Developing the science, technology, and knowledgebase necessary to enable sustainable, cost-effective production of advanced biofuels and bioproducts from nonfood plant biomass in support of a new biobased economy.
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Joint BioEnergy Institute (JBEI)
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U.S. Department of Energy

Bioenergy Research Centers

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Introduction

Multiple societal benefits underlie U.S. Department of Energy (DOE) research efforts to support a viable and sustainable domestic biofuel and bioproducts industry from nonfood lignocellulosic plant biomass. These benefits include ensuring future energy security, lowering greenhouse gases to mitigate climate impacts, diversifying the range of available biobased products, producing fewer toxic chemicals and byproducts, creating jobs in rural areas, and improving the trade balance.

Cellulose—the most abundant biological material on Earth—is contained within plant cell walls in the form of long, tightly bound chains of sugars (polysaccharides) that can be converted into biofuels and bioproducts by microbes. Physically accessing these sugars, however, is difficult because the cellulose is embedded within a matrix of other polymers including hemicellulose and lignin, making it resistant to degradation. This resistance, called recalcitrance, along with a lack of efficient methods to convert lignocellulose to useful products are major impediments to the cost-effective production of biofuels and bioproducts from plant biomass. Innovation stemming from advanced biotechnology-based research is key to accelerating needed improvements in the sustainable production of lignocellulosic biomass, its deconstruction into sugars and lignin, and conversion (see figure, From Biomass to Advanced Biofuels and Bioproducts, this page).

Over 10 years (2007–2017), three Bioenergy Research Centers (BRCs), supported by the Genomic Science program within DOE’s Office of Science Office of Biological and Environmental Research (BER), made significant advances toward this new biobased economy (see sidebar, Genomic Science to Advance DOE Missions, p. 2). The BRCs produced multiple breakthroughs in the form of deepened understanding of sustainable biomass production practices, targeted re-engineering
of biomass feedstocks, development of new methods for deconstructing feedstocks, and engineering of microbes for more effective production of a diverse range of biofuels. More specifically, advances included (1) the demonstration that lignin composition and deposition can be genetically engineered to reduce plant cell wall recalcitrance without affecting plant viability; (2) development of effective biomass pretreatments that can be adapted commercially to lower costs; (3) discoveries of novel microbes and enzymatic pathways for more efficient deconstruction of lignocellulosic biomass; (4) proof-of-concept research for consolidated bioprocessing (i.e., simultaneous breakdown and conversion to ethanol and other biofuels by naturally cellulolytic microbes); (5) metabolic engineering of microbes and plants for the biological production of numerous advanced biofuels or their immediate precursors; and (6) identification of hundreds of new plant genes and an improved understanding of their role in cell wall biosynthesis.

In all, the three original BRCs produced 2,696 peer-reviewed publications, 619 invention disclosures, 397 patent applications, 199 licenses or options, 101 patents, and 14 company startups. Through this work, they transferred substantial insight and expertise to industry through cooperation with both large and small companies and knowledge to other researchers.

From Cellulosic Ethanol to Advanced Biofuels and Bioproducts

These successes are being leveraged in the next phase of DOE bioenergy research and expanded from a focus on ethanol and biofuels beyond ethanol (i.e., advanced biofuels) to include the development of bioproducts. These bioproducts are nonpharmaceutical chemicals that directly replace or substitute for chemicals currently derived from petroleum or natural gas (see figure, Flowchart Comparing Potential Biomass- and Petroleum-Derived Products, p. 3). They also may be novel chemicals that cannot be efficiently produced from petroleum.

In this research endeavor, there are four BRCs based in the geographically diverse East, Midwest, Southeast, and West Coast regions. BRC locations correspond to the geographic distribution of potential dedicated biomass crops under development (see figure, Approximate Geographic Distribution of Potential Dedicated Biomass Crops, p. 4). BRC partners include universities, private companies, nonprofit organizations, and DOE national laboratories (see DOE Bioenergy Research Centers and Partners map, on back). These four BRCs take distinctive approaches toward the common goal of accelerating the pathway to improving and scaling up advanced biofuel and bioproduct production processes.
**Flowchart Comparing Potential Biomass- and Fossil Fuel–Derived Products.** Today, fossil fuel–derived products are found in virtually all facets of human life, including transportation, recreation, communications, health, housing, safe food supply, and textiles. Lignocellulosic biomass has the potential to (1) replace petroleum and natural gas as the raw material for producing these products and (2) provide new and improved properties that could enable new products and applications. As commercial-scale production of advanced biofuels and bioproducts derived from lignocellulose is realized, synthetic biology and metabolic engineering can be applied to convert lignocellulosic biomass into any number of chemical intermediates, building blocks, and final products. By no means exhaustive, this figure represents some examples of chemical intermediates and building blocks that could be gleaned from lignocellulosic biomass to make the same products currently derived from fossil feedstocks.
• **Center for Advanced Bioenergy and Bioproducts Innovation** (CABBI; University of Illinois at Urbana-Champaign) seeks to enable the production of drop-in (i.e., could be produced from oil) fuels and chemicals directly in plants as sustainable biofactories for a range of bioproducts.

• **Center for Bioenergy Innovation** (CBI; Oak Ridge National Laboratory) is accelerating the domestication of sustainable bioenergy crops and targeted consolidated bioprocessing innovations to improve cost efficiencies within the bioenergy supply chain.

• **Great Lakes Bioenergy Research Center** (GLBRC; University of Wisconsin–Madison) aims to develop the science and technological advances to ensure sustainability at each step in the process of creating biofuels and bioproducts from lignocellulose.

• **Joint BioEnergy Institute** (JBEI; Lawrence Berkeley National Laboratory) is broadening and maximizing production of economically viable biofuels and bioproducts from plant biomass to enable biorefinery development.

**Breaking Down Remaining Scientific Barriers**

Using an integrative approach, BER is uniquely well positioned to address the basic research challenges associated with establishing an economically competitive and sustainable domestic biofuel and bioproducts industry. This approach ranges from sustainably growing new engineered energy crops and developing novel methods for deconstructing lignocellulosic material into chemical building blocks to creating new metabolic pathways inserted into microbial hosts to produce biofuels and bioproducts.

Significant advances in plant breeding, molecular genetics, and genomic technologies provide unique opportunities to build on existing knowledge of plant biology and more confidently predict and manipulate functional properties of biomass feedstock crops. Similarly, continuing advances in omics-enabled technologies and synthetic biology approaches for microorganisms provide opportunities to further develop nonmodel microorganisms for...
applications in industrial biotechnology and for conversion of biomass into biofuels and bioproducts. Most importantly, integrating plant and microbial systems biology with cutting-edge research in chemical engineering, synthetic biology, and computational biology facilitates the scientific breakthroughs needed to foster the development of a sustainable bioeconomy.

Remaining basic science challenges that continue to limit the cost-effective conversion of plant biomass to advanced biofuels and bioproducts fall into four scientific focus areas: (1) sustainability, (2) feedstock development, (3) lignocellulosic deconstruction and separation, and (4) conversion to advanced biofuels and bioproducts (see sidebar, DOE Bioenergy Research Center Strategies at a Glance, this page).

**Sustainability.** Designing sustainable biofuel and bioproduct production systems requires knowledge about the interactions between

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**DOE Bioenergy Research Center Strategies at a Glance**

Overcoming the critical basic science challenges to cost-effective production of biofuels and bioproducts from plant biomass 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. These BRC strategies are listed briefly below.

<table>
<thead>
<tr>
<th>Sustainability</th>
<th>Feedstock Development</th>
<th>Deconstruction and Separation</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CABBI</td>
<td>Integrate economic and environmental analyses for biomass supply.</td>
<td>Develop “plants as factories” concept for biofuels and bioproducts.</td>
<td>Establish automated biofoundry concept for fuels and bioproducts.</td>
</tr>
<tr>
<td>CBI</td>
<td>Optimize water and nutrient use in dedicated bioenergy crops.</td>
<td>Create multiomics tools for developing high-yield bioenergy crops.</td>
<td>Generate drop-in biofuels and bioproducts from biomass and lignin residues.</td>
</tr>
<tr>
<td>JBEI</td>
<td>Study environmental resilience of engineered bioenergy crops.</td>
<td>Engineer plants for atom-economical conversion into biofuels and bioproducts.</td>
<td>Develop high-throughput synthetic biology tools and hosts for scalable, atom-economical biofuels and bioproducts.</td>
</tr>
</tbody>
</table>

* CABBI: Center for Advanced Bioenergy and Bioproducts Innovation; CBI: Center for Bioenergy Innovation; GLBRC: Great Lakes Bioenergy Research Center; JBEI: Joint BioEnergy Institute
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 lignocellulosic biofuels and bioproducts industry.

A large portion of the plant cell wall contains long chains of sugars (polysaccharides) that can be converted to fuels and chemicals. Cellulose, the major polysaccharide, 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 and bioproducts 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 coating that shields 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 coproducts that can inhibit sugar conversion to biofuels. Finding ways to control lignin formation in plants is a major focus of bioenergy research, along with developing a suite of tools to create valuable bioproducts from lignin.

Approximate distribution of the three primary components of herbaceous perennial plant cell walls—cellulose, hemicellulose, and lignin.
crops and their environment, impacts of crop choice and management systems, and key plant-microbe-environment interactions that provide a range of ecosystem services. Linking these advances to breakthroughs in ecosystem science enables the use of systems biology approaches for the fundamental design of sustainable biofuel and bioproduct production systems.

BER goals for this scientific focus area include:

- Gaining a mechanistic understanding of how bioenergy crop interactions with biotic and abiotic environmental factors influence crop growth, yield, and quality.
- Identifying the most impactful research areas through process integration and techno-economic evaluation of biomass-to-fuels technologies that address the economics of biofuels and bioproducts production.
- Using multiscale modeling to advance predictive understanding of biofuel cropping ecosystems.

**Feedstock Development.** Establishing a sustainable, lignocellulosic bioeconomy will require a fundamental shift in how feedstocks are produced, processed, and transported to mills and biorefineries. New bioenergy feedstocks—including dedicated crops for biofuels and bioproducts and nonfood crops for oils or other nonpharmaceutical in planta–produced products—need to be engineered for sustainable production and efficient conversion to biofuels and bioproducts.

BER goals for this scientific focus area include:

- Enhancing bioenergy feedstocks with improved traits for yield, water use, nutrient uptake and recycling, resilience to biotic and abiotic stress, and conversion to biofuels and bioproducts.
- Developing high-throughput analytical tools to promote bioenergy feedstock crop creation, evaluation, and production.
- Conducting field testing of new potential bioenergy feedstock crops under environmentally relevant conditions across multiple geographic regions to assess viability and robustness (see figure, Approximate Geographic Distribution of Potential Dedicated Biomass Crops, p. 4).
- Developing quantitative models informed by experimentation to predict how bioenergy feedstock genotypes perform under different geographic and environmentally relevant conditions.

**Lignocellulosic Deconstruction and Separation.** Further research is needed to make deconstruction processes low cost, low energy, more efficient, with minimal environmental impact, and capable of converting a range of lignocellulosic biomass types into hydrolysates that contain as much cellulosic or hemicellulosic sugars as possible for conversion into biofuels and bioproducts. Additionally, technologies are required to convert the relatively large fraction of carbon found in the lignin portion of lignocellulosic biomass into biofuels and bioproducts (see sidebar, Plant Cell Wall Recalcitrance, p. 6). Stronger linkages between advances in biomass development and fuels production will strengthen these deconstruction efforts.

BER goals for this scientific focus area include:

- Developing feedstock-agnostic deconstruction processes capable of efficiently fractionating biomass into targeted output streams with minimal inhibitor formation.
- Gaining a detailed understanding of plant cell wall biosynthesis, composition, structure, and properties during deconstruction.
- Improving enzymes and approaches for biomass breakdown and cellulose, hemicellulose, and lignin processing.
• Developing quantitative understanding and multiscale modeling of plant cell wall deconstruction to improve efficiency.

**Conversion to Advanced Biofuels and Bioproducts.** Advances in metabolic engineering have resulted in an expanded suite of microbially produced molecules beyond ethanol to potentially serve as biofuels. For these biofuels to be sustainable and economically viable, advances must be made in platform organism development, pathway efficiency, yield, rate, and metabolite tolerance.

This focus also recognizes the environmental and economic benefits to be gleaned from using biomass to produce chemicals currently derived from petroleum, as well as the potential unbounded diversity of new molecules that could be produced from biomass. The synergies between the methods and approaches for biofuel and bioproduct synthesis create an opportunity to leverage basic research in biofuels development with broader possibilities toward advancing a biobased economy.

**BER goals for this scientific focus area include:**

• Developing high-throughput methods to screen or select high-performance strains to improve product formation rates, titers, yields, and selectivity (i.e., the ability to produce only the desired product while minimizing byproducts).

• Establishing a broader set of platform microorganisms suitable for metabolic engineering to produce biofuels and bioproducts, as well as high-throughput methods for experimental validation of gene function.

• Developing new approaches and models to predict optimal production pathways, metabolic models that fully articulate the metabolic complexity from genomic and metabolomic data, and models that can predict behavior and yields to inform scaled-up applications.

• Developing techniques to enhance microbial robustness for tolerating toxins to improve fermentation yields and to gain a better understanding of the cellular and molecular bases of tolerance for major chemical classes of inhibitors found in these processes.

• Advancing technologies for consolidated bioprocessing.

• Identifying, creating, and optimizing microbial and chemical pathways to produce promising, atom-economical intermediates and final bioproducts from biomass that are less toxic and more environmentally benign compared to current products produced from petroleum or natural gas. Atom-economical processes minimize atoms lost from the starting material to attain the highest possible yield.

• Developing high-throughput, real-time, in situ analytical techniques to understand and characterize the pre- and post-bioproduct separation streams in detail.

• Creating methodologies for efficiently identifying viable target molecules, identifying high-value bioproducts in existing biomass streams, and utilizing current byproduct streams.

• Identifying and improving plant feedstocks with enhanced higher extractable levels of desired bioproducts or bioproduct precursors, including lignin streams that are homogeneous and consistent.

The next-phase DOE BRCs are initiating new research to address these challenges and provide a broad scientific underpinning for producing biofuels and bioproducts from sustainable biomass resources and speeding the translation of basic research results to industry.
Center for Advanced Bioenergy and Bioproducts Innovation

cabbi.bio

CABBI Overview

The Center for Advanced Bioenergy and Bioproducts Innovation (CABBI), led by the University of Illinois at Urbana-Champaign, is developing efficient ways to grow, transform, and market biofuels and other bioproducts by integrating recent advances in genomics, synthetic biology, and computational biology to increase the value of biomass crops. CABBI represents a unique, game-changing research model designed to accelerate bioproduct development while retaining the flexibility to assimilate new disruptive technologies, regardless of their source. The center aims to develop the predictive capability to determine which feedstock combinations, regions and land types, market conditions, and bioproducts have the potential to support the ecologically and economically sustainable displacement of fossil fuels.

CABBI is working to develop transformative technologies for the economic and sustainable production of biofuels and bioproducts from plants. Over 5 years, CABBI aims to provide:

- A regionally adaptive, yet national-scale platform for grass-based biorefining using feedstocks with improved yield and resource-use efficiency.
- A broad set of platform microorganisms, as well as automated tools to engineer them, to develop value-added products from plant-produced feedstocks or substrates.
- An integrated economic and environmental framework for determining feedstock supply and its sustainability.

Another of CABBI’s fundamental objectives is to ensure translation and commercial deployment of its research results—whether in the form of new plant breeds; new biofuels and other biobased chemicals, lubricants, and adhesives; newly tested processes and applications; or new understanding about economic or ecological impacts.

Research Focus Areas

CABBI’s research is organized into three focus areas: feedstock development, conversion, and sustainability.

Feedstock Development: Growing the Right Crops. CABBI is founded on the “plants as factories” paradigm, in which biofuels, bioproducts, high-value molecules, and foundation molecules for conversion are synthesized directly in plant stems (see image, Plants as Factories, this page). This
approach would circumvent the challenges of developing efficient lignocellulose deconstruction methods, while still retaining residual biomass for deconstruction by traditional or emerging methods. CABBI researchers are focusing on sorghum, sugarcane, energy cane, and Miscanthus, high-yielding grasses that grow throughout the rain-fed eastern United States, including on marginal soils. Sorghum and sugarcane are the world’s highest biomass producers, with demonstrated potential for accumulation of oil in vegetative biomass after successful metabolic engineering (see image, High-Yielding Sorghum Grass, this page). CABBI research centers on increasing the yield efficiency and resiliency of these grasses to minimize environmental impacts; shifting stem carbon to more versatile and easily converted carbon forms than recalcitrant lignocellulose; and building high levels of oils and specialty fatty acids in vegetative tissues—all with the goal of making the plant factory more efficient.

Conversion: Turning Plants into High-Value Chemicals. CABBI is working to gain a better understanding of how native metabolism and physiology constrains the production of non-natural compounds, as well as learning to identify compounds that can be produced efficiently in living organisms. As part of this research effort, CABBI scientists are further developing a versatile, automated “biofoundry” for rapidly engineering microbial strains that can efficiently produce diverse, high-value bioproducts such as biodiesel, organic acids, jet fuels, lubricants, and alcohols (see image, Automated Biofoundry, p. 11). CABBI also is developing ways to accelerate the design-build-test-learn framework to overcome challenges associated with designing biological systems to produce non-natural compounds.
Automated Biofoundry. Engineered conventional and nonconventional yeasts can transform one plant-based product into another. To speed up the process, CABBI’s automated biofoundry, called the Illinois Biological Foundry for Advanced Bio-manufacturing (iBioFAB), can rapidly alter microbes genetically for the job.

Sustainability: Improving the Environmental and Economic Bottom Line. CABBI is developing new techno-economic, lifecycle analysis, and integrated systems-level modeling approaches to examine economic and ecological tradeoffs of outcomes from the feedstock and conversion themes. Key points of emphasis are on obtaining a mechanistic understanding of the plant, soil, microbe, and climate interactions that underlie the productivity and ecosystem services of different feedstocks and on investigating the technological and economic pathways to a sustainable and resilient bioeconomy. Also key to this work are the improvement and integration of ecosystem and economic models, which can show how biofuel mandates and other policies can be designed to meet energy and multidimensional environmental goals without lowering food production.

By developing this overarching framework for a closed-loop integration of research and outcomes among the feedstock, conversion, and sustainability themes, CABBI experts are engaged in the innovative research needed for a sustainable bioeconomy.
Center for Bioenergy Innovation

The Center for Bioenergy Innovation (CBI), led by Oak Ridge National Laboratory (ORNL), is pursuing a variety of new technologies to cost effectively create fuels and products currently made from petroleum. Through basic science research on nonfood crops or dedicated bioenergy crops such as poplar and switchgrass, CBI researchers are discovering and designing plants and microbes to produce advanced biofuels and bioproducts.

Specifically, the CBI team is accelerating progress toward identifying and using key plant genes for growth, yield, composition, and sustainability traits to lower feedstock costs and improve year-round feedstock supplies. Additionally, CBI is developing consolidated bioprocessing (CBP), a process in which microbes simultaneously digest the biomass and convert it to biofuels and bioproducts without added enzymes. CBP combines multiple approaches and tools to overcome industrially relevant barriers to using microbes in biomass deconstruction and conversion.

Finally, translating CBI’s research results into the testing of applications and potential commercial deployment is an important step toward reaching DOE’s bioenergy objectives. Through economic and sustainability analysis, CBI is assessing the impact its research will have on cost reduction and scale-up risks for the integrated production of biomass planting and harvest all the way through conversion to fuels.

Ultimately, CBI aims to:

- Create high-yielding bioenergy crops, which display uniform productivity and increased sustainability, by harnessing natural diversity via genomic selection in two perennial feedstocks, poplar and switchgrass (see image, Natural Variation in Biomass Yield, this page).
- Engineer CBP microbes to produce commercially relevant quantities of advanced biofuels and bioproducts.
- More completely utilize all plant cell wall components, specifically lignin, to funnel and improve biological production of coproduct chemicals and novel materials.

Research Focus Areas

CBI’s research targets three research focus areas: improving sustainable biomass feedstocks, enhancing biomass deconstruction and
conversion through CBP, and transforming lignin residues into valuable bioproducts. An underlying theme is to accelerate the domestication of bioenergy-relevant plants and microbes to enable innovations across the bioenergy supply chain by understanding and manipulating complex traits controlled by multiple genes.

**Sustainable Biomass Feedstocks.** CBI plans to simultaneously improve feedstock traits and sustainability in poplar and switchgrass. Using native perennial plants such as poplar and switchgrass provides immediate advantages in sustainability, including less erosion, lower soil compaction, and fewer chemical inputs. CBI is harnessing the vast natural diversity in these two target species using genomic selection and genome-wide association study (GWAS) approaches. Expected results include:

- **Higher Yields:** Increase in biomass productivity (tons per acre per year) by more than 50% in these feedstocks over agronomically relevant landscapes. Feedstock yield is a major cost contributor in mature biorefinery supply chain scenarios.

- **Increased Sustainability:** Increase in water use efficiency by 10%, preventing major yield loss under drought, and nutrient use efficiency by 20%. Pest and pathogen resistance also will increase, while concurrently favoring beneficial microbial associations.

- **Improved Feedstock Uniformity:** More uniform biomass composition over time and across environments, allowing more reliable processing, and discovery of the underlying genes controlling biomass variability.

**Consolidated Bioprocessing.** CBP should eliminate the need for added enzymes and pretreatment, which are the two largest processing cost components in fuel production (see image, Consolidated Bioprocessing, this page). CBP microbes (e.g., *Clostridium thermocellum*) are being engineered to produce commercially...
relevant quantities of butanol, isobutanol, or esters. Butanol and isobutanol can be used either as biofuels or bioproducts. The esters are compatible with the current fuel supply chain (i.e., their properties are similar to those of gasoline), allowing them to be mixed at a refinery. CBI is targeting these chemical compounds because they have efficient metabolic pathways, enabling their unique traits (e.g., suitability in CBP or ability to function at high temperatures) to be utilized. Currently, such traits are difficult to transfer from one organism to another. CBI researchers also are working to accelerate the domestication and engineering of these microbes as well as other novel microbes with beneficial characteristics or traits.

A novel element, co-treatment or light milling during the deconstruction process, is being added to the CBP approach to enable a reduction in facility scale. Together, CBP and co-treatment are expected to transform the deconstruction and conversion steps through significantly lower capital and processing costs by essentially eliminating pretreatment. Moreover, an economic analysis by CBI indicates that the anaerobic

C. thermocellum system is the most energy-, electron-, and carbon-efficient way to convert biomass to biofuels. Expected results include:

- Over 80% carbohydrate solubilization at industrially relevant solids loading (i.e., greater than 120 g biomass/L) through accelerated domestication approaches, metabolic engineering, and synthetic biology.
- Production of fuels at more than 30 g/L isobutanol or n-butanol.
- Quality process residues suitable for further conversion into a portfolio of valuable lignin bioproducts (see image, Creating Value-Added Products, this page).

**Valuable Lignin Bioproducts.** Using a three-pronged approach, CBI is developing methods to transform lignin-rich residues remaining after CBP into valuable bioproducts, including chemical feedstocks such as propanol guaiacol and propanol catechol. First, CBI researchers are modifying lignin in planta to maximize the number of carbon-oxygen bonds via genetic diversity and engineering. This will enable the production of lignin designed for deconstruction. Second, reductive catalytic fractionation is being used to further optimize the deconstruction process. This technique solubilizes and partially depolymerizes the lignin by targeting the carbon-oxygen bonds utilizing natural diversity in and modifications of plant cell walls. Finally, CBI researchers are employing biological funneling to produce the chemical precursors for plastics. In this process, there are microbial biocatalysts that (1) exhibit ligninolytic, aromatic-catabolic activities; (2) funnel heterogeneous aromatic monomers to central aromatic intermediates; and (3) produce target chemical feedstocks from lignin via atom-efficient transformations. Expected results include:

- Proof-of-concept demonstration of more than five commercially attractive lignin-derived products from CBP residues.
- Lignin more easily converted into valuable products (monomers or polymers) by modifying the lignin in the plant feedstock.
Great Lakes Bioenergy Research Center
glbrc.org

GLBRC Overview
The Great Lakes Bioenergy Research Center (GLBRC), led by the University of Wisconsin–Madison, seeks to generate the knowledge needed to sustainably produce advanced biofuels and bioproducts from lignocellulosic bioenergy crops. In close partnership with Michigan State University and other collaborators, GLBRC is working to address identified knowledge gaps in the production of advanced biofuels and bioproducts from dedicated bioenergy crops grown on marginal, nonagricultural lands. These research advances will help to enable a new generation of biorefineries that produce a profitable mix of fuels and products from as much of the plant biomass as possible.

Research Focus Areas
The techno-economic success of lignocellulosic biorefineries hinges on converting as much of the biomass as possible to fuels and products. GLBRC’s research teams are cross-linked among various scientific domains to develop solutions to these priority research focus areas: sustainable biomass production and feedstock yield optimization, efficient deconstruction and conversion, and integration of these processes into industrial field-to-product pipelines.

Sustainable Cropping Systems. GLBRC’s efforts are centered on improving dedicated bioenergy crops grown on marginal, nonagricultural lands. GLBRC crop targets include poplar, switchgrass, energy sorghum, and mixed perennial species such as restored prairie. The center’s goals are to maximize ecosystem performance, as well as crop yield and quality under nutrient-limiting or other stressful conditions found on marginal lands. This effort combines field and greenhouse studies to understand plant-soil-microbiome interactions that can be leveraged to improve switchgrass productivity, and includes engineering plants with lignin and polysaccharides that can readily be turned into advanced biofuels and bioproducts (see image, Zip-Lignin™, this page).

Efficient Biomass Conversion. GLBRC’s conversion efforts seek to improve the efficiency and economic value of producing fuels and products from biomass. Looking at cost- and atom-economical deconstruction and fractionation techniques, GLBRC researchers are testing the ability of gamma-valerolactone (GVL) to perform as a feedstock-agnostic... 

Zip-Lignin™. Lignin is a key obstacle to extracting sugars from biomass. Researchers have redesigned lignin to include weak bonds, or “zips,” which make it much easier to break apart. Here, the feruloyl-coenzyme A monolignol transferase is expressed following introduction of weak bonds into the lignin of poplar tissue. Zip-Lignin™ technology, already licensed to an international leader in woody biomass processing, can reduce the cost of producing fuels and chemicals from many energy crops and enable pathways to new products from biomass. [Courtesy Shawn Mansfield, University of British Columbia]
deconstruction method for plant biomass (see image, GVL Fractionation, this page). Simultaneously, researchers are benchmarking its performance against other industry-accepted deconstruction techniques.

Another effort in this focus area is to enhance the ability of broad-spectrum enzymes that degrade cellulose, xylan, and mannann (classified as CMX), using them to deconstruct and separate biomass fractions for subsequent conversion.

Other GLBRC researchers are reprogramming microbes to produce advanced, drop-in biofuels such as isobutanol, a jet-fuel precursor that has not yet been made from lignocellulosic sugars at industrial scale. To improve the economics of lignocellulosic biorefineries, the center also is evaluating the ability of isolated microbes, synthetic consortia, and native microbiomes to produce valuable hydrocarbons, fatty acids, and isoprenoids from the conversion residue left over after biofuel fermentation.

Field-to-Product Integration. GLBRC research teams are building, modeling, and evaluating field-to-product activities that will occur in the next-generation lignocellulosic bioindustry. These efforts aim to optimize the field-to-product pipeline for future industry use (see figure, Integrated Field-to-Product Pipeline, p. 17).

One cross-cutting team is working to understand and mitigate the negative impact of biomass variability on the performance of
conversion microbes. Another team brings together plant biologists, chemists, and synthetic biologists to create novel pathways for developing valuable products from lignin. GLBRC also has a team of plant and microbial biologists to understand and optimize economical pathways for converting biomass into terpenes and other isoprenoid compounds for industry. Each of these activities will include techno-economic and lifecycle analyses and metabolic modeling as a way to optimize the value of this pipeline.

Together, these integrated and iterative activities are advancing understanding and mitigation of the impact of biomass variability on conversion, creating products from lignin, optimizing plant and microbial production of terpenoid fuels and products, and modeling and optimizing field-to-product activities.
JBEI Overview

The Joint BioEnergy Institute (JBEI), led by Lawrence Berkeley National Laboratory (LBNL), is working to convert nonfood bioenergy crops into economically viable, carbon-neutral biofuels and bioproducts currently derived from petroleum, as well as many other bioproducts that cannot be efficiently produced from petroleum. Ultimately, JBEI aims to:

- Advance basic understanding of plant cell walls, biomass recalcitrance, and microbial physiology.
- Establish predictive biosystems design tools for plants, microbes, and enzymes.
- Develop technologies for feedstock-agnostic deconstruction that liberate high yields of sugars and lignin-derived intermediates suitable for bioconversion.
- Make possible the production of drop-in biofuels at or less than $2.50 per gallon.
- Make possible the production of novel bioproducts.
Research Focus Areas

JBEI’s research is establishing the scientific knowledge and new technologies in sustainability, feedstock development, deconstruction and separation, and conversion that are needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts. When fully scaled, these advances will enable the production of replacements for petroleum-derived gasoline, diesel, jet fuel, and bioproducts.

**Sustainability.** JBEI seeks to ensure that bioenergy crops are robust and sustainable. Researchers are using techno-economic assessment and lifecycle analysis models to (1) predict the impact of research results on the biofuel selling price and carbon efficiencies and (2) assess economic and environmental performance at the U.S. national scale over multiple decades.

**Feedstock Development.** In developing fundamental understanding of cell wall biology, JBEI will have the knowledgebase to engineer and field test bioenergy crops for low susceptibility to disease and drought. These bioenergy crops also are being tailored for facile biomass deconstruction into sugars and lignin-derived intermediates and near full utilization by engineered biofuel- and bioproduct-producing microorganisms. Most of JBEI’s research in this focus area targets sorghum, but other JBEI work examines switchgrass and poplar (see image, Developing Better Plants for Biofuels, p. 18).

**Deconstruction and Separation.** JBEI is developing an integrated, feedstock-agnostic deconstruction process using renewable and biocompatible ionic liquids and optimized enzyme mixtures. The deconstruction process will liberate high yields (as much as 90% or more) of the sugars and lignin-derived intermediates from bioenergy crops suitable for biological conversion (see figure, Crystallography Study Benefits Bioenergy Industry, this page). This work includes the discovery and

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*Crystallography Study Benefits Bioenergy Industry.* During the deconstruction process, lignin is partially converted to molecules like stilbenes, which occur naturally in plants and some bacteria and may play a role in plant pathogen resistance. Enzymes such NOV1 could be used to produce valuable bioproducts from the breakdown of stilbenes and similar molecules. JBEI and GLBRC researchers recently reported the atomic-level structure of NOV1 (pictured above), gaining insight into how the enzyme breaks down a stilbene substrate into two smaller compounds. **(A)** This protein fold view highlights the placement of iron (yellow), dioxygen (red), and resveratrol, a representative substrate (blue), in the active site of the enzyme. **(B)** This surface slice representation shows the shape of the active site cavity and the arrangement of iron, dioxygen, and resveratrol. [Courtesy Ryan McAndrew, JBEI and LBNL]
optimization of enzymes through the exploration of targeted ecosystems and microbes that are the natural sources of these enzymes. Further JBEI research in this focus area involves developing predictive biomass deconstruction tools that will enable the efficient design of deconstruction processes based on these discoveries.

**Conversion.** JBEI is engineering microbes (e.g., *Pseudomonas putida*) with metabolisms to simultaneously use the sugars and aromatics resulting from the deconstruction process and produce a variety of the targeted biofuels and bioproducts at industrially relevant titers, rates, and yields. These are products that otherwise would be made from petroleum using traditional chemistry.

Achieving these goals requires integration across all four research focus areas. Biomass deconstruction technology can be improved with engineered crops, the composition of which is matched as closely as possible with the metabolism of the microbes to maximize conversion. Ionic liquids are chosen to maximize product yield and minimize toxicity, which in turn enables process integration and consolidation. Finally, techno-economic and lifecycle analysis are needed to optimize the sustainability and affordability of the entire process.

Additionally, JBEI is developing new analytical technologies and methods to meet current and future needs in biofuels research to increase sample throughput, decrease reagent use, increase measurement fidelity, and reduce assay time (see image, Microfluidic Tools, this page).

**JBEI Partners**

- Lawrence Berkeley National Laboratory (Berkeley, California; lead institution)
- Brookhaven National Laboratory (Upton, New York)
- Iowa State University (Ames)
- Lawrence Livermore National Laboratory (Livermore, California)
- Pacific Northwest National Laboratory (Richland, Washington)
- Sandia National Laboratories (Livermore, California; Albuquerque, New Mexico)
- University of California (Berkeley)
- University of California (Davis)
- University of California (San Diego)
- University of California (Santa Barbara)
- University of California Agriculture and Natural Resources (Parlier)

**Microfluidic Tools.** JBEI is developing microfluidic (i.e., lab-on-a-chip) tools for biomass deconstruction and synthetic biology applications. This droplet generator and digital microfluidics device offers potential for automating molecular biology experiments and significantly reducing cost, time, and variability while improving throughput. [Courtesy LBNL and Roy Kaltschmidt]
Understanding the instructions for life encoded in the DNA sequence, or genome, of natural systems offers a wealth of potential for advancing biological solutions to many of today’s energy and environmental challenges. To harness this potential, the U.S. Department of Energy (DOE) Genomic Science program supports fundamental research to understand the systems biology of plants and microbes as they respond to and modify their local environments. Systems biology is the holistic, multidisciplinary study of complex interactions that specify the function of an entire biological system—whether single cells or multicellular organisms—synthesizing decades of reductionist studies that identified and characterized individual components.

As a leader in systems biology research, the Genomic Science program uses genome sequences as the blueprint for understanding the common principles that govern living systems. Knowledge of these common principles revealed by studying organisms relevant to one DOE mission facilitates breakthroughs in the basic biology important to other DOE and national needs.

By examining the translation of genetic codes into functional proteins, biomolecular complexes, metabolic pathways, and regulatory networks, Genomic Science program research focuses on the grand challenge of developing a mechanistic, predictive understanding of plant and microbial system behavior across a range of scales, from genes to small ecosystems. Scientific insights achieved in pursuit of this challenge will enable, for example, the design and re-engineering of plants and microbes for DOE missions in sustainable advanced biofuels and bioproducts, improved carbon storage capabilities, and controlled biological transformation of materials such as nutrients and contaminants in the environment.

The Genomic Science program is part of the Office of Biological and Environmental Research within DOE’s Office of Science.

**Genomic Science Approaches**

Addressing extremely complex science questions that span all scales of biology, research supported by the 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, including genomics and metagenomics, analytical “omics,” molecular imaging and structural analysis, predictive modeling, and genome-scale engineering.

**Genomics and Metagenomics.** Sequencing and analyzing DNA from individual organisms (genomics) or microbial communities in environmental samples (metagenomics) form the foundation for systems biology research. The DOE Joint Genome Institute is an important scientific user facility that generates high-quality sequences and analysis techniques for diverse microbes, plants, and other organisms relevant to DOE energy and environmental missions.

**Analytical Omics.** Transcriptomics, proteomics, metabolomics, and other analyses—collectively described as “omics”—identify and measure the abundance and fluxes of key
RESEARCH PORTFOLIO

BIOENERGY RESEARCH CENTERS
Provide technologies and scientific insights across four multi-partnership centers laying the groundwork for sustainable, cost-effective advanced biofuels and bioproducts from lignocellulosic biomass.

SYSTEMS BIOLOGY FOR BIOENERGY
Improves fundamental understanding of potential plant feedstocks and microbes capable of deconstructing biomass and synthesizing biofuels and bioproducts.

PLANT FEEDSTOCKS GENOMICS
Accelerates breeding for improved, dedicated bioenergy crops by characterizing the genes, proteins, and molecular interactions influencing biomass production (a joint effort with the U.S. Department of Agriculture).

SUSTAINABLE BIOENERGY
Investigates plant-microbe interactions underpinning development of dedicated, high-yield bioenergy crops that require few external inputs, grow on marginal soils, and withstand changing conditions.

BIOSYSTEMS DESIGN
Develops knowledge for engineering useful traits into plants and microbes to produce biofuels and bioproducts and to advance biotechnology.

CARBON CYCLING AND ENVIRONMENTAL MICROBIOLOGY
Links structure and function of microbial communities in the field with key environmental or ecosystem processes.

COMPUTATIONAL BIOLOGY
Provides hypothesis-generating analysis techniques, data, and simulation capabilities within the DOE Systems Biology Knowledgebase (KBase) to accelerate collaborative, reproducible systems science.

GOAL
Achieve a predictive, systems-level understanding of plants, microbes, and biological communities to enable biobased solutions to DOE mission challenges in energy and the environment.

OBJECTIVES

1. Determine the molecular mechanisms, regulatory elements, and integrated networks needed to understand genome-scale functional properties of biological systems.
2. Develop omics experimental capabilities and enabling technologies needed to achieve dynamic, systems-level understanding of organism and community function.
3. Flexibly scale understanding of biological processes from defined subsystems to individual organisms, consortial assemblies of multiple organisms, or complex communities operating at ecosystem scales.
4. Understand the principles governing living systems and develop tools for more sophisticated biosystems design, enabling the targeted modification of functional properties at the genome scale.
5. Develop the knowledgebase, computational infrastructure, and modeling capabilities to advance predictive understanding and manipulation of biological systems.

SYNERGIES WITH ENABLING BER PROGRAMS AND USER FACILITIES

DOE JOINT GENOME INSTITUTE
jgi.doe.gov
Provides high-quality sequence data and analysis techniques for plants, microbes, and their communities in support of bioenergy and environmental research.

BIOIMAGING TECHNOLOGY
science.energy.gov/ber/bioimaging-technology/
Develops imaging, measurement, and characterization platforms to visualize the spatial and temporal relationships of key plant and microbial metabolic processes.

STRUCTURAL BIOLOGY INFRASTRUCTURE
www.berstructuralbiportal.org
Provides specialized instruments at light and neutron facilities to understand the properties and structures of biological molecules and link this information to function.

ENVIRONMENTAL MOLECULAR SCIENCES LABORATORY
www.emsl.pnl.gov
Provides tools for characterizing molecules to organisms, including the chemical constituents and dynamics of complex natural systems (e.g., soil microbiome).
molecular species indicative of organism or community activity. Global analyses of important cellular components such as RNA transcripts, proteins, and metabolites inform scientists about organisms’ physiological status. This research, along with chemical and structural analytical technologies including stable isotope tracking and nano secondary ion mass spectrometry (NanoSIMS), also provides insights into gene function and indicates which genes are activated and translated into functional proteins as organisms and communities develop or respond to environmental cues. Methods that analyze DNA, RNA, proteins, and other molecules extracted directly from environmental communities enable discovery of new biological processes and provide novel insights into relationships between the composition of communities and the functional processes that they perform.

**Molecular Imaging and Structural Analysis.** Genomic Science program investigators are developing and using new methods for characterizing the chemical reaction surfaces, organization, and structural components in molecular complexes and tracking molecules to view cellular processes as they are occurring. Depending on the spatial scale, a variety of imaging technologies can be used to visualize the complex molecular choreography within biological systems. Some of these tools (e.g., synchrotrons, neutron sources, and electron microscopes) are available at DOE Office of Science user facilities that provide state-of-the-art spatial, temporal, and chemical measurement sensitivity.

**Predictive Modeling.** Computational models are used to capture, integrate, and represent current knowledge of biology at various scales. Researchers are using genome sequences and molecular, spatial, and temporal data to build models of signaling networks, gene regulatory circuits, and metabolic pathways that can be iteratively tested and validated to refine system understanding.

**Genome-Scale Engineering.** Genomes and systems-level understanding are uncovering the principles that govern system behavior, enabling genome-scale redesign of organisms. This research approach may involve building entirely new microbes from a set of standard parts—genes, proteins, and metabolic pathways—or radically redesigning existing biological systems to enable capabilities that the systems would not possess naturally.
The DOE Office of Science manages fundamental research programs in basic energy sciences, high-energy physics, fusion, biological and environmental sciences, and computational science. It 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, nanoscale science research centers, genome sequencing centers, and advanced resources in imaging and analysis for biological and environmental systems.

The Office of Biological and Environmental Research (BER) within the DOE Office of Science supports transformative science and scientific user facilities to achieve a predictive understanding of complex biological, Earth, and environmental systems for energy and infrastructure security and resilience. This research, conducted across DOE national laboratories, universities, and research institutions, focuses on interconnections between energy and the environment. BER aims to understand fundamental biological, biogeochemical, and physical principles to be able to predict processes occurring at scales ranging from the molecular and genomics-controlled smallest scales to environmental and ecological processes at the scale of planet Earth.

Starting with the genetic information encoded in organisms’ genomes, biological research, housed within BER’s Biological Systems Science Division, seeks to discover the principles that guide translation of the genetic code into the functional proteins and metabolic and regulatory networks underlying the systems biology of plants and microbes as they respond to and modify their environments. This predictive understanding will enable the design and re-engineering of microbes and plants for improved energy resilience and sustainability, including advanced biofuels and bioproducts, enhanced carbon storage capabilities, and controlled biological transformation of materials such as nutrients and contaminants in the environment.

Earth and environmental systems research, housed within BER’s Climate and Environmental Sciences Division, advances fundamental understanding of the dynamic, physical, and biogeochemical processes required to systematically develop and validate Earth system models (ESMs) that integrate across the atmosphere, land masses, oceans, sea ice, and subsurface. These ESMs are required for predictive tools and approaches needed to inform future energy and resource needs.

To facilitate world-class research in these areas, BER also supports three user facilities—Joint Genome Institute, Environmental Molecular Sciences Laboratory, and Atmospheric Radiation Measurement Research Facility—that enable observations and measurements of biological, biogeochemical, and atmospheric processes using the latest technologies.
For More Information

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

- Center for Advanced Bioenergy and Bioproducts Innovation (CABBI)
cabbi.bio

- Center for Bioenergy Innovation (CBI)
cbi.ornl.gov

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

- Joint BioEnergy Institute (JBEI)
  jbei.org

- DOE Genomic Science Program (GSP)
genomicscience.energy.gov

- GSP: Systems Biology for Bioenergy
genomicscience.energy.gov/biofuels/

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

- Lignocellulosic Biomass for Advanced Biofuels and Bioproducts (report)
genomicscience.energy.gov/biofuels/lignocellulose/

- DOE Office of Biological and Environmental Research
  science.energy.gov/ber/

- DOE Office of Science
  science.energy.gov