidated bioprocessing	PNNL	Pacific Northwest National Laboratory
CESD	Climate and Environmental Sciences Division	RELATCH	RELATive CHange
CSE	caffeoyl shikimate esterase	SNL	Sandia National Laboratories
DOE	U.S. Department of Energy	SNP	single-nucleotide polymorphism
DSRS	dynamic sensor-regulator system	SSCF	simultaneous saccharification and cofermentation
GLBRC	Great Lakes Bioenergy Research Center	SynBERC	Synthetic Biology Engineering Research Center
GVL	gamma-valerolactone	TAIR	The Arabidopsis Information Resource
HT-NIMS	high-throughput nanostructure-initiator mass spectrometry	UBC	University of British Columbia
HTP	high-throughput	UC	University of California
iCLEM	Introductory College-Level Experience in Microbiology	UCLA	University of California–Los Angeles
IIT	Informatics and Information Technology	UGA	University of Georgia
IP	intellectual property	UNH	University of New Hampshire
JBEI	Joint BioEnergy Institute	UNT	University of North Texas
JGI	DOE Joint Genome Institute	USDA	U.S. Department of Agriculture
KBase	DOE Systems Biology Knowledgebase	UT	University of Tennessee
LAO	linear alpha olefin	UW	University of Wisconsin
LBNL	Lawrence Berkeley National Laboratory	WARF	Wisconsin Alumni Research Foundation
		WVU	West Virginia University

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