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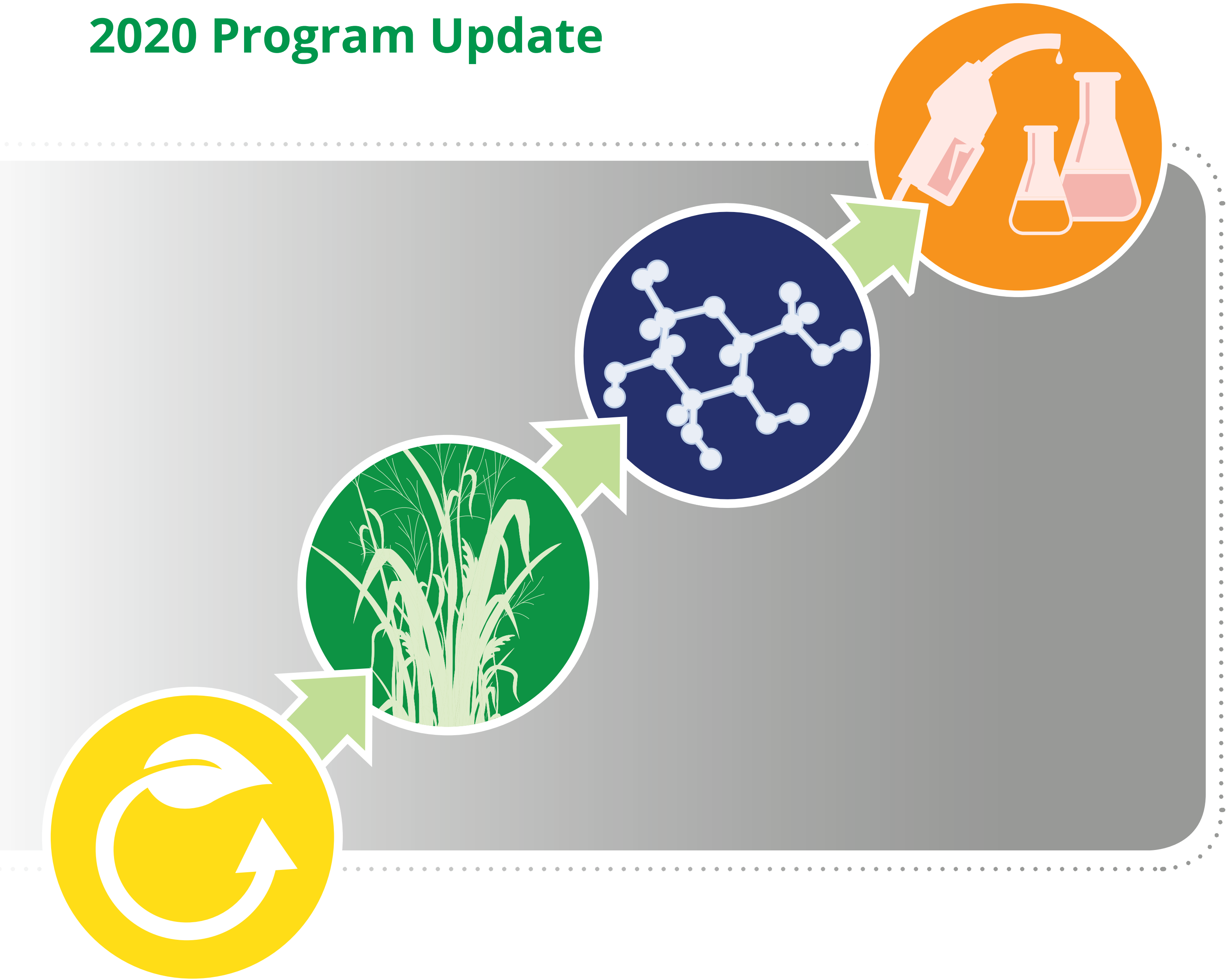
U.S. DEPARTMENT OF ENERGY

Bioenergy Research Centers

2020 Program Update



U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

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Websites for DOE Bioenergy Research 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

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U.S. Department of Energy

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2020 Program Update

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U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

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U.S. Department of Energy

Bioenergy Research Centers

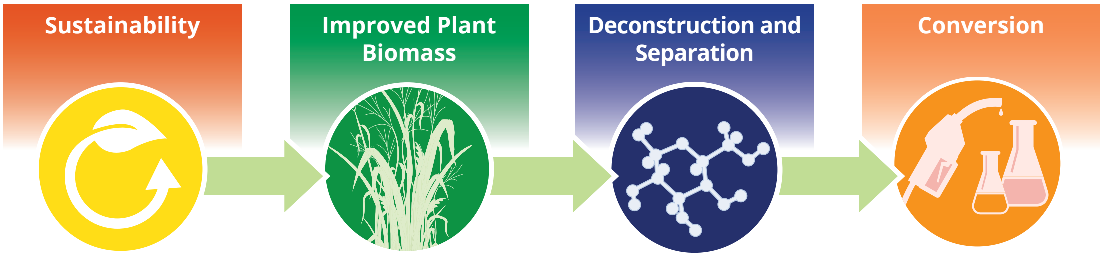
Introduction

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

Lignocellulose is the most abundant biological material on Earth. Most often contained in plant cell walls, it is made up of long, tightly bound chains of sugars (polysaccharides) that can be converted into biofuels and bioproducts by microbes. However, cellulose in cell walls is resistant to degradation because it is embedded within a matrix of other polymers including hemicellulose and lignin. This resistance or 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 research based on advanced biotechnology 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).

Since 2007, the Genomic Science program within DOE's Office of Science Office of Biological and Environmental Research (BER) has supported the Bioenergy Research Center (BRC) program, whose mission is to break down the barriers to actualizing a domestic bioenergy industry (see sidebar, Genomic Science to Advance DOE Missions, p. 2). Toward this end, the BRCs have made significant advances in making this new biobased economy a reality. The BRCs have produced multiple breakthroughs in the form of deepened understanding of sustainable biomass production practices, targeted re-engineering

From Biomass to Advanced Biofuels and Bioproducts



Sustainability

Produce biomass for biofuels and bioproducts with minimal to positive impacts on the environment.

Biomass Development

Develop crops with cell walls optimized for deconstruction and conversion to biofuels and bioproducts.

Biomass Breakdown

Improve enzymes and microbes that break down biomass into sugars and lignin.

Biofuel and Bioproduct Synthesis

Engineer metabolic pathways in microbes to produce biofuels and bioproducts.

Genomic Science to Advance DOE Missions

For more than 35 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 predictive, whole-system 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 and the environment. For more information on the Genomic Science program as well as BER and DOE's Office of Science, see p. 44 and p. 47, respectively.

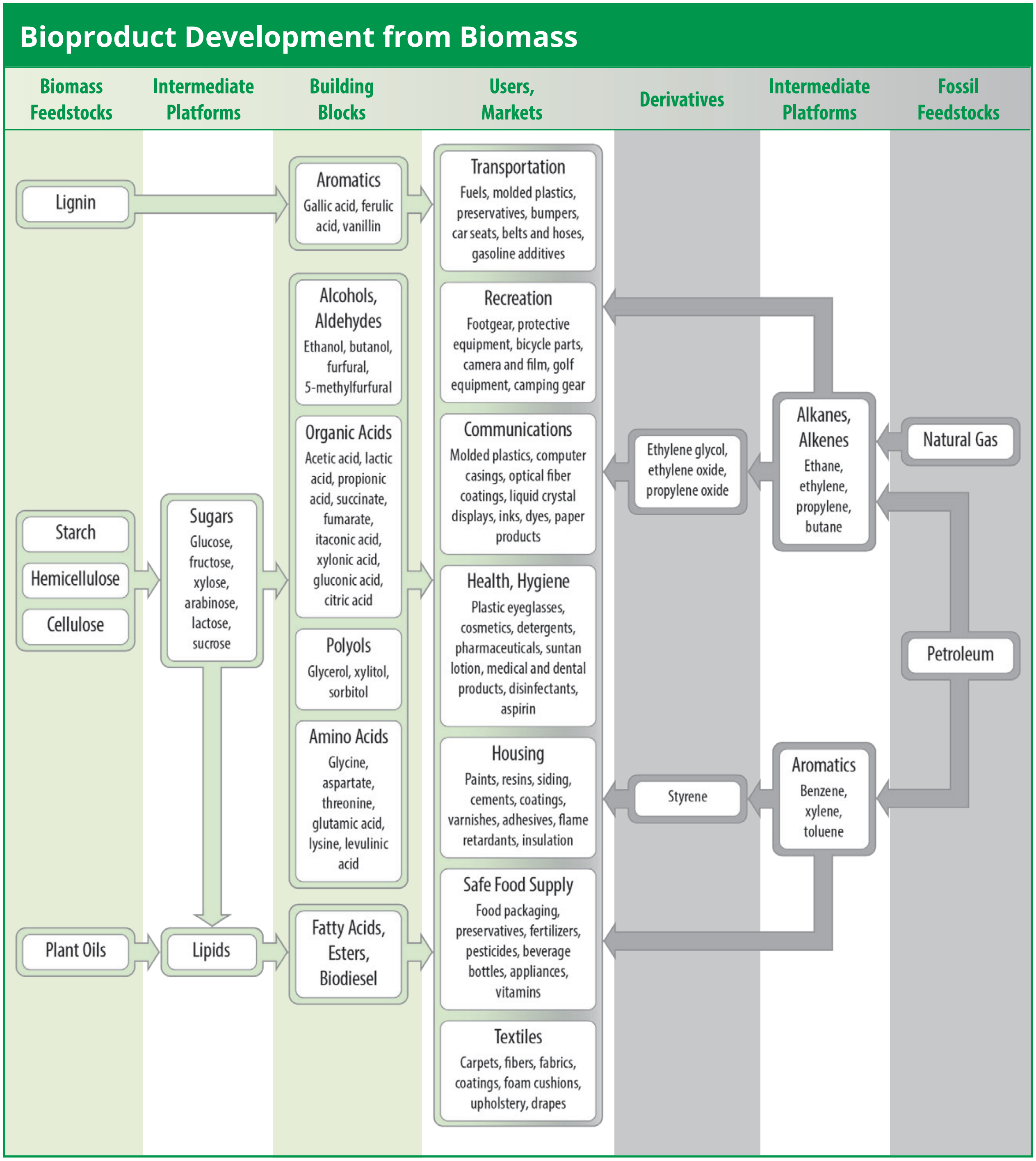
of biomass feedstocks, development of new methods for deconstructing feedstocks, and engineering of microbes for more effective production of a diverse range of biofuels. More specifically, advances included (1) engineering of lignin composition and deposition to reduce plant cell wall recalcitrance without affecting plant viability and to facilitate lignin valorization; (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) research for the refinement of consolidated bioprocessing (i.e., simultaneous breakdown and conversion to biofuels and bioproducts by naturally cellulolytic microbes); (5) metabolic engineering of microbes and plants for the biological production of numerous advanced biofuels, bioproducts, or their immediate precursors; (6) identification of new plant genes and an improved understanding of their role in cell wall biosynthesis; and (7) refinement of technoeconomic and lifecycle analyses.

Advanced Biofuels and Bioproducts

These BRC technological advancements and successes are being leveraged by the BRCs to further improve the production efficiencies of biofuels and bioproducts. 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 Fossil Fuel–Derived Products, p. 3). They also may be novel chemicals that cannot be efficiently produced from petroleum.

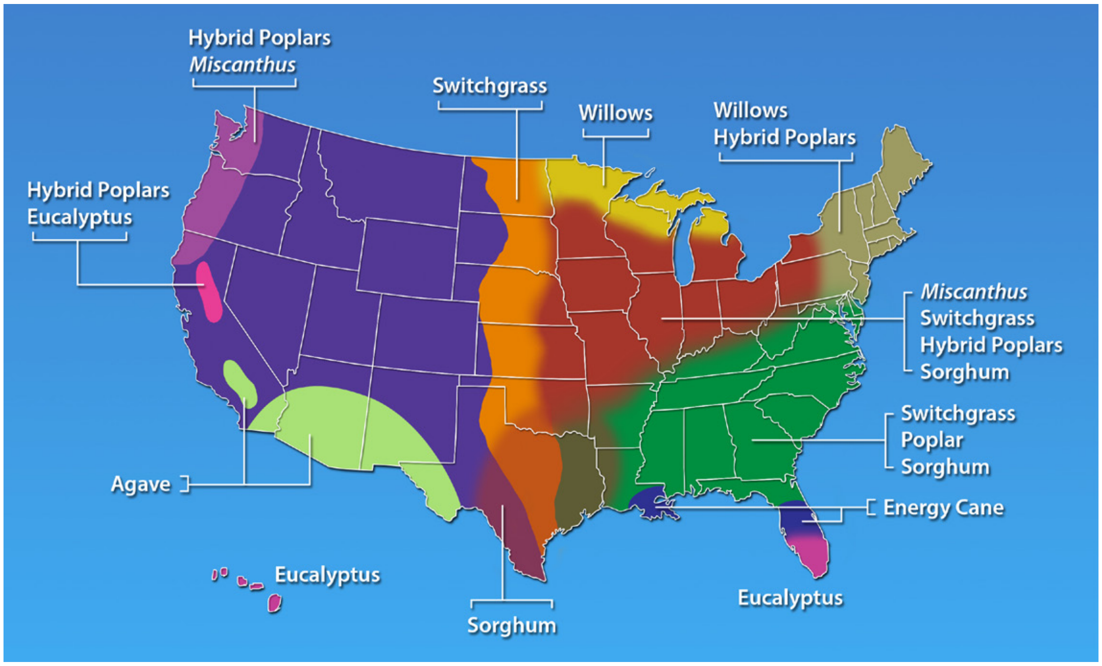
The four BRCs in this research endeavor are based in the geographically diverse Midwest, Southeast, and West Coast regions. BRC locations correspond to the geographic ranges of biomass crops that are being developed (see figure, Approximate Geographic Distribution of Potential Dedicated Biomass Crops, p. 4). BRC partners include universities, private companies, nonprofit organizations, and DOE national laboratories (see DOE Bioenergy Research Centers and Partners map, back cover). 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.

- **Center for Advanced Bioenergy and Bioproducts Innovation** (CABBI; University of Illinois at Urbana-Champaign) is integrating recent advances in agronomics, genomics, biosystems design, and computational biology to increase the value of energy crops, using a "plants as factories" approach to grow fuels and chemicals in plant stems and an automated foundry to convert biomass into valuable chemicals that are ecologically and economically sustainable.



Bioproduct Development from Biomass

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, biosystems design 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.



Approximate Geographic Distribution of Potential Dedicated Biomass Crops. Multiple crops designed for various agroecosystems will require continued development to realize biomass yields for large-scale production of biofuels and bioproducts. As research progresses, new crops could be added and the boundaries of their potential ranges could change. Agricultural residues (e.g., wheat straw, rice hulls, and corn stover) are not included on this map.

- **Center for Bioenergy Innovation** (CBI; Oak Ridge National Laboratory) is accelerating the domestication of bioenergy-relevant plants and microbes to enable high-impact, value-added coproduct development at multiple points in the bioenergy supply chain.
- **Great Lakes Bioenergy Research Center** (GLBRC; University of Wisconsin–Madison) is developing 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 using the latest tools in molecular biology, chemical engineering, and computational and robotics technologies to transform biomass into biofuels and bioproducts.

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 biofuels 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 plant or 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 biosystems design 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, biosystems design, and computational biology

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.

	Sustainability	Feedstock Development	Deconstruction and Separation	Conversion
CABBI	Integrate economic and environmental analyses for a sustainable biomass supply.	Develop "plants as factories" for biofuels and bioproducts.	Develop process and extraction technologies for plant oils and sugars.	Establish an automated biofoundry for fuels and bioproducts.
CBI	Optimize water and nutrient use in dedicated, high-yielding bioenergy crops.	Create genomic tools for accelerating the domestication of bioenergy crops.	Advance integrated and consolidated thermophilic bioprocessing.	Generate drop-in biofuels and bioproducts from biomass and lignin residues.
GLBRC	Conduct long-term studies of growing energy crops on nonagricultural land.	Design productive and high-value energy cropping systems.	Develop cost-effective biomass deconstruction and separation strategies.	Identify and engineer novel biomass conversion microbes.
JBEI	Study environmental resilience of engineered bioenergy crops.	Engineer plants for atom-economical conversion into biofuels and bioproducts.	Develop feedstock-agnostic biomass deconstruction processes using renewable ionic liquids.	Develop high-throughput biosystems design tools and hosts for scalable, atom-economical biofuels and bioproducts.

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

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 production systems for biofuels and bioproducts requires knowledge about the interactions between 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 technoeconomic 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.
- Determining biomass crop and crop management systems needed to ensure sustainably produced feedstocks.

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 genetic tools and biosystems design approaches to advance bioenergy feedstock crop creation and production.
- 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. 7). Stronger linkages between advances in biomass development and fuels production will strengthen these deconstruction efforts.

BER goals for this scientific focus area include:

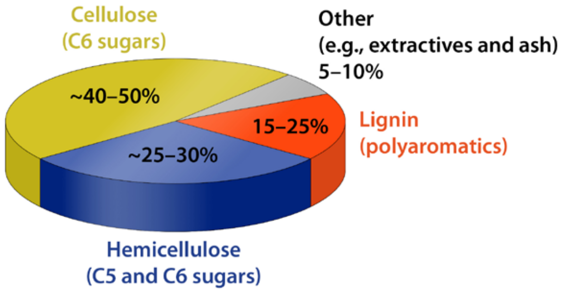
- Developing feedstock-agnostic deconstruction processes capable of efficiently fractionating biomass into targeted output streams with minimal inhibitor formation.
- Gaining a detailed understanding of plant cell wall biosynthesis, composition, structure, and properties during deconstruction.
- Improving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

Plant Cell Wall Recalcitrance: A Key Scientific Challenge

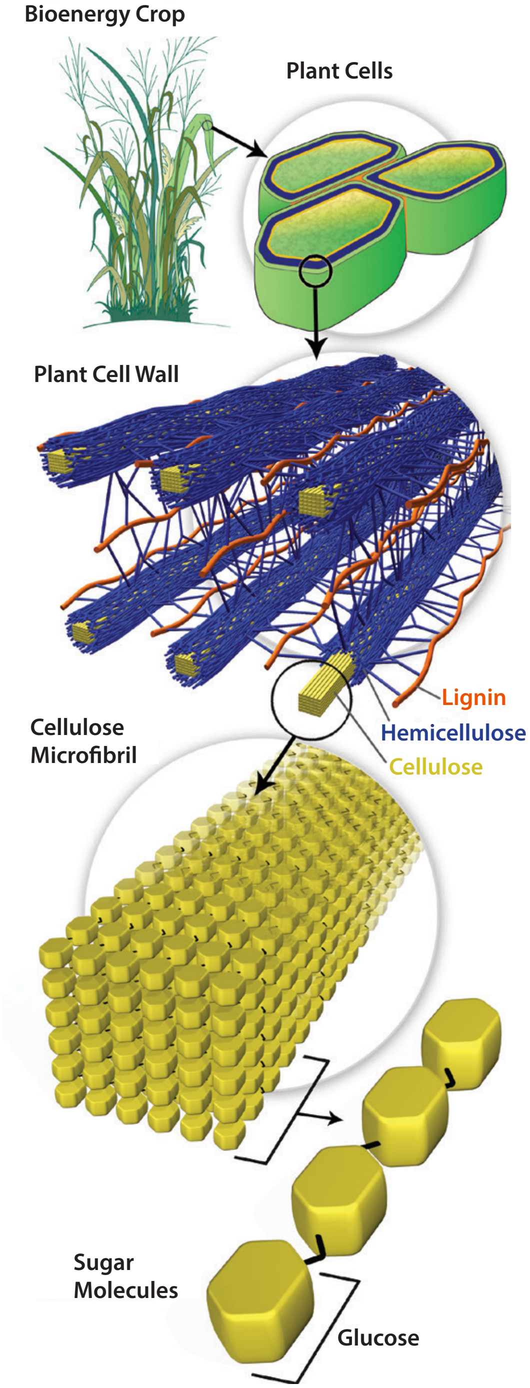
The tough structural materials in plant cell walls form a complex composite exquisitely designed to support plant structure and resist biological and chemical assaults. This natural resistance to degradation is called "recalcitrance" and represents one of the greatest challenges to attaining a viable,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



- 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 (see sidebar, Lignin Valorization, this page).

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Lignin Valorization

Lignin valorization—the conversion of lignin to higher-value products—has been pursued for more than a century. Besides niche markets in the pulp and paper industry, however, lignin valorization remains a major challenge and is an exciting frontier for biomass conversion. In current designs of pioneer biorefineries, which aim to convert polysaccharides to fuels, residual lignin is slated for combustion to heat and power. However, recent technoeconomic analyses and lifecycle assessments of various lignin valorization processes have suggested both major economic and sustainability benefits associated with lignin conversion to a range of valuable coproducts in the biorefinery, even when importing fossil-based power into the biorefinery for heat and power needs.

The scientific challenges in lignin valorization result primarily from its inherent reactivity and heterogeneity. Standard pretreatment approaches, such as dilute acid or organosolv methods, often cleave labile carbon-oxygen linkages at high temperature and form reactive intermediates that condense into more recalcitrant lignin, creating a substrate that is difficult to convert into valuable products. The Department of Energy's Bioenergy Research Centers (BRCs) are collectively taking a holistic approach to overcome this challenge and ultimately realize the potential for industrial-scale lignin valorization processes.

The first decade of BRC research definitively demonstrated that lignin *in planta* exhibits significant plasticity and that nature still holds surprises in terms of natural variation in lignin chemistry. Current BRC approaches for lignin engineering *in planta* include strategies to identify and harness the diversity of lignin in undomesticated energy crops through genome-wide association studies (GWAS), as well as engineering non-native monolignols into lignin as a complementary approach to GWAS studies. By connecting genotype-to-phenotype with GWAS methods, genetic engineering approaches using CRISPR-Cas technologies can then rapidly generate domesticated energy crops that exhibit advantageous lignin phenotypes with high valorization potential.

In terms of lignin processing, the BRCs are pursuing a synergistic portfolio of biological and catalytic approaches toward lignin valorization in the biorefinery that are closely linked to *in planta* engineering.

- Researchers at the Center for Bioenergy Innovation are pursuing a process concept to convert polysaccharides via consolidated bioprocessing with cotreatment, which operates at temperatures that do not modify lignin. This approach produces a native lignin stream that can undergo facile depolymerization via reductive or oxidative chemocatalysis or by further biocatalysis. The resulting lignin-derived intermediates are fed to engineered strains of *Pseudomonas putida* for biological funneling of aromatic compounds to atom-efficient products that can be used for performance-advantaged bioproducts.
- Researchers at the Great Lakes Bioenergy Research Center are pursuing economic harvesting and use of cell wall "clip-offs" (simple aromatics that

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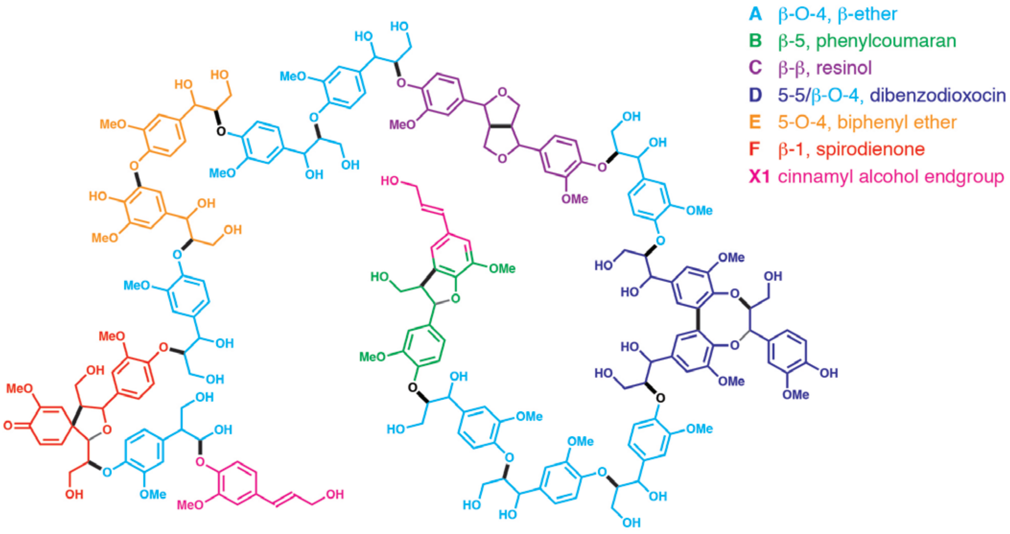
are easily released). Another goal is an innovative flow-through pretreatment technology using gamma-valerolactone that also fractionates biomass into native-like lignin and high yields of monomeric sugars. The resulting lignin streams are then processed through oxidative or hydrogenolytic catalytic chemistry that leads to high yields of aromatic intermediates. An emerging, promising aromatic-catabolic microbe, *Novosphingobium aromaticivorans*, is then engineered to funnel the lignin-derived intermediates to atom-efficient endproducts that can be used for novel polymers and other formulated products.

- Researchers at the Joint BioEnergy Institute are pursuing a novel ionic liquid–based "one pot" strategy that will enable simultaneous depolymerization of both polysaccharides and lignin, resulting in a mixed, liquid stream of monomeric sugars and aromatic compounds. These intermediates are then fed to engineered strains of *P. putida*, *Rhodosporidium toruloides*, or other novel funneling microbes to produce hydrocarbon fuels and other valuable coproducts from carbohydrates and aromatic compounds simultaneously.

- At the Center for Advanced Bioenergy and Bioproducts Innovation, researchers are attempting to shift grass stem carbon to more versatile and easily converted carbon forms than recalcitrant lignin and to integrate biological and chemical catalytic approaches to produce a diverse portfolio of advanced fuels and bioproducts.



Molecular Lignin Model. Lignin derives from radical coupling reactions of monolignols, mainly by endwise coupling of the monomer onto the growing polymer. Lignins are racemic polymers with no defined structural sequence, with the various types of dimeric units shown here characterized by their diagnostic interunit linkages: β-ether units (β–O–4-aryl ether linkages), phenylcoumarans (β–5), resinols (β–β), biphenyl ethers (5–O–4 or 4–O–5), dibenzodioxocins (from 5–5-coupling of two oligomers followed by 4–O–β-coupling with a monomer), and spirodienones (β–1). Most methods of degrading lignins to low–molecular weight compounds operate by cleaving the β-ether linkages in β-ether units that are, fortunately, the most prevalent. A model of softwood lignin is shown above. [GLBRC]

Continued from page 8

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.

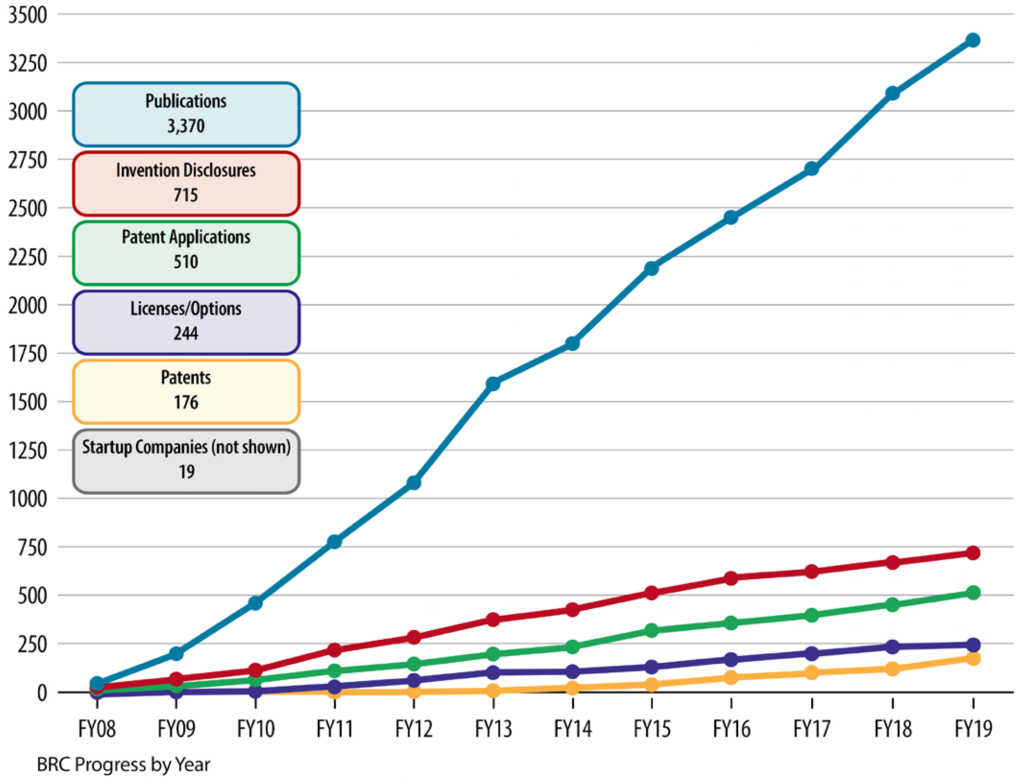
BER goals for this scientific focus area include:

- 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 DOE BRCs are addressing these challenges to provide a broad scientific underpinning for producing biofuels and bioproducts from sustainable biomass resources and the building blocks of new technological advances and speeding the translation of basic research results to industry. As of the end of fiscal year 2019, the BRCs have produced 3,370 peer-reviewed publications, 715 invention disclosures, 510 patent applications, 244 licenses or options, 176 patents, and 19 company startups. Through this work, they transferred substantial insight and expertise to industry via cooperation with both large and small companies and knowledge to other researchers (see sidebar, Accelerating Deployment of Biofuel and Bioproduct Science and Commercialization, p. 11).

Accelerating Deployment of Biofuel and Bioproduct Science and Commercialization

Scientific advances made by the Department of Energy's Bioenergy Research Centers (BRCs) are providing crucial knowledge needed to develop new biobased fuels and products, methods, and tools that industry can use. Through intellectual property licensing agreements, partnerships, and targeted collaborative affiliations, the BRCs are helping to speed the translation of basic research results to industry, contributing to clean energy.



Summary of Bioenergy Research Center (BRC) Scientific and Technological Output Through Fiscal Year (FY) 2019. Collectively, the four BRCs are producing a portfolio of diverse and complementary scientific strategies that address the challenges of biomass conversion to biofuels and bioproducts. The resulting knowledgebase is providing new insights to help industry meet the broad challenges of reducing the cost of and meeting the demand for advanced biofuels and bioproducts.

CABBI
CENTER FOR ADVANCED BIOENERGY
AND BIOPRODUCTS INNOVATION

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 novel ways to grow, transform, and market biofuels and other bioproducts by integrating recent advances in genomics, biosystems design, and computational biology to increase the value of biomass crops. CABBI represents a unique, game-changing research model designed to accelerate bioproduct development from the bench to industrial scale, while retaining the flexibility to assimilate 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 advancing transformative technologies for the economic and sustainable production of biofuels and bioproducts from plants. Over five 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 production, conversion, and sustainability.

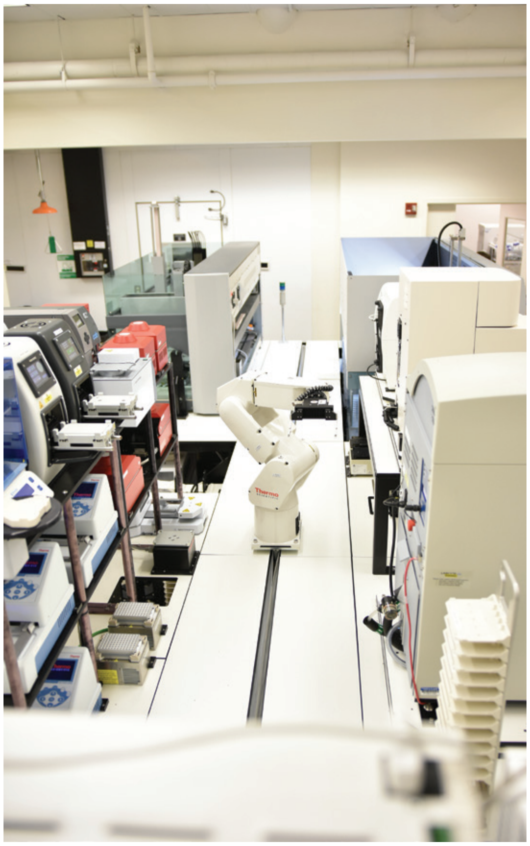
Feedstock Production: Growing the Right Crops. CABBI is founded on the "plants as factories" paradigm, in which biofuels, bioproducts, high-value molecules, and foundation molecules for conversion are synthesized directly in plant stems. This approach circumvents the challenges of developing efficient methods to deconstruct lignocellulose, 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. These grasses are the world's highest biomass producers (see image, High-Yielding Sorghum Grass, p. 14), with demonstrated potential in transgenic sugarcane for oil accumulation in vegetative biomass (Huang et al. 2017). CABBI research centers on increasing the yield efficiency and resiliency of these grasses to minimize

High-Yielding Sorghum Grass. Sorghum, a key bioenergy and bioproduct feedstock, is harvested at the 320-acre Illinois Energy Farm. [CABBI]

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 enhancing understanding of native yeast (e.g., *Saccharomyces cerevisiae*) metabolism and physiology and applying this knowledge to engineer organisms for production of natural and non-natural compounds. As part of this research effort, CABBI scientists are further developing a versatile, automated "biofoundry" (see image, Illinois Biological Foundry for Advanced Biomanufacturing, this page) for rapidly engineering microbial strains that can efficiently produce diverse, high-value bioproducts such as biodiesel, organic acids, jet fuels, lubricants, and alcohols. CABBI is also accelerating the design-build-test-learn framework to overcome challenges associated with designing biological systems to produce non-natural compounds.

Sustainability: Improving the Environmental and Economic Bottom Line. CABBI is developing new technoeconomic and lifecycle analyses and integrating systems-level modeling to examine economic and ecological tradeoffs associated with products and processes generated under the feedstock production and conversion themes. Using production-scale field experiments, CABBI is developing a mechanistic understanding of the plant, soil, microbe, and climate interactions that underlie the productivity and ecosystem services of different feedstocks (see image, Collecting *Miscanthus* Root Samples, p. 15) and investigating 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 are used to show



Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB). iBioFAB is a fully integrated computational and physical infrastructure that supports rapid design, fabrication, validation, and quality control, as well as analysis of genetic constructs and organisms. [CABBI]

CABBI Partners

- **University of Illinois at Urbana-Champaign** (lead institution)
- **Archbold Biological Station** (Venus, Florida)
- **Boston University** (Massachusetts)
- **Brookhaven National Laboratory** (Upton, New York)
- **Colorado State University** (Fort Collins)
- **HudsonAlpha Institute for Biotechnology** (Huntsville, Alabama)
- **Institute for Systems Biology** (Seattle, Washington)
- **Iowa State University** (Ames)
- **Lawrence Berkeley National Laboratory** (Berkeley, California)
- **Mississippi State University** (Starkville)
- **Princeton University** (New Jersey)
- **Texas A&M University** (College Station)
- **University of California** (Berkeley)
- **University of Florida** (Gainesville)
- **University of Idaho** (Moscow)
- **University of Nebraska** (Lincoln)
- **University of Wisconsin** (Madison)
- **U.S. Department of Agriculture Agricultural Research Service** (Houma, Louisiana; Peoria and Urbana, Illinois)
- **West Virginia University** (Morgantown)

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.

Industry Partnerships

One of CABBI's objectives is to translate its research results to commercial deployment. CABBI and the Integrated Bioprocessing Research Laboratory have an Industrial Affiliates program to engage industry in cutting-edge bioprocessing techniques and derisk new intellectual property (IP) for transition to commercialization. Industrial Affiliates get a first look at newly developed technologies in bioprocessing and bioenergy, as well as the associated IP, through an annual biotechnology showcase. Industry representatives also sit on CABBI's strategic advisory board, bringing a corporate perspective to its research directions.

Collecting *Miscanthus* Root Samples. *Miscanthus* and sorghum root samples are analyzed for biomass and distribution patterns at the Illinois Energy Farm, and those data are evaluated alongside carbon and nitrogen cycling data. [CABBI]

Education and Outreach

CABBI's outreach efforts help students from grade school to undergraduate levels better understand bioenergy feedstock production; conversion methods to produce valuable fuels and chemicals; and economic and environmental sustainability in the field, the laboratory,

CABBI-Sponsored Students Present Their Research. University of Illinois undergraduate students earned a silver medal at the 2019 International Genetically Engineered Machine competition. [CABBI]

and the world. Two such hands-on efforts included: (1) in collaboration with the Carl R. Woese Institute for Genomic Biology, presenting the annual World of Genomics held in 2019 at the National Academy of Sciences in Washington, D.C.; and (2) sponsoring and advising University of Illinois undergraduates in the annual International Genetically Engineered Machine (iGEM) competition (see image, CABBI-Sponsored Students Present Their Research, this page). The Illinois iGEM research project, conducted in summer 2019, explored how glyphosphate, an ingredient in many commercial herbicides, can be degraded into nontoxic components using a genetically modified bacterium.

CABBI Early Career Investigator Spotlight

"We have studied the response to nitrogen fertilizer in *Miscanthus*, which is rapidly gaining in value to the Midwest ecosystem. CABBI gives us the opportunity to expand this research to a wider range of field conditions. Overwhelmingly, the optimal assortment of crops trends in favor of perennials like *Miscanthus*: hardy, self-sufficient plants that live for more than two growing seasons. Transforming just 15% of annual cropland to perennials protects the soil's natural nutrient levels, fostering helpful organisms and minimizing the need for synthetic fertilizers and pesticides."

— Emily Heaton, Associate Professor of Agronomy, Iowa State University

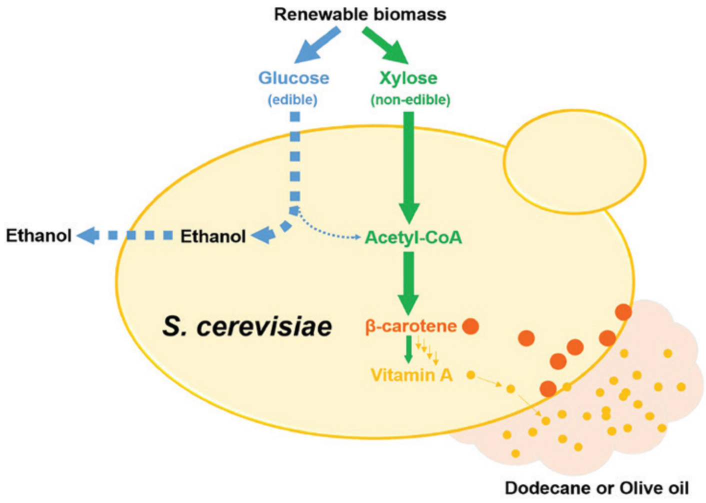
CABBI Research Highlights

Field Data Improve Model Performance

Archbold Biological Station in Venus, Florida. Researchers install an eddy covariance tower to measure and compare gas fluxes over pasture and sugarcane fields. [U.S. Department of Agriculture Agricultural Research Service]

With the Illinois Energy Farm, Iowa Sustainable Advanced Bioeconomy Research Farm, and partners and collaborators from a wide range of geographic locations (Mississippi, Wisconsin, and Florida), CABBI is collecting field-scale data on carbon, nitrogen, and water cycling. These data are being used to strengthen models for analyzing the environmental and economic sustainability of bioenergy cropping systems. An example is data gleaned from research examining nitrogen response across *Miscanthus* × *giganteus* established over multiple years and at multiple sites (Tejera et al. 2019; see also CABBI Early Career Investigator Spotlight, this page).

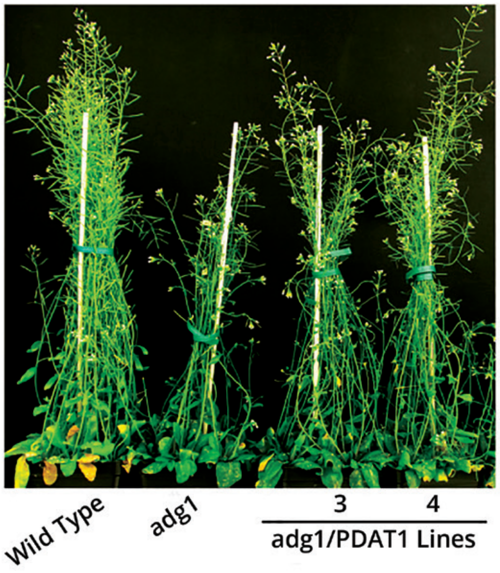
Converting Biomass into Useful Bioproducts



Vitamin A Production from Xylose. Vitamin A is an essential human micronutrient. CABBI researchers engineered *Saccharomyces cerevisiae* to produce this vitamin from xylose, using dodecane or olive oil as an extractive agent to increase production. [Reprinted with permission from Sun, Y., et al. 2019. "Vitamin A Production by Engineered *Saccharomyces cerevisiae* from Xylose via Two-Phase *In Situ* Extraction," *ACS Synthetic Biology* **8**(9), 2131–40. Copyright 2019 American Chemical Society.]

CABBI is harnessing metabolic engineering to create bioproducts using xylose, a major component of lignocellulosic biomass. Xylose stimulates production of branched-chain higher alcohols, such as isobutanol and 2-methyl-1-butanol, in *S. cerevisiae* (Zhang, Y., et al. 2019). Engineered *S. cerevisiae* can convert xylose into valuable bioproducts such as vitamin A (Sun et al. 2019). Additionally, CABBI researchers are exploring bioproducts from novel yeasts such as *Yarrowia lipolytica*, which naturally produces lipids and citric acid. One study showed that a higher pH drives production of citric acid, while lower pH maximizes total lipids and culture density (Zhang, S., et al. 2019). The oleaginous yeast *Rhodosporidium toruloides* has also shown promise in the production of multiple value-added chemicals such as galactitol, which can be used to produce polymers with applications in medicine and as a precursor for anti-cancer drugs (Jagtap et al. 2019).

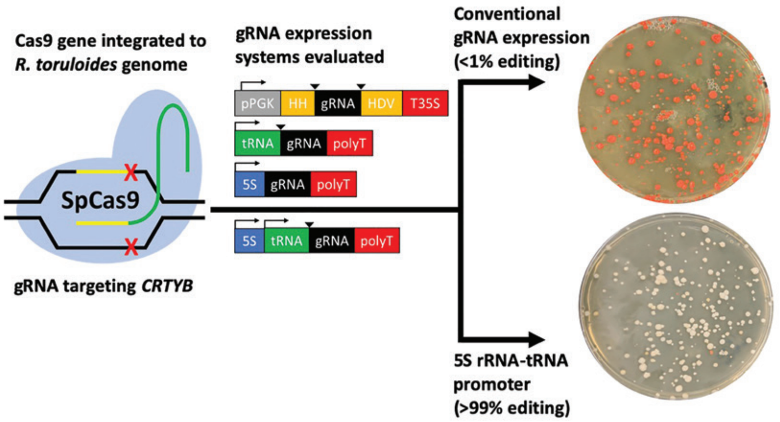
Laying the Foundation for Plants as Factories



Overexpression of Phospholipid:Diacylglycerol Acyltransferase1 (PDAT1) Improves Growth of *Arabidopsis* Line. Overexpression of PDAT1 caused an increase in both fatty acid synthesis and turnover and increased the accumulation of triacylglycerol at the expense of sugars. [Reprinted under a Creative Commons license (CC-BY-4.0) from Fan, J., et al. 2019. "Diversion of Carbon Flux from Sugars to Lipids Improves the Growth of an *Arabidopsis* Starchless Mutant," *Plants* **8**(7), 229. DOI:10.3390/plants8070229.]

CABBI research findings are creating a basis for next-generation feedstock varieties that accumulate ready-to-use oils in their stems. Work to increase (Yu et al. 2019) and tailor (Bansal et al. 2018) the types of triacylglycerol fatty acids in *Camelina* seed and to divert carbon from sugar to lipids in *Arabidopsis* while minimizing growth impacts (Fan et al. 2019) contributes to the knowledge needed to design new lines of sugarcane, *Miscanthus*, and sorghum. As a result, newly engineered feedstocks will contain high levels of fatty acids at low cost. To more easily implement these improvements, other researchers are increasing the efficiency of gene editing in sugarcane and sorghum. Zhao et al. (2019) described the development of a highly expressed single-copy locus as a landing pad for transgene stacking in sugarcane using site-specific recombination or genome-editing tools. Additionally, CRISPR technology has been used to successfully edit sorghum within specific gene families (Li et al. 2018).

New Biosystems Design Tools and Techniques to Enable High-Throughput Strain Development



Development of a CRISPR/Cas9 System for Gene Editing. A 5S ribosomal RNA (rRNA)-transfer RNA (tRNA) fusion promoter enables guide RNA (gRNA) expression and high-efficiency CRISPR/Cas9 gene editing in *Rhodosporidium toruloides*. [Reprinted with permission from Schultz, J. C., et al. 2019. "Development of a CRISPR/Cas9 System for High Efficiency Multiplexed Gene Deletion in *Rhodosporidium toruloides*," *Biotechnology and Bioengineering* **116**(8), 2103–109. Copyright 2019, John Wiley and Sons.]

New CABBI tools are improving the efficiency of biosystems design in a variety of yeasts. CABBI reported the first CRISPR/Cas9-based system for gene disruption in the yeast *Issatchenkia orientalis* (Tran et al. 2019), and the first CRISPR/Cas9 system for modular, targeted gene knockouts in *R. toruloides*, a promising platform yeast that grows on lignocellulosic sugars (Schultz et al. 2019). These editing systems pave the way for further genome and metabolic engineering for bioproduct production. New laboratory techniques are being aided by machine learning and model-guided strain design. CABBI researchers helped develop web-based design software that improves the efficiency of Golden Gate assembly, a fundamental tool for biosystems design and genetic engineering (HamediRad et al. 2019a), and a novel strain design algorithm that incorporates transcriptional regulation and will facilitate the design of new engineered yeast strains (Shen et al. 2019). The center has also integrated its biofoundry with a machine-learning algorithm that designs experiments, executes them, analyzes the resulting data with a probabilistic model, and determines the best target points to address for achieving an improved biosystem (HamediRad et al. 2019b).

New Processing Techniques to Maximize Feedstock Value

Feedstock Conversion into Valuable Chemicals. Ming-Hsun Cheng, a CABBI postdoctoral researcher, is developing new processing techniques in the Integrated Bioprocessing Research Laboratory at the University of Illinois at Urbana-Champaign. He is using one of the few continuous pretreatment reactors operated at public institutions. [CABBI]

Getting the most value out of CABBI feedstocks requires new methods for processing the resulting biomass into useful chemicals and demonstrating the cost effectiveness of these new methods. To support the "plants as factories" paradigm, techniques must be developed to extract the plant oils and sugars, as well as to valorize the lignocellulosic biomass, without compromising the bioproduct quality. While feedstocks are under continued development, researchers have gotten a head start evaluating processing approaches for sugarcane and sorghum. Technoeconomic analysis of a process comprised of sequential deacetylation, followed by hot water pretreatment and disk refining, found that improved steam recovery and decreased enzyme costs can make cellulosic ethanol production from sugarcane bagasse economically feasible (Cheng et al. 2019a). Another study showed that the combination of improved novel pretreatment steps with engineered yeast strains can incrementally improve ethanol yields from sugarcane bagasse (Wang et al. 2019). For sorghum, the center found that chemical-free, continuous hydrothermal pretreatment of biomass can minimize inhibitor formation and be successfully scaled up from the bench to pilot plant, suggesting a promising industrial application (Cheng et al. 2019b).

Center for Bioenergy Innovation

cbi.ornl.gov

CBI Overview

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 dedicated bioenergy crops (e.g., poplar and switchgrass) and a suite of engineered microbes, CBI researchers are advancing the domestication and design of plants and microbes to produce advanced biofuels and bioproducts, including hydrocarbons for jet fuel and chemical feedstocks for plastic precursors.

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, including brief milling during deconstruction (i.e., cotreatment).

Natural Variation in Biomass Yield. Logs harvested from a CBI research plot show that poplar trees with different individual genotypes grew at varying rates. CBI researcher Wellington Muchero is identifying genes from naturally occurring trees that produce more biomass to create new tree progeny with uniform biomass under varying conditions. [ORNL]

Finally, CBI is committed to translating its research results into applications and potential commercial deployment to meet DOE's bioenergy objectives. Through economic and sustainability analyses, CBI is assessing how its research on new supply chains and process configurations will reduce cost and scale-up risk across the bioenergy supply chain, from biomass planting and harvest all the way through conversion to fuels and products.

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.
- More completely utilize all plant cell wall components, specifically lignin, to funnel and improve biological production of coproduct chemicals and novel materials.

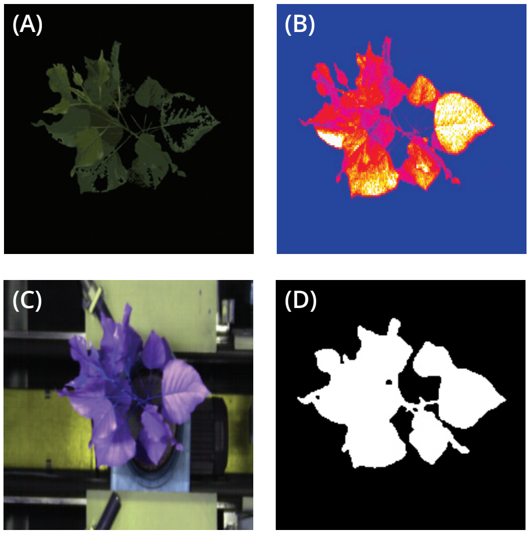
CBI Partners

- **Oak Ridge National Laboratory** (Oak Ridge, Tennessee; lead institution)
- **Colorado State University** (Fort Collins)
- **Dartmouth College** (Hanover, New Hampshire)
- **GreenWood Resources, Inc.** (Portland, Oregon)
- **Massachusetts Institute of Technology** (Cambridge)
- **National Renewable Energy Laboratory** (Golden, Colorado)
- **Noble Research Institute** (Ardmore, Oklahoma)
- **The Pennsylvania State University** (State College)
- **University of California** (Riverside)
- **University of Colorado** (Boulder)
- **University of Georgia** (Athens)
- **University of North Texas** (Denton)
- **University of Tennessee** (Knoxville)
- **University of Wisconsin** (Madison)
- **West Virginia University** (Morgantown)

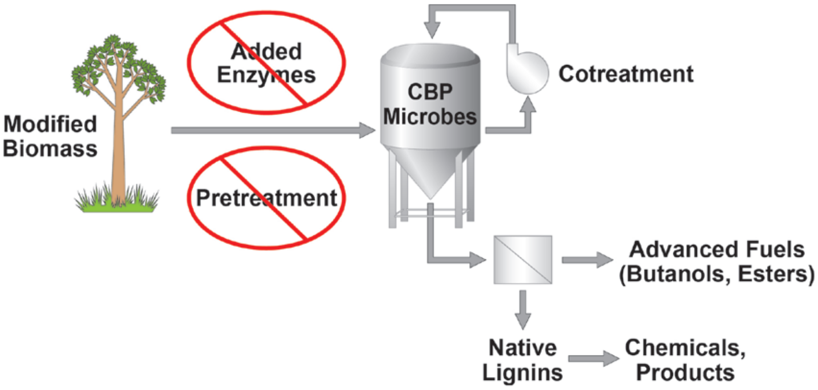
Research Focus Areas

CBI's research targets three research focus areas: improving sustainable biomass feedstocks, enhancing biomass deconstruction and conversion through CBP into specialty fuels, 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. In the last two years, CBI has identified, characterized and utilized key plant genes for yield, cell wall composition, and sustainability traits as a means of achieving lower feedstock costs. Focusing on native perennial plants, such as poplar and switchgrass, provides immediate advantages in sustainability, including fewer chemical inputs and better soil conservation. CBI researchers are harnessing the vast natural diversity in these two dedicated bioenergy crops to uncover the genetic determinants of complex traits related to cell wall chemistry, disease resistance, favorable mycorrhizal colonization, and drought tolerance via genome-wide association studies (GWAS), genomic selection, and genomic editing approaches to design uniform high-yielding, high-quality, resource-efficient feedstocks. CBI's overarching sustainability goals are to discover and develop poplar and switchgrass genotypes with superior water-use efficiency, nitrogen-use efficiency, and pathogen resistance. CBI researchers are using these genotypes in genomic selection programs to discover and test key genes and mechanisms underlying these important sustainability traits and to characterize and deploy beneficial plant microbes to increase the yield stability of plants under low-input and environmentally challenging agricultural conditions. Recent advancements in plant phenotyping (see image, High-Throughput Phenotyping, this page) are being combined with



High-Throughput Phenotyping. Multiple plant phenotypic data can be gathered at the same time for computational analyses. **(A)** Normal image estimates leaf area and growth. **(B)** Chlorophyll fluorescence measures photosynthetic and stress performance. **(C)** Hyperspectral image indicates leaf water, nitrogen, and biochemistry. **(D)** "Mask" composite image eliminates the background. [ORNL]



Consolidated Bioprocessing (CBP) and Cotreatment Paradigm. CBP combines the three biologically mediated steps for biomass processing (cellulase production, enzymatic hydrolysis, and microbial fermentation) into a single operation, thereby eliminating the need for added enzymes and pretreatment. CBP implementation requires microbes that can produce a functional cellulase system while generating advanced fuels at high yields and concentrations. [ORNL]

artificial intelligence–based genomic selection approaches and CRISPR gene editing tools to provide innovative systems biology platforms for identifying unique biotechnological traits and developing improved, sustainable nonfood bioenergy crops.

Consolidated Bioprocessing. CBP eliminates the need for added enzymes and pretreatment, which are the two largest processing cost components in fuel production (see figure, CBP and Cotreatment Paradigm, this page). CBI scientists are focused on robust microbial platforms for converting targeted feedstocks to jet fuel and other specialty fuels and developing consolidated, one-step saccharification and fermentation processes at high rates, titers, and yields. CBI is similarly evaluating CBP combined with a brief milling during fermentation (i.e., cotreatment) for enhanced deconstruction efficacy, lignin valorization potential, and economic viability. CBI technoeconomic analyses estimate that the process intensification associated with combining saccharification and fermentation will reduce both capital and operating costs to scale up the technology. Also being addressed are the fundamental and broadly enabling science questions that arise during the domestication process for new CBP microbes. CRISPR-based gene editing tools for rapid domestication of nonmodel microorganisms are providing the foundational science for a promising and relatively unexplored branch of biotechnology that is applicable to a wide range of host organisms and relevant to the new bioeconomy. CBI researchers are extending the CBP work into hybrid processes to catalytically upgrade alcohols and aromatics into liquid biohydrocarbons functionally equivalent to petroleum (i.e., drop-in biofuels). In the past two years, the sugar residues most recalcitrant to CBP solubilization have been identified, and CBI researchers have shown that the solid residues remaining after CBP are enriched in the industrially exploitable aromatic lignin polymer. In addition, using super high-resolution microscopy, CBI researchers recently uncovered the unique interfacial environment where cellulolytic microbes interact with the biomass surface.

Valuable Lignin Bioproducts. As an example of accelerated domestication and convergent design of plants and microbes, 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 (see image, Creating Value-Added Products, this page). First, CBI researchers are modifying lignin *in planta* to maximize the number of carbon-oxygen bonds via genetic diversity and engineering, enabling the production of lignin designed for deconstruction. Second, reductive catalytic

Creating Value-Added Products. CBI is generating commercially attractive products from lignin residues, thereby increasing the cost effectiveness of biofuels and bioproducts. One example is these lignin-derived pellets, which can be used to create three-dimensional objects through computer-controlled printing. [ORNL] fractionation (RCF) 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 and modifications of plant cell walls. RCF is also proving to be a useful analytical tool for characterizing and examining lignin composition and degradation. Finally, CBI researchers are employing biological funneling to produce the chemical precursors for plastics. In this process, microbial biocatalysts are designed to (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. CBI targets three lignin-derived products (cis,cis-muconic acid; 2-pyrone-4,6-dicarboxylic acid; and α-ketoadipate) that can be cost effectively converted into precursors for commodity polymers such as adipic and terephthalic acids.

Industry Interactions

CBI seeks to form important industrial relationships, disseminate CBI research results, gain feedback on industrial bottlenecks and concerns, and generate information about commercial opportunities including collaborations. Significant interactions have occurred with various companies including DSM; Forage Genetics International; Gevo; Commercial Aviation Alternative Fuels Initiative (CAAFI); White Dog Labs, Inc.; and Phenotype Screening Corporation. In addition, CBI, DOE's Joint Genome Institute, and LanzaTech are completing a collaboration to release the genome sequences of 200 to 300 industrial clostridial species.

CBI IP is disclosed to CBI's Commercialization Council, which consists of technology transfer representatives from each CBI partner institution. The council reviews new CBI-funded inventions by considering their technical merit and commercial potential in order to develop an IP strategy and share licensing leads. Each owner institution protects its CBI inventions according to its standard practices and coordinates any joint IP. In fiscal year 2019, CBI partners submitted 10 invention disclosures, six provisional patent applications, four utility patent applications, and five issued patents.

Bioenergy Lesson Plans. Students measure the sugar content of various liquids using a refractometer. Connections are made between the chemical composition of biomass and the simple sugars used to make ethanol. [ORNL]

Education and Outreach

CBI offers interdisciplinary research opportunities for graduate students, postdoctoral researchers, and visiting scientists and also seeks to broaden public understanding of bioenergy and the pipeline of future bioeconomy workers. Working with the Creative Discovery Museum in Chattanooga, Tennessee, the CBI-supported "Farming for Fuels" hands-on or distance-learning programs have reached more than 290,000 students, parents, and teachers nationwide over 11 years through hubs at multiple science centers or museums across 14 states (see image, Bioenergy Lesson Plans, this page). These materials incorporate Next Generation Science Standards (K-12 science content standards) and are available at learnbioenergy.org. The lessons were downloaded by ~29,000 users last year. The ongoing program is self sustaining with direct costs only in training, distance learning, and curricula development.

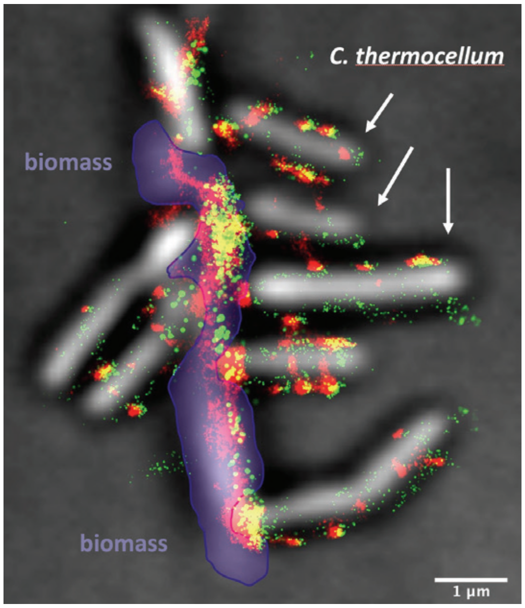
CBI Early Career Investigator Spotlight

"The visibility, training, and connections of early career researchers are important for career advancement. Working with the multidisciplinary CBI team has helped me build and expand my research program at the University of Georgia in ways that would not be possible without the support and collaborative environment of CBI. My experience working in a CBI Early Career assignment at ORNL expanded my training in large-scale grant management, which is a unique experience that many people at my career stage do not receive. Further, living and working at ORNL opened up even more opportunities to contribute to the annual review process, interact with many DOE staff members, speak at important conferences, and publish in high-profile journals."

— Breeanna Urbanowicz, Assistant Professor, University of Georgia

CBI Research Highlights

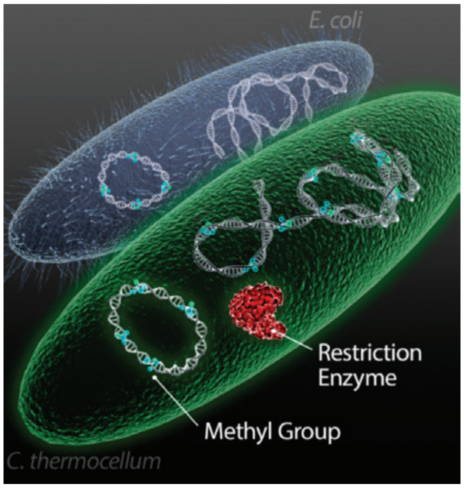
Using State-of-the-Art Microscopy to Understand Biomass Deconstruction



New Insights into Microbial Biomass Deconstruction. Enhanced optical techniques pinpoint the location of "scaffoldin" proteins and biomass-degrading enzymes in the cellulosome of *Clostridium thermocellum* interacting with plant biomass (purple/center). [National Renewable Energy Laboratory]

Clostridium thermocellum is the most efficient microorganism known for solubilizing lignocellulosic biomass. CBI researchers are using super high-resolution optical microscopy for imaging these dedicated biomass-deconstructing microorganisms. The technique requires a simple sample preparation, allows high-resolution fluorescence microscopy to detect the location of proteins, and is leading to a better understanding of biomass deconstruction by *C. thermocellum*. Ongoing experiments show that the distribution of the microbial multienzyme complexes that break down cellulose, known as cellulosomes, depends on the biomass substrate. CBI scientists have shown that in the absence of biomass, these cellulosomes are regularly distributed along the bacterial surface. When microbes are grown on biomass, however, the cellulosomes are targeted to specific interaction regions that serve as anchoring points to bind biomass (Yarbrough et al. In preparation). These techniques, applied to a combination of single and mixed bacterial cultures, are expected to help to elucidate models for microbial population dynamics in other microbial consortia or biofilms. Further research is using these pioneering techniques to uncover the molecular mechanisms underlying cellulosome formation during microbial growth on CBI-targeted biomass feedstocks. This mechanistic understanding should lead to improvements in biomass solubilization rates for CBP.

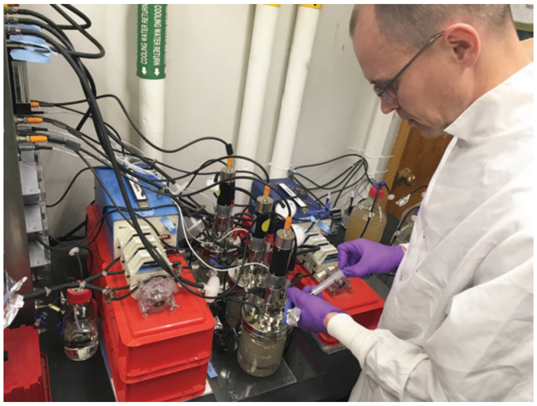
Customizing Microbes for Better Biofuel Production



Genetic Engineering in Microbes. A new method uses *Escherichia coli* to generate DNA with methylation patterns that targeted microbes recognize and accept as their own, facilitating customization of microbes for biofuels production. [ORNL]

To increase the efficiency of the conversion process, microbes are needed that can break down cellulose and ferment it into biofuels in a single set of reactions. However, enhancing or introducing target traits in nondomesticated microbes can be challenging. Few tools are available for engineering nonmodel microbes, and the organisms have developed defense mechanisms that can foil attempts to insert new genes. These defense mechanisms guard microbes against unintentionally copying foreign DNA. To distinguish their own DNA from foreign DNA, each microbe places a methyl group on a handful of specific DNA sequences. These methylated sequences are unique to the organism and act like a signature. Special enzymes called restriction enzymes patrol the cell and digest any DNA that lacks methyl groups on the signature sequences. CBI scientists have developed a method to leverage this defense system to coax microbes into accepting bioengineered DNA as their own. Using two sequencing methods, CBI scientists first identified a microbe's signature sequences and the enzymes that methylate them. Then these enzymes, known as methyltransferases, are expressed in *Escherichia coli.* With the right methyltransferases in place, *E. coli* are able to make copies of DNA with the expected methylation patterns, ensuring the target microbe would accept and use the new DNA (Riley et al. 2019). This rapid domestication method offers numerous benefits for applied and basic research, especially in identifying gene function. With this approach, scientists can remove or overexpress genes of interest in microbes to determine the effect on the organisms' traits. In addition to bioenergy, the method can be employed in biomedical and other fundamental research.

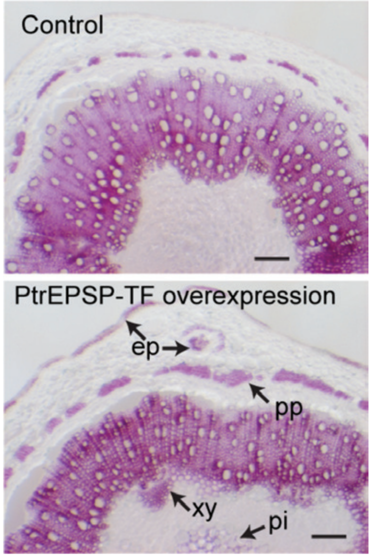
Overcoming Biomass Recalcitrance



Consolidated Bioprocessing (CBP). CBI researcher Evert Holwerda uses a microbial bioreactor with precise measurement tools to optimize biomass deconstruction and conversion to advanced biofuels and bioproducts. Ultimately, this CBP approach will eliminate the need for added enzymes and pretreatment. [Dartmouth College]

Recalcitrance is a key barrier to biological processing of biomass to fuels and chemicals, but the relative impacts of physical, chemical, and genetic interventions to improve biomass processing have yet to be evaluated systematically. CBI researchers tested total carbohydrate solubilization (TCS) with combinations of three biocatalysts (i.e., *C. thermocellum*, *Caldicellulosiruptor bescii*, and commercial Novozyme enzymes) in poplar and switchgrass feedstocks of varying recalcitrance. They used CBP alone, CBP with cotreatment, or CBP preceded by cosolvent-enhanced lignocellulosic fractionation (CELF) pretreatment (Holwerda et al. 2019). Results showed that some form of nonbiological treatment will be needed to achieve high TCS for most feedstocks. However, this need to augment biological solubilization does not necessarily require harsh thermochemical pretreatment, nor must this pretreatment occur prior to biological conversion. The relative magnitudes for increasing TCS were affected first by the cotreatment approach, followed by the biocatalysts (CBP microbe or enzymes), the plant species tested, and, lastly, their genetic modification. Importantly, the use of cotreatment with CBP can overcome most plant feedstock variation in recalcitrance and achieve high TCS.

Key Gene in Lignin Biosynthesis Moonlights as Regulator



Lignin Deposition Altered by Overexpression of the EPSP Gene. Ectopic lignin is deposited (indicated by black arrows) in the stems of poplars overexpressing PtrEPSP-TF. Lignin shows red color after staining. [Image used with permission of American Society of Plant Physiologists from Xie, M., et al. 2018. "A 5-Enolpyruvylshikimate 3-Phosphate Synthase Functions as a Transcriptional Repressor in *Populus*," *The Plant Cell* **30**, 1645–60. Permission conveyed through Copyright Clearance Center, Inc.]

To identify gene functions, GWAS mapping is being used to link phenotypic measurements (e.g., composition, growth, and metabolites) to the sequenced genotypes of about a thousand poplar individuals in several common gardens. One study (Xie et al. 2018) found a new function of a gene [5-enolpyruvylshikimate 3-phosphate (EPSP)] synthase previously known to catalyze the production of aromatic amino acids as part of the synthesis of lignin and other compounds. In this study, the GWAS data led to the discovery of a binding motif that endows transcription activity to one poplar EPSP synthase. This new function was validated by assessing EPSP overexpression poplars for lignin deposition, secondary metabolism, and gene expression levels, as well as through biochemical studies. The discovery provides a new strategy to manipulate and control lignin biosynthesis for better biofuel products. This study also validates the GWAS approach using this poplar population to identify new gene functions. CBI is collecting additional phenotypes of interest. Researchers from two other BRCs (GLBRC and CABBI) are also measuring phenotypes related to their interests in these same common gardens.

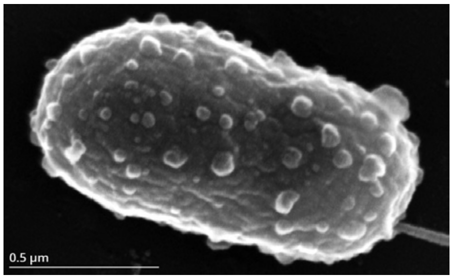
Biosynthesizing Pectic Polysaccharides

Cell Wall Biosynthesis. Graduate student Robert Amos characterizes the enzyme kinetics of the cell wall biosynthetic protein complex GAUT1:GAUT7. [University of Georgia]

Pectins are structural polysaccharides that contribute to plant growth and development and cell wall structure and function. Their presence—even at relatively low levels—provides a barrier to the deconstruction of native biomass feedstocks such as poplar and switchgrass and partially obstructs degradative enzymes from accessing cellulose and hemicelluloses during processing and saccharification. The most abundant pectic glycan, homogalacturonan (HG), is synthesized by members of the galacturonosyltransferase (GAUT) gene family. CBI researchers determined the mechanism of HG synthesis using multiple enzyme assays and product analysis techniques to measure the rates of HG elongation and the product distributions catalyzed by GAUT proteins in the presence of HG acceptors of varying chain lengths, respectively. A two-phase distributive elongation model was proposed in which the rapid elongation of HG acceptors to high–molecular weight products occurs only after the product reaches a critical chain length of about 11 monomers. Amos et al. (2018) reported the first *in vitro* demonstration of the nonprocessive mechanism by which GAUT proteins synthesize long HG polysaccharides. Other cell wall matrix polysaccharides, including xylan, mannans, and xyloglucan, are also long polymers and may be synthesized through similar mechanisms. These findings on plant cell wall biosynthesis should provide insights into plant cell wall structure and breakdown.

Understanding Microbial Catabolism for Lignin Valorization

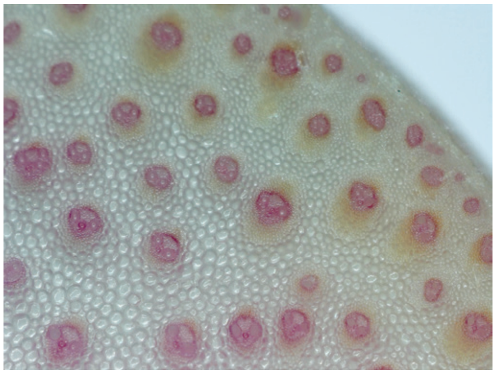
Pseudomonas putida KT2440 is a soil bacterium that rapidly catabolizes lignin-derived aromatics and high–molecular weight lignin, which can then be quickly engineered for performance-advantaged chemical production. CBI researchers discovered that *P. putida* secretes abundant outer membrane vesicles (OMVs) in the presence of lignin and that proteins with known and putative roles in lignin degradation are selectively and temporally enriched into OMVs. Further, *in vivo* and *in vitro* assays with enriched OMVs demonstrate breakdown of model lignin-derived aromatics (Salvachua et al. Submitted). Together, these results suggest a new model for bacterial breakdown of lignin in the extracellular environment via OMVs. Ongoing work is investigating the spatiotemporal organization and enzymatic constituents of lignin utilization by *P. putida*. The discovery of this new mechanism for lignin breakdown and depolymerization should enable new approaches for improving lignin valorization.



***Pseudomonas putida*: Outer Membrane Vesicle (OMV) Secretion During Lignin Breakdown.** Scanning electron microscopy image of *P. putida* after 72 hours of cultivation in lignin-rich media showing OMVs blebbing from the outer membrane. [National Renewable Energy Laboratory]

New Enzyme Identified in the Lignin Pathway

Lignin is an abundant plant cell wall polymer important for bioproduct production. The biosynthesis of lignin is highly conserved among higher plant species. However, increasing evidence questions the established enzymatic pathway to lignin formation in vascular plants. CBI used biochemical and molecular genetic approaches to prove that a coumarate 3-hydroxylase (C3H) enzyme provides an alternate route to lignin monomer production from the phenolic precursor 4-coumarate. The enzyme catalyzing the direct 3-hydroxylation of 4-coumarate to caffeate is a bifunctional peroxidase that oxidizes ascorbate and 4-coumarate at comparable rates (Barros et al. 2019). This finding finally uncovers the missing enzyme in the lignin pathway that was proposed over 40 years ago. Moreover, this conclusive evidence for C3H's role and existence indicates that the previously characterized pathways may be less important. The bifunctional nature of this enzyme suggests close links between lignin synthesis and abiotic stress through reactive oxygen species metabolism, which may be important for engineering lignin in the face of a more variable climate.



Reviewing Lignin Biosynthesis. As this cross section of a corn stem reveals, lignin (stained red) accounts for a significant portion of the plant tissue. CBI researchers have uncovered a new enzyme in the biosynthesis of this important cell wall polymer. [University of North Texas]

Great Lakes Bioenergy Research Center

glbrc.org

GLBRC Overview

The Great Lakes Bioenergy Research Center (GLBRC) is a cross-disciplinary research center led by the University of Wisconsin (UW)–Madison. With Michigan State University (MSU) and other collaborators, GLBRC is developing biobased fuels and products that are economically viable and environmentally sustainable.

GLBRC scientists envision a future in which dedicated energy crops grown on nonagricultural land provide the raw materials for major portions of society's liquid transportation fuels and chemicals that are currently derived from petroleum. This future will create new economic opportunities for biorefineries, farmers, and rural communities and provide climate benefits without diverting land from food production. To fulfill this vision, the center is addressing key knowledge gaps that currently limit the industrial-scale production of specialty fuels and products from such purpose-grown energy crops.

Research Themes

The technoeconomic success of lignocellulosic biorefineries hinges on maximizing conversion of biomass into a profitable mix of fuels and products. GLBRC's research teams span multiple scientific domains to develop innovative solutions in three crosscutting research themes: sustainable biomass production, efficient biomass deconstruction and conversion, and integration of these processes into industrial field-to-product pipelines.

Sustainable Cropping Systems. GLBRC is improving systems for growing dedicated energy crops on lands not currently used for agricultural purposes. Using nonagricultural land for nonfood crops such as poplar, switchgrass, energy sorghum, and mixed perennial species reserves arable U.S. farmland for food production, while simultaneously providing potential environmental benefits such as climate change mitigation and increased biodiversity. The center's goals are to maximize ecosystem performance and crop yield and quality under nutrient-limited or other stressful conditions found on nonagricultural lands. To achieve these goals, GLBRC research teams are:

- Engineering plants with lignin and polysaccharides that can readily be turned into specialty fuels and products (see image, Digestible Lignin, p. 29).
- Identifying and developing plant and microbiome traits that improve energy crop productivity and tolerance to cold, drought, and nitrogen stress.
- Investigating micro- and landscape-scale controls on soil carbon sequestration, nitrous oxide emissions, and nitrogen fixation in energy cropping systems.

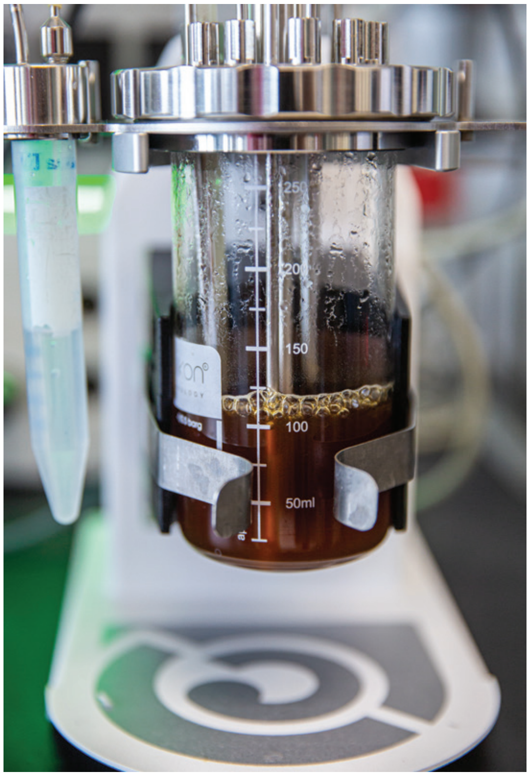
Efficient Biomass Conversion. GLBRC's conversion efforts are focused on enabling a new generation of biorefineries that are both economically viable and environmentally sustainable. The center's research seeks to boost industry economics by finding new ways of processing biomass at low cost, producing fuels

Digestible Lignin. GLBRC researcher Rebecca Smith focuses on how to make lignin more digestible. Lignin is the notorious hard-to-process "glue" that lends plant tissues their structure and sturdiness. [GLBRC]

compatible with multiple engine types, and converting as much plant material as possible into products that are valuable to industry. To achieve these goals, GLBRC research teams are:

- Improving methods for feedstock-agnostic biomass deconstruction and separation by the renewable solvent gamma-valerolactone and broad-specificity glycosyl hydrolase enzymes.
- Identifying metabolic burdens and lignocellulosic hydrolysate stresses and how they pose barriers to efficient production of isobutanol (IBA) by industrially accepted microbes (see image, Hydrolysate Fermenters, this page).
- Generating the knowledge needed to design new industry-ready platform microbes capable of producing targeted products from conversion residues derived from specialty fuel production.

Field-to-Product Integration. The path from farm field to products consists of several interdependent phases, including crop production, biomass deconstruction, and conversion into



Hydrolysate Fermenters. A hydrolysate experiment in process in GLBRC's Experimental Fermentation Facility. [GLBRC]

GLBRC Partners

- **University of Wisconsin—Madison** (lead institution)
- **Michigan State University** (East Lansing)
- **Michigan Technological University** (Houghton)
- **Texas A&M University** (College Station)
- **University of British Columbia** (Vancouver, Canada)

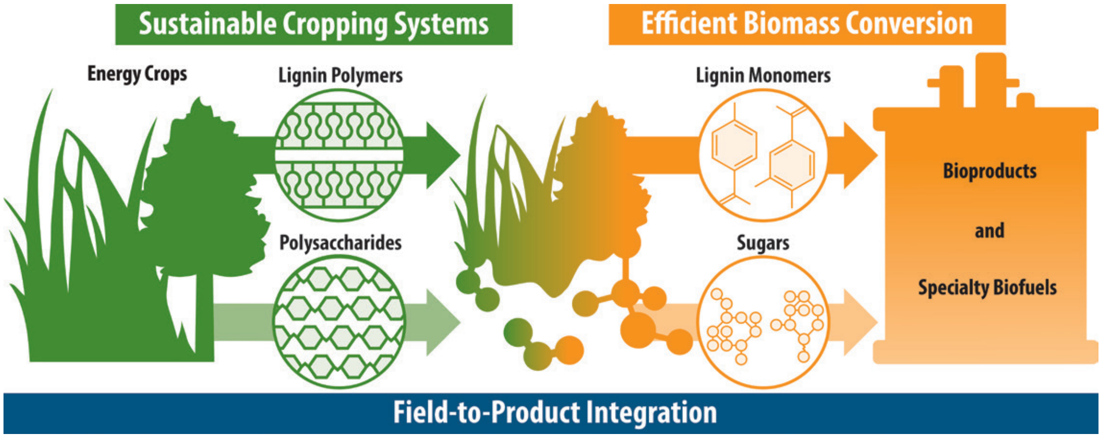
targeted products that are of value to industry. GLBRC's multidisciplinary research teams are building, modeling, and evaluating strategies for the next-generation lignocellulosic bioindustry to improve these individual steps while integrating them into an optimized field-to-product pipeline (see figure, Research Integration, this page). To achieve these goals, GLBRC research teams are:

- Creating novel and robust plant, landscape, and biorefinery models to predict the sustainability, lifecycle, and economic outcomes of alternative field-to-product pipelines.
- Laying the groundwork to understand how the performance of conversion microbes is affected by seasonal or environmental changes in energy crops.
- Developing capacities for improved microbial conversion of lignin into products.
- Enabling the efficient production of terpenes by plants and microbes.

Industry Partnerships

GLBRC works closely with companies and licensing agents to anticipate industrial needs, move new technologies into the marketplace, and advance the overall economics of biorefining. Industry collaborations help the center focus its research on critical industry bottlenecks and more quickly develop new technologies for commercial use, while industry representatives on GLBRC's scientific advisory board provide valuable perspective and guidance on research directions.

GLBRC IP is protected by and commercialized through the Wisconsin Alumni Research Foundation, a nonprofit entity that manages and licenses UW–Madison IP, and MSU Technologies, MSU's technology transfer and commercialization office. These two organizations provide companies with opportunities to acquire rights in GLBRC inventions and copyrights to drive commercialization and create new economic opportunities for biorefiners, farmers, and rural communities.



Research Integration. GLBRC is developing sustainable biofuels and bioproducts from all usable portions of dedicated energy crops grown on marginal, nonagricultural lands. [GLBRC]

Education and Outreach

The mission of GLBRC's outreach team is to inform various audiences—including the general public, undergraduate students, and educators—about bioenergy research, energy concerns, and sustainability issues affecting the planet. Engaging the community with fun, informative, hands-on activities and events, GLBRC's outreach efforts are designed to pique curiosity and promote discussion. In addition, GLBRC trains future scientists and offers opportunities for undergraduate students to gain research experience on both the UW–Madison and MSU campuses through the Research Experience for Undergraduates (REU) program (see image, REU Program, this page) and iGEM team sponsorship.

Research Experience for Undergraduates (REU) Program. Participants in GLBRC's 2019 REU program at Michigan State University and the University of Wisconsin–Madison. [GLBRC]

GLBRC Early Career Investigator Spotlight

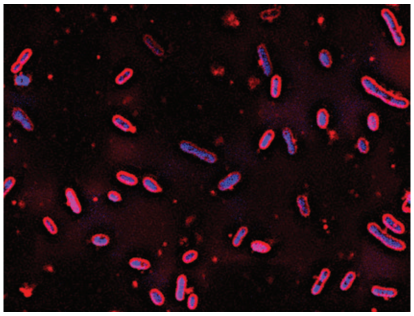
"GLBRC is a rich scientific environment for a young investigator. The value of having a center to tackle a big societal challenge is that it brings together teams of people from a wide variety of disciplines to work together toward a common goal. This experience has accelerated my research and enabled me to pursue questions I would not otherwise be able to."

— Daniel Amador-Noguez, Assistant Professor of Bacteriology, University of Wisconsin–Madison

GLBRC Research Highlights

Producing a Potential Plastic Precursor from Lignin

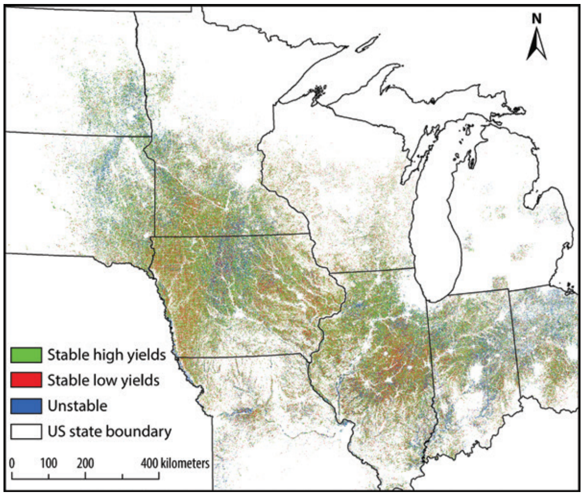
GLBRC researchers have engineered a soil bacterium that could turn a renewable material—lignin from plant cells—into a replacement for petroleum-based plastics. Lignin is an abundant source of aromatic compounds—second on the planet only to petroleum—but is notoriously resistant to efficient conversion into useful products. Breaking down lignin, whether through biological or chemical means, invariably produces heterogeneous mixtures of aromatic (ring-containing) compounds. Despite recent advances in plant biomass deconstruction and lignin depolymerization strategies, the heterogeneity of resulting product mixtures presents a major challenge for conversion into commodity chemicals. Microbes that can metabolize lignin-derived aromatics have evolved pathways that funnel these heterogeneous mixtures into a few common intermediates, which simplifies further processing.



Novosphingobium aromaticivorans. GLBRC scientists' genetic tweaks to this microbe, first isolated from soil contaminated by petroleum products, could jumpstart a renewable plastics industry and transform the economics of biorefining. [GLBRC]

GLBRC scientists engineered a strain of *Novosphingobium aromaticivorans* that converts mixed lignin-derived aromatics into a single chemical called 2-pyrone-4,6-dicarboxylic acid (PDC), a potential precursor for making polyester, epoxy adhesives, and other bioplastics. This work represents a valuable advance in using bacteria to funnel mixtures of aromatic compounds into defined single commodities. It also shows that *N. aromaticivorans* could be an ideal microbial platform for valorization of lignin and other plant-derived aromatics. Ultimately, the information and strategies developed here and in future optimization of PDC production by *N. aromaticivorans* may help engineer this and other microbes to produce a wide range of additional valuable compounds from lignin (Perez et al. 2019).

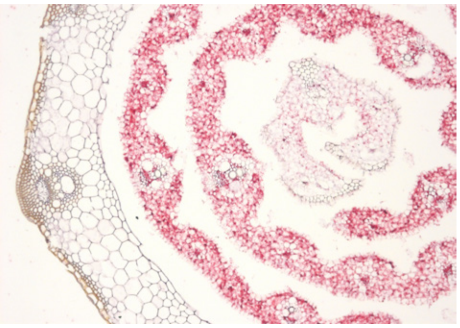
Regional Yield Stability Analysis: Revealing Opportunities for Cellulosic Biofuel Placement



Yield Stability Map of Midwest. Using eight years of satellite imagery, field boundaries, and crop data, GLBRC researchers determined locations of high, variable, and low yields across areas of Midwest farms to determine nitrogen fertilizer waste. [GLBRC]

The United States contains some of the most productive farmland in the world, but large swaths of lower-productivity, nonagricultural land could be used to grow energy crops. GLBRC researchers used eight years of satellite imagery, field boundaries, and crop data to assess the temporal stability of corn and soybean yields at a resolution of 30 × 30 m for 30 million hectares of 10 midwestern states. Researchers were able to identify portions of individual fields with stable high yields, stable low yields, or temporally inconsistent yields. On average, 26% of subfields in the region could be classified as stable low yield, 28% as unstable, and 46% as stable high yield. Moreover, stable low-productivity areas were responsible for most of the region's nitrogen fertilizer loss to the environment, the cost of which is substantial. The study showed that nitrogen loss from these 10 midwestern states totals hundreds of millions of dollars of wasted fertilizer and 6.8 million metric tons of greenhouse gas emissions annually. Conversion of these stable low-yield acreages to perennial cellulosic crops could provide large quantities of biomass, increase the economic footprint of a future lignocellulosic industry, and deliver major ecological and environmental benefits while reserving farmland for food production. With smart management, these crops could benefit the ecosystem; help preserve water, air, and environmental quality; and provide a revenue source for local communities (Basso et al. 2019).

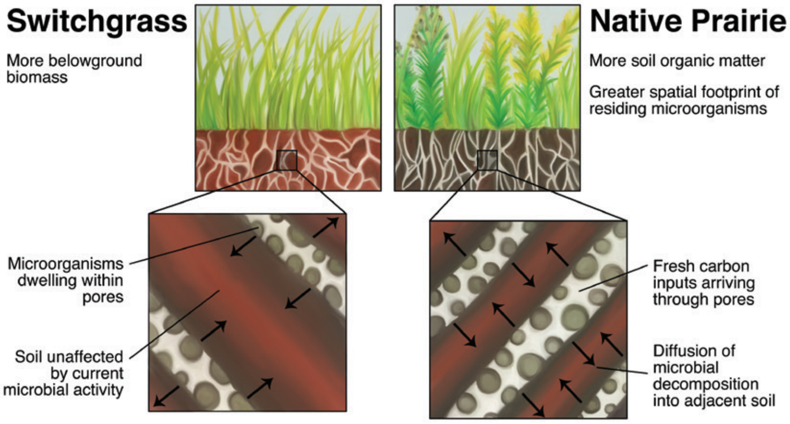
Increasing Plant Glucan Production for More Productive Conversion



BdTHX1 Expression in *Brachypodium*. GLBRC researchers determined where mixed-linkage glucan, a polymer of glucose, is produced in grasses and which genes are responsible for that production. Researchers expressed BdTHX1 (red, immunolabeling) inside the elongating leaf and leaf sheath of the model bioenergy grass *Brachypodium*. [GLBRC]

Engineering bioenergy crops to accumulate large amounts of easy-to-use sugars can increase the yield of fuels and products that can be created from them. One such sugar, glucose, is an important feedstock of advanced biorefineries because it can be transformed into a variety of biofuels and bioproducts. GLBRC researchers set out to determine where mixed-linkage glucan (MLG), a polymer of glucose, is produced in grasses and which genes are responsible for that production. Using the model bioenergy grass *Brachypodium*, GLBRC researchers demonstrated that MLG is present primarily in the Golgi apparatus. However, they also found that overproduction of MLG caused development and growth defects, an undesirable outcome. To provide further insight into how plants make MLG, the team examined downstream genes responsible for these outcomes. The researchers identified a transcription factor (BdTHX1) that plays an important role in the production and restructuring of MLG by regulating the expression of two genes. This discovery will be instrumental for engineering the bioenergy grass sorghum to produce and accumulate large amounts of MLG without inhibiting plant growth (Kim et al. 2018; Fan et al. 2018).

Identifying Soil Pores as Key to Carbon Fixation

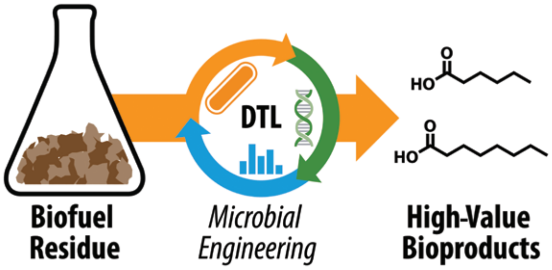


Soil Pores Schematic. The microbial footprint defines the soil volume available for carbon protection. This schematic representation shows the effect that the abundance of 30 to 150-µm pores has on the size of the spatial footprint of microorganisms residing in such pores in **(left)** a perennial switchgrass monoculture system versus **(right)** a biodiverse native vegetation system. [GLBRC]

Increasing the potential of soil to store carbon is a well-established strategy for capturing atmospheric carbon dioxide. Some promising methods of increasing carbon accumulation in the ground, including reducing soil disturbance, increasing biomass inputs, and enhancing plant diversity, have not achieved the carbon gains that ecosystem models anticipate, suggesting that this process is not properly understood. GLBRC researchers have designed a method to more accurately establish how carbon is stored in agricultural lands, showing that soil pore structure has a notable impact on carbon accumulation and fortification. Over nine years, GLBRC researchers studied five different cropping systems and found that the two systems with more plant diversity—poplar and native prairie—developed higher soil porosity and higher levels of soil carbon. Researchers used X-ray microtomography and microscale enzyme mapping to show how pore structures affect microbial activity and carbon protection in these systems, as well as how plant diversity impacts the development of soil pores conducive to greater carbon storage. A major source of stable soil carbon comes from microbes producing organic compounds that are then absorbed onto soil mineral particles.

Analysis revealed that cropping systems with many different plant species had many more pores of the right size for stable carbon storage. Ultimately, this work could improve the climate resilience of cropping systems and may prompt farmers to focus on plant diversity when attempting to increase soil carbon storage (Kravchenko et al. 2019).

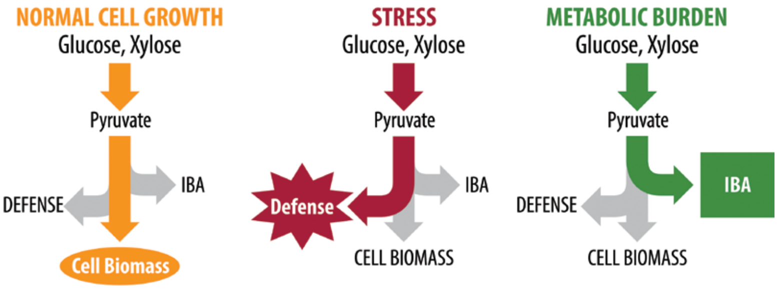
Using Metabolic Network Analysis to Identify Distinct Microbial Roles in Producing Preferred Bioproducts



Biofuel Residue Medium-Chain Fatty Acids (MCFAs). GLBRC researchers are using an iterative design-test-learn (DTL) cycle to engineer mixed microbial communities designed to convert biofuel residue **(left)** into MCFAs **(right)**, which can be used to make a variety of industrial chemicals and pharmaceuticals. [GLBRC]

The organic matter left over after biofuel production is a rich potential feedstock for making additional high-value bioproducts. Developing methods to convert a larger percentage of this carbon into useful products is crucial for improving the economics of biorefining and reducing dependence on fossil fuels. GLBRC researchers are using mixed microbial communities to produce medium-chain fatty acids (MCFAs), which can be used to make a variety of industrial chemicals and pharmaceuticals, from the conversion residue remaining after lignocellulosic biofuel production (Scarborough et al. 2018a). The researchers are analyzing the composition and metabolic characteristics of an MCFA-producing microbiome with a goal of engineering microbial communities to optimize production of specific bioproducts. The team identified the 10 most abundant bacterial strains in the microbiome, then used gene expression patterns and metabolic capabilities of these strains to predict distinct metabolic roles within the multistep conversion process. The findings provide insights into how microbial communities with defined metabolic networks could drive preferential production of specific bioproducts, such as MCFAs, for maximum carbon conversion and improved efficiency (Scarborough et al. 2018b).

Identifying Optimal Enzyme Expression Levels to Maximize Biofuel Production

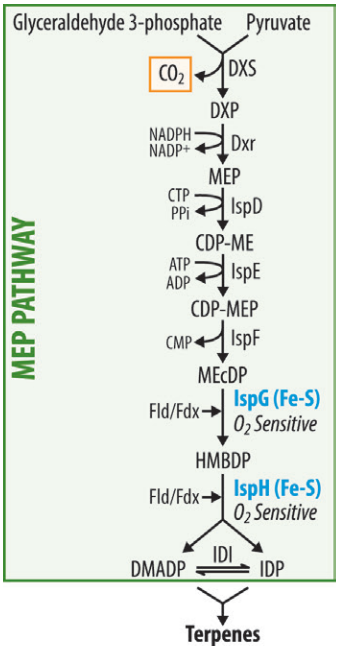


Microbial Carbon Flux. Microbes naturally direct energy and carbon toward cell biomass **(left)**. In stress-inducing environments like lignocellulosic hydrolysates, microbes redirect energy and carbon to defense mechanisms **(middle)**. A major GLBRC goal is to reprogram microbes to direct carbon and energy to specialty biofuels such as isobutanol (IBA) rather than cell biomass or stress responses **(right)**. [GLBRC]

OptSSeq (Optimization by Selection and Sequencing) is a way to determine the optimal balance of enzymes in a biosynthetic pathway to maximize flux through the pathway. This method, developed by GLBRC researchers, is a versatile biosystems design tool applicable to any pathway whose function can be linked to cell growth or survival. Applied to synthetic biofuel production pathways, it can help identify and ameliorate potential flux bottlenecks, as well as help identify constraints *in vivo* that limit maximum product formation (Ghosh and Landick 2016). GLBRC researchers have used OptSSeq to optimize enzyme levels for maximal IBA production in *E. coli*. Using models that predict impacts of enzyme synthesis costs on cellular growth rates, the researchers found that optimum levels of pathway enzymes led to maximal IBA production and that additional limitations lie in the *E. coli* metabolic network. This work provides a useful starting point for optimizing IBA production in diverse microbes and fermentation conditions (Ghosh et al. 2019).

Overcoming Metabolic Bottlenecks in Bioproduct Synthesis

GLBRC researchers have identified metabolic changes in a prolific, ethanol-producing bacterium that could allow it to produce higher levels of isoprenoids, a class of natural products that includes many industrial precursors and commodity chemicals. This finding could guide efforts to engineer microbes to produce renewable alternatives to a broad range of petrochemicals. The researchers focused on a key isoprenoid biosynthesis pathway known as the methyl erythritol-4-phosphate, or MEP, pathway. The final catalyzed step in the MEP pathway requires an iron-sulfur cluster cofactor and is known to be the rate-limiting step in isoprenoid synthesis. Oxygen exposure disrupts the iron-sulfur cluster and induces a large bottleneck in the MEP pathway. *Zymomonas mobilis* was able to resolve this bottleneck almost completely within an hour through upregulation of a relatively small number of enzymes, including several involved in the formation and maintenance of the iron-sulfur cluster. The results suggest that a small number of regulatory changes may allow *Z. mobilis* to shunt much more carbon through the MEP biosynthesis pathway than it does under normal anaerobic conditions. Enzymes that maintain iron-sulfur cluster activity may be good targets for engineering the microbe to produce higher levels of isoprenoids (Martien et al. 2019; see also GLBRC Early Career Investigator Spotlight, p. 31).



MEP Pathway Schematic. Work by GLBRC researchers has identified potential enzyme targets in the MEP pathway to drive higher microbial production of terpenes, a large and diverse class of compounds with potential as specialty biofuels and bioproducts. [GLBRC]

JBEI
Joint BioEnergy Institute

Joint BioEnergy Institute

jbei.org

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 technoeconomic and lifecycle assessment models to (1) predict the impact of JBEI's scientific and technological breakthroughs on the biofuel selling price and carbon efficiency of conversion to fuels and products and (2) assess the long-term economic and environmental performance of scaled-up production at the U.S. national scale.

Feedstock Development. In developing fundamental understanding of cell wall biology and a suite of plant biosystems design tools, JBEI is creating a knowledgebase for engineering and field testing bioenergy crops. These crops are tailored for facile biomass deconstruction into sugars and lignin-derived intermediates and near full utilization by microbes engineered to produce biofuels and bioproducts. The field tests are also validating low susceptibility to disease and drought. 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. 37).

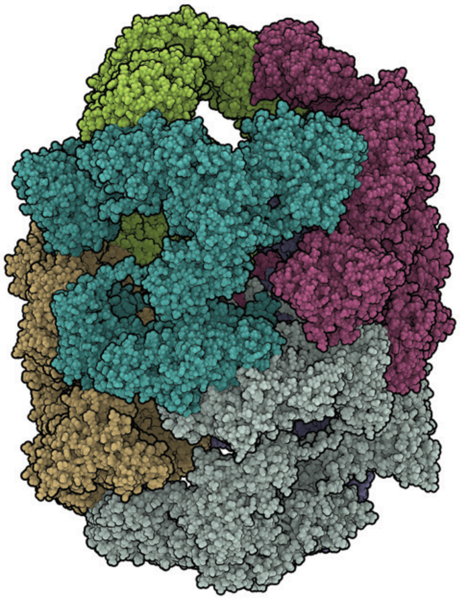
Deconstruction and Separation. JBEI is developing an integrated, feedstock-agnostic deconstruction process that uses renewable and biocompatible ionic liquids to pretreat the biomass, as well as optimized enzyme mixtures that depolymerize the polysaccharides and lignins into bioavailable oligomers. This integrated deconstruction process liberates high yields (as much as 90% or more) of the

Developing Better Plants for Biofuels. Building a successful lignocellulosic biofuels industry depends, in part, on developing specialized biofuel crops that are optimized for deconstruction into sugars and fermentation into biofuels and bioproducts. Pictured is sorghum, a bioenergy crop being grown at the University of California, Davis, a JBEI partner. The sorghum flowers are bagged to prevent pollen exchange. [LBNL]

sugars and lignin-derived intermediates from bioenergy crops suitable for conversion into biofuels and bioproducts. This work includes the discovery and optimization of enzymes through the exploration of targeted ecosystems and microbes that are the natural sources of these enzymes (see image, Fatty Acid Synthase, this page). Further JBEI research in this focus area involves developing predictive biomass deconstruction tools that will enable the efficient design of affordable and scalable deconstruction processes based on these discoveries.

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

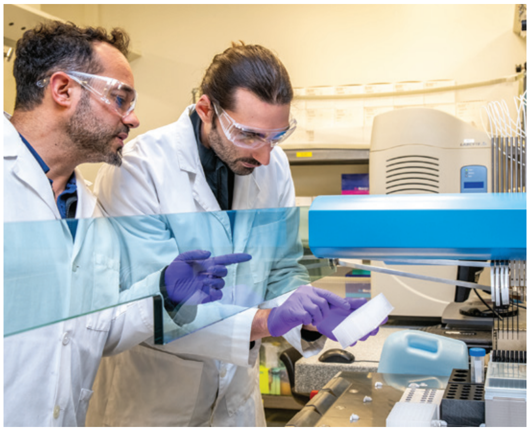
Achieving these goals requires integration across all four research focus areas. Biomass deconstruction technology can be improved with engineered crops, the composition of which is matched as closely as possible with the metabolism of the microbes to maximize conversion. Ionic liquids are chosen to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



Fatty Acid Synthase. Fatty acids mimic some of the high–energy density properties that characterize hydrocarbon components of petroleum-derived fuels. Pictured is a computational model of the crystal structure of a fatty acid synthase. JBEI research has facilitated the discovery of a variety of enzymes and metabolic pathways that enable the biochemical conversion of fatty acids to a range of industrially relevant compounds. [LBNL]

JBEI Partners

- **Lawrence Berkeley National Laboratory** (Berkeley, California; lead institution)
- **Argonne National Laboratory** (Argonne, Illinois)
- **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)



BioLector Microbioreactor. JBEI scientists work with the BioLector instrument to understand the microbial production of biofuels and bioproducts. [LBNL]

consolidation. Finally, technoeconomic and lifecycle analyses 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, BioLector Microbioreactor, this page). These analytical technologies provide the high-quality data needed to feed machine-learning algorithms that can systematically direct the metabolic engineering process.

Industry Partnerships

JBEI is committed to transformative research and innovation that results in economic and performance step changes for biomanufacturing. The institute cultivates relationships with industry thought leaders through its advisory committee, biobased targets council, and continual outreach efforts. Robust communication with the private sector ensures that JBEI's IP is both cutting edge and industrially compelling, resulting in a rigorous commercialization pipeline consisting of multiple startup companies, licensing agreements, and strategic partnerships.

LBNL manages all JBEI-related partnership agreements and IP, regardless of which partner institution owns the IP. In addition, JBEI employs a director of commercialization who coordinates all JBEI industry interactions. These structures enable industry to work with a single institution and point of contact to access all that JBEI has to offer.

Education and Outreach

The mission of JBEI's outreach efforts is to keep the nation at the forefront of scientific discovery by providing educational experiences and resources to students and teachers to enable future generations of scientists and encourage a diverse science, technology, engineering, and mathematics (STEM) workforce. JBEI scientists mentor high school and undergraduate students, providing them with an opportunity to work in the laboratory on projects related to JBEI's research program and to learn about industry's current challenges and opportunities. JBEI also supports students, teachers, instructors, and professors with quality resources including state-of-the-art activities, lesson plans, and information for all levels of education. JBEI collaborated in the development of bioenergy-related materials featured in *Biotechnology: Science for the New Millennium, 2017*, which is used at over 1,200 high schools and 300 colleges to teach the concepts and hands-on laboratory procedures for preparing students for bioscience majors and careers in the rapidly growing biotechnology industry. The gem in JBEI's broad outreach efforts is the Introductory College Level Experience in Microbiology (iCLEM; see image, JBEI's 2019 iCLEM Program Participants, this page), an eight-week paid summer research program for high-potential, low-income high school students and the teachers serving under-resourced schools.

JBEI's 2019 Introductory College Level Experience in Microbiology (iCLEM) Program Participants. The iCLEM program has been nationally recognized, and 98% of its alumni attend college with more than 80% majoring in science, technology, engineering, and mathematics (STEM). [LBNL]

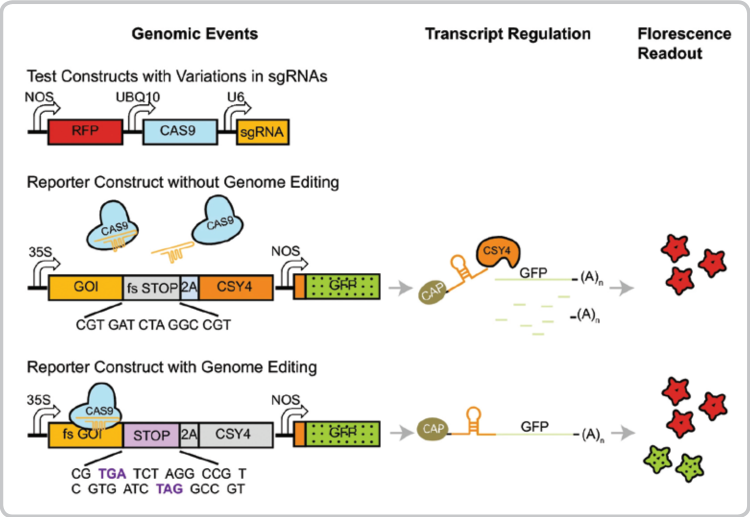
JBEI Early Career Investigator Spotlight

"Being part of JBEI for the past five years has meant being part of a highly collaborative (and supportive) team of interdisciplinary scientists. I have learned to use techniques such as lifecycle analysis to guide my research and been encouraged to push discoveries from the lab out into the field and industry. This experience has provided me with a platform to develop a career as a national lab scientist and do science that would not be possible otherwise."

— Jenny Mortimer, Director of Plant Systems Biology and Deputy Vice President of the Feedstocks Division, JBEI

JBEI Research Highlights

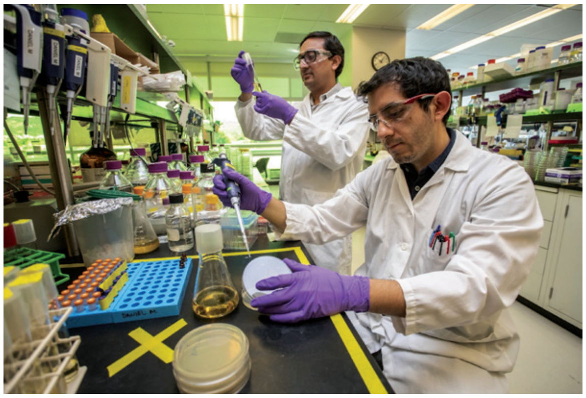
Genome Editing in Plants for Modified Lignin Content



Schematic Illustration of a Transient Assay to Test sgRNA Efficiency for CRISPR Editing *In Vivo*. The model shows assay components and their predicted interactions. The symbols for the corresponding genomic sequence, transcript sequence, and protein product of the same gene are drawn in the same color. Gray designates deactivated modules in the reporter construct before or after CRISPR editing events. Editing in the gene of interest (GOI) results in expression of the CSY4 nuclease and allows the green fluorescent protein (GFP) to be expressed. Guide RNA efficiency is determined by the GFP/red fluorescent protein (RFP) expression ratio. fs: frame-shifted; 2A: 2A peptide. [Reprinted under a Creative Commons license (CC-BY-4.0) from Liang, Y., et al. 2019. DOI:10.1186/s13068-019-1467-y.]

Lignin is the most important contributor to biomass recalcitrance. Obtaining plants with lower lignin content requires the transformation of a lignin-deficient mutant, an approach that can be difficult to apply to a range of bioenergy crops. With CRISPR, single-guide RNA (sgRNA) can be used to direct the Cas9 protein to bind and cleave a particular DNA sequence for genome editing. However, in plants, the rules governing sgRNA selection are not well established. To develop a screening assay to test sgRNA efficiency *in vivo*, JBEI researchers targeted *hct*, a gene in the lignin biosynthesis pathway, in a tissue-specific manner. They developed a transient assay that is widely applicable for evaluating sgRNA efficiency before applying CRISPR genome editing in stable transgenic plants. Using the highly efficient sgRNA, the researchers generated chimeric Hct plants with decreased lignin content and increased saccharification rate, while still maintaining the plant's integrity (Liang et al. 2019). The use of highly efficient sgRNA can accelerate the process of expanding germplasm for both molecular breeding and research to generate plants with optimized bioenergy traits, such as lignin that is easier to break down and convert into biofuels and bioproducts.

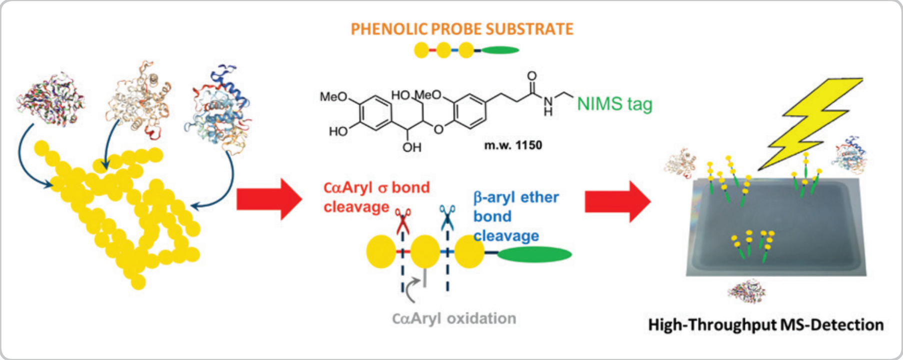
Modeling Jet Fuel Production Costs



Optimizing the Production Pipeline for Biofuels. JBEI scientists Nawa Baral **(left)** and Daniel Mendez-Perez **(right)** work on biojet fuel samples. Their research team is exploring how advances in production could make the plant-based jet fuels, currently under development at JBEI, price competitive with conventional fossil jet fuels. [LBNL]

Developing alternative energy sources for the air transportation sector remains one of the most challenging hurdles to reducing reliance on fossil fuels. JBEI researchers used a technoeconomic analysis to estimate the minimum selling price and lifecycle greenhouse gas mitigation costs for five different production pathways to four potential biojet fuel molecules—limonane via limonene; limonane via 1,8-cineole; tetrahydromethylcyclopentadiene dimer (RJ-4); bisabolane; and *epi*-isozizaane. The simulation results showed that all five pathways could indeed create fuel products at the target price of $2.50 per gallon if producers are able to convert the leftover lignin into a valuable chemical—something JBEI researchers are currently working toward—that could be sold to offset the cost of biofuels. Moreover, the researchers found that because of the attractive properties of these molecules, including higher volumetric energy density, commercial airlines may also be willing to pay as much as a $0.50 per gallon premium for these biojet fuels (Baral et al. 2019).

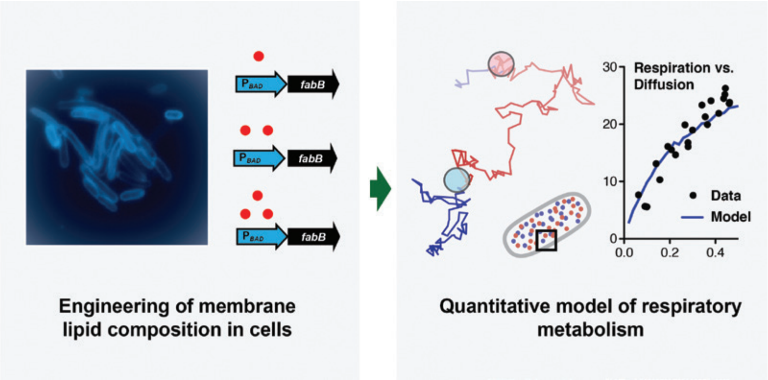
Characterizing Lignin-Modifying Enzyme Activities



Lignin Analytics. Probes are constructed to span major lignin linkages, enabling detailed examination of reaction mechanisms using nanostructure-initiator mass spectrometry (NIMS). The high-throughput and low-sample requirements of this platform are enabling the discovery of new high-performance enzymes that could ultimately decrease biofuel and bioproduct production costs. [LBNL]

Valorization of lignin is central to cost-competitive biofuels and bioproducts, but still lacking is detailed understanding of how lignin is enzymatically depolymerized. Development of suitable enzymatic assays for characterizing lignin-modifying enzymes (LMEs) is an important step. Toward this goal, JBEI has extended its cellulase assay platform, which uses substrates containing a charged perfluorinated tag and nanostructure-initiator mass spectrometry (NIMS), into a reliable and rapid assay for examining detailed mechanisms of LMEs. Two LMEs, laccase from the polypore mushroom *Trametes versicolor* and manganese peroxidase (MnP) from the white rot fungus *Nematoloma frowardii*, were tested for catalytic activity against two model substrates: phenolic and nonphenolic. Findings showed that the reaction of laccase and MnP with the phenolic substrate yields products that arise from the cleavage of the carbon-carbon single bond between the α-carbon and the adjacent aryl carbon, which is consistent with the mechanism for producing phenoxy radicals as reaction intermediates. Interestingly, the nonphenolic substrate catalyzed a different reaction pathway, producing an α-oxidation product as well as the cleavage of the β-aryl ether bond. This assay provides a wealth of information on bond cleavage events not available using conventional colorimetric assays and is performed using microliter volumes; quantitative analysis of product formation and kinetics is rapidly achieved by NIMS (Deng et al. 2018). Given the success of this approach, JBEI researchers are extending it to examine enzyme activities against all major lignin linkages. Together with the substrates already prepared for studying the activities of cellulases and hemicellulases, this substrate pool forms the basis for a powerful mass spectrometry–based multiplexing assay, which has the ability to simultaneously detect multiple functions of enzymes (or enzyme cocktails) responsible for deconstructing lignocellulosic biomass. Ultimately, this approach could aid in identifying more efficient, low-cost enzyme cocktails useful for converting all biomass polymers into valuable bioproducts.

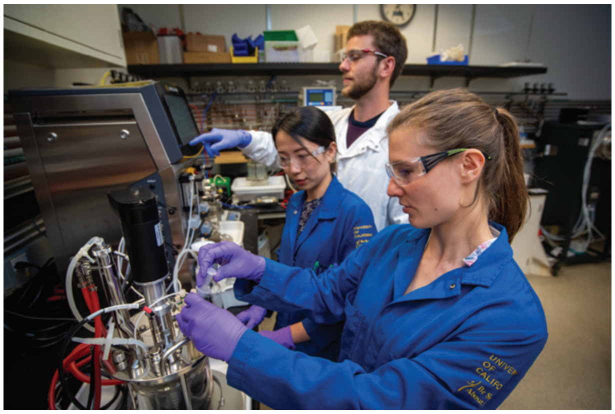
Determining How Membrane Viscosity Affects Cellular Respiration



Systems-Level Model for Cellular Respiration. Metabolic engineering leads to a fundamental discovery on how the lipid composition of cellular membranes affects levels of cellular respiration. [LBNL]

Lipids, which consist of fatty acid molecules, determine the physical properties of biological membranes, and their composition can vary substantially between and within organisms. JBEI researchers examined the specific role that the viscosity of energy-transducing membranes plays in cellular respiration. Viscosity, which refers to how fluid a membrane is, can fluctuate depending on which fatty acids are present. Using metabolic engineering, the researchers modulated fatty acid biosynthesis in the model bacterium *E. coli* and titrated inner membrane viscosity across a 10-fold range by controlling the abundance of unsaturated or branched lipids. They found that these fluidizing lipids tightly controlled respiratory metabolism, an effect that can be explained with a quantitative model of the electron transport chain (ETC) that features diffusion-coupled reactions between enzymes and electron carriers (quinones). They also found that lipid unsaturation modulated mitochondrial respiration in engineered budding yeast strains. Thus, diffusion in the ETC may serve as an evolutionary constraint for lipid composition in respiratory membranes (Budin et al. 2018). As lipid synthesis in bacteria and yeast hosts is often engineered to produce molecules, these findings suggest new ways by which the pathways to produce biofuels and bioproducts could be optimized to maintain proper respiratory function, thereby increasing production.

Creating a Biological Production Platform for Biofuels and Bioproducts

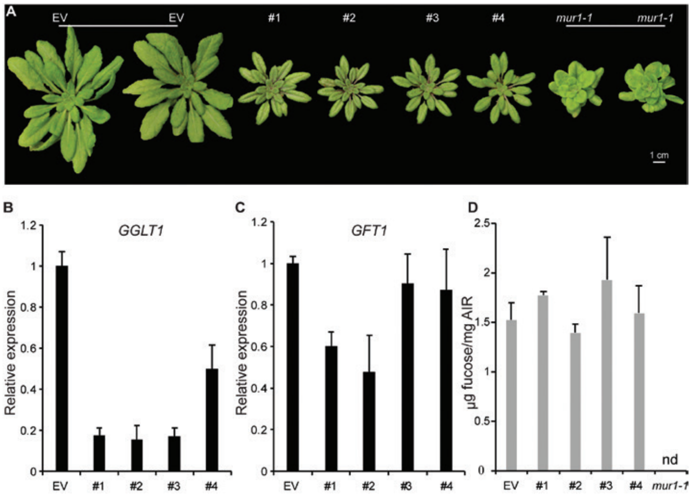


Blue Pigment from Engineered Fungi. JBEI researchers work in the laboratory on the biological production of indigoidine by *Rhodosporidium toruloides*. [LBNL]

Renewable feedstocks can be converted to a range of useful fuels and bioproducts. JBEI researchers engineered the versatile fungal host *R. toruloides* to test how well the species could produce nonribosomal peptide (NRP) synthases, large enzymes that bacteria and fungi use to assemble important compounds. Specifically, they used two bacterial genes, from *Bacillus subtilis* and *Streptomyces lavendulae*, to convert the cellular amino acid glutamine to a bipyridyl compound, indigoidine. Indigoidine, an NRP, is a deep blue pigment similar to indigo, a synthetic dye used to color denim and many other items. This fungi-produced indigoidine could provide a more sustainable alternative to indigo. Moreover, along with earlier studies, this work demonstrates *R. toruloides* as a valuable biotech host that can catabolize a range of carbon sources and be used in a separation-free mode for bioproduction. It also established a heterologous pathway for NRPs, an important biosynthetic space, in this fungal host. Additionally, the researchers found that the fermentation conditions could be optimized to significantly increase titers, rates, and yields and be implemented in a fed-batch bioreactor mode. This study represents the highest levels of a bacterial NRP generated heterologously in a fungal host (Wehrs et al. 2019).

Understanding the Biosynthesis of Plant Cell Walls

JBEI researchers are exploring how plant cell walls are made and how they might be engineered to make better bioenergy crops for biofuel and bioproduct production. As part of this work, they recently completed characterizing a group of sugar nucleotide transporters (GDP-linked sugars), proteins that provide substrate for glycosylation reactions. One of these proteins, GGLT1 (Sechet et al. 2018) was identified as the first GDP-L-galactose transporter and was found to play a critical role in the production of the complex pectin rhamnogalacturonan-II (RG-II). Loss of this protein was lethal, and these plants will be useful in understanding the importance of RG-II in cell wall architecture. Another protein, GONST2, has been identified as a GDP-mannose transporter with a specific role in glycosylating membrane lipids. An unexpected finding of this work was that while *gonst2* mutants appeared to grow normally, they were twice as resistant to the powdery mildew pathogen. Using biosystems design tools to control the expression of these genes will be the next important step to channel substrate as part of metabolic engineering strategies (see also JBEI Early Career Investigator Spotlight, p. 39).



Better Understanding of GGLT1. To understand the importance of GGLT1, JBEI researchers generated RNA interference (RNAi) transgenic lines with a hairpin (hp) RNA construct, which specifically targeted GGLT1. Forty independent hpGGLT1 transformants were screened, and four were selected for characterization. **(a)** Empty vector control (EV) and four hpGGLT1 lines grown on soil for 1 month. **(b)** Relative expression of GGLT1 and **(c)** its closest homologue, GFT1, in the four hpRNAi lines selected determined by quantitative polymerase chain reaction (PCR). **(d)** The cell wall fucose content of the selected lines. [Reprinted under a Creative Commons license (CC-BY-NC) from Sechet, J., et al. 2018. "Suppression of *Arabidopsis* GGLT1 Affects Growth by Reducing the L-Galactose Content and Borate Cross-Linking of Rhamnogalacturonan-II," *The Plant Journal* **96**(5), 1036–50. DOI:10.111/tpj.14088.]

DOE Genomic Science Program

genomicscience.energy.gov

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

GENOMIC SCIENCE PROGRAM

Systems Biology for Energy and the Environment

Fundamental Genomic Science research includes single-investigator projects, multi-institutional collaborations, and research centers at universities and national laboratories across the country.

RESEARCH PORTFOLIO

BIOENERGY RESEARCH CENTERS

Advance basic research to underpin production of biofuels and bioproducts from inedible lignocellulosic plant biomass.

SYSTEMS BIOLOGY FOR BIOENERGY

Improves fundamental understanding of microbes with bioenergy-relevant traits for deconstructing biomass and synthesizing biofuels and bioproducts.

PLANT SCIENCE FOR BIOENERGY

Elucidates and validates the functional role of genes, gene families, and associated pathways to enhance understanding of critical processes in DOE-relevant plant systems.

SUSTAINABILITY RESEARCH FOR BIOENERGY

Investigates plant-soil-microbe interactions in laboratory and field settings to enhance biomass productivity under changing biotic and abiotic conditions.

BIOSYSTEMS DESIGN

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

ENVIRONMENTAL MICROBIOME SCIENCE

Links structure and function of microbial communities in the field with key environmental or ecosystem processes.

COMPUTATIONAL BIOLOGY

Provides new computational approaches and hypothesis-generating analysis techniques, data, and simulation capabilities such as 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.**

genomicscience.energy.gov

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 RESEARCH

science.osti.gov/ber/bioimaging-research/

Develops imaging, measurement, and characterization platforms to visualize the spatial and temporal relationships of key plant and microbial metabolic processes.

STRUCTURAL BIOLOGY INFRASTRUCTURE

berstructuralbioportal.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.

DOE Office of Science and Office of Biological and Environmental Research

science.energy.gov

science.energy.gov/ber/

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 facilities, 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,

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/
- ***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

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.

Cited References

Amos, R. A., et al. 2018. "A Two-Phase Model for the Non-Processive Biosynthesis of Homogalacturonan Polysaccharides by the GAUT1:GAUT7 Complex," *Journal of Biological Chemistry* **293**(49), 19047–63. DOI:10.1074/jbc.RA118.004463.

Bansal, S., et al. 2018. "Towards the Synthetic Design of *Camelina* Oil Enriched in Tailored Acetyl-Triacylglycerols with Medium-Chain Fatty Acids," *Journal of Experimental Botany* **69**(18), 4395–402. DOI:10.1093/jxb/ery225.

Baral, N. R., et al. 2019. "Techno-Economic Analysis and Life-Cycle Greenhouse Gas Mitigation Cost of Five Routes to Bio-Jet Fuel Blendstocks," *Energy & Environmental Science* **12**(3), 807–24. DOI:10.1039/cBee03266a.

Barros, J., et al. 2019. "4-Coumarate 3-Hydroxylase in the Lignin Biosynthesis Pathway is a Cytosolic Ascorbate Peroxidase," *Nature Communications* **10**. DOI:10.1038/s41467-019-10082-7.

Basso, B., et al. 2019. "Yield Stability Analysis Reveals Sources of Large-Scale Nitrogen Loss from the US Midwest," *Scientific Reports* **9**, 5774. DOI:10.1038/s41598-019-42271-1.

Budin, I., et al. 2018. "Viscous Control of Cellular Respiration by Membrane Lipid Composition," *Science* **362**(6419), 1186–89. DOI:10.1126/science.aat7925.

Cheng, M. H., et al. 2019a. "Economic Analysis of Cellulosic Ethanol Production from Sugarcane Bagasse Using a Sequential Deacetylation, Hot Water and Disk Refining Pretreatment," *Processes* 7(10), 642. DOI:10.3390/pr7100642.

Cheng, M., et al. 2019b. "Sugar Production from Bioenergy Sorghum by Using Pilot-Scale Continuous Hydrothermal Pretreatment Combined with Disk Refining," *Bioresource Technology* **289**, 121663. DOI:10.1016/j.biortech.2019.121663.

Deng, K., et al. 2018. "Rapid Characterization of the Activities of Lignin-Modifying Enzymes Based on Nanostructure-Initiator Mass Spectrometry (NIMS)," *Biotechnology for Biofuels* **11**, 266. DOI:10.1186/s13068-018-1261-2.

Fan, J., et al. 2019. "Diversion of Carbon Flux from Sugars to Lipids Improves the Growth of an *Arabidopsis* Starchless Mutant," *Plants* **8**(7), 229. DOI:10.3390/plants8070229.

Fan, M., et al. 2018. "A Trihelix Family Transcription Factor is Associated with Key Genes in Mixed-Linkage Glucan Accumulation," *Plant Physiology* **178**, 1207–21. DOI:10.1104/pp.18.00978.

Ghosh, I. N., et al. 2019. "OptSSeq Explores Enzyme Expression and Function Landscapes to Maximize Isobutanol Production Rate," *Metabolic Engineering* **52**, 324–40. DOI:10.1016/j.ymben.2018.12.008.

Ghosh, I. N., and R. Landick. 2016. "OptSSeq: High-Throughput Sequencing Readout of Growth Enrichment Defines Optimal Gene Expression Elements for Homoethanologenesis," *ACS Synthetic Biology* **5**, 1519–34. DOI:10.1021/acssynbio.6b00121.

HamediRad, M., et al. 2019a. "Highly Efficient Single-Pot Scarless Golden Gate Assembly," *ACS Synthetic Biology* **8**(5), 1047–54. DOI:10.1021/acssynbio.8b00480.

HamediRad, M., et al. 2019b. "Towards a Fully Automated Algorithm Driven Platform for Biosystems Design," *Nature Communications* **10**, 5150. DOI:10.1038/s41467-019-13189-z.

Holwerda, E. K., et al. 2019. "Multiple Levers for Overcoming the Recalcitrance of Lignocellulosic Biomass," *Biotechnology for Biofuels* **12**, 15. DOI:10.1186/s13068-019-1353-7.

Huang, H., et al. 2017. "Evaluation of the Quantity and Composition of Sugars and Lipid in the Juice and Bagasse of Lipid Producing Sugarcane," *Biocatalysis and Agricultural Biotechnology* **10**, 148–55. DOI:10.1016/j.bcab.2017.03.003.

Jagtap, S. S., et al. 2019. "Production of Galactitol from Galactose by the Oleaginous Yeast *Rhodosporidium toruloides* IFO0880," *Biotechnology for Biofuels* **12**, 250. DOI:10.1186/s13068-019-1586-5.

Kim, S.-J., et al. 2018. "In the Grass Species *Brachypodium distachyon*, the Production of Mixed-Linkage (1,3;1,4)-β-Glucan (MLG) Occurs in the Golgi Apparatus," *The Plant Journal* **93**(6), 1062–75. DOI:10.1111/tpj.13830.

Kravchenko, A. N., et al. 2019. "Microbial Spatial Footprint as a Driver of Soil Carbon Stabilization," *Nature Communications* **10**, 3121. DOI:10.1038/s41467-019-11057-4.

Li, A., et al. 2018. "Editing of an Alpha-Kafirin Gene Family Increases Digestibility and Protein Quality in Sorghum," *Plant Physiology* **177**(4), 1425–38. DOI:10.1104/pp.18.00200.

Liang, Y., et al. 2019. "A Screening Method to Identify Efficient sgRNAs in *Arabidopsis*, Used in Conjunction with Cell-Specific Lignin Reduction," *Biotechnology for Biofuels* **12**, 130. DOI:10.1186/s13068-019-1467-y.

Martien, J. I., et al. 2019. "Systems-Level Analysis of Oxygen Exposure in *Zymomonas mobilis*: Implications for Isoprenoid Production," *mSystems* **4**, e00284–18. DOI:10.1128/mSystems.00284-18.

Perez, J. M., et al. 2019. "Funneling Aromatic Products of Chemically Depolymerized Lignin into 2-Pyrone-4-6-Dicarboxylic Acid with *Novosphingobium aromaticivorans*," *Green Chemistry* **21**(6), 1340–50. DOI:10.1039/c8gc03504k.

Riley, L. A., et al. 2019. "Rational Development of Transformation in *Clostridium thermocellum* ATCC 27405 via Complete Methylome Analysis and Evasion of Native Restriction-Modification Systems," *Journal of Industrial Microbiology and Biotechnology* **46**(9–10), 1435–43. DOI:10.1007/s10295-019-02218-x.

Salvachua, D., et al. "Outer Membrane Vesicles Catabolize Lignin-Derived Aromatic Compounds in *Pseudomonas putida* KT2440." Submitted.

Scarborough, M. J., et al. 2018a. "Increasing the Economic Value of Lignocellulosic Stillage Through Medium-Chain Fatty Acid Production," *Biotechnology for Biofuels* **11**, 200. DOI:10.1186/s13068-018-1193-x.

Scarborough, M. J., et al. 2018b. "Metatranscriptomic and Thermodynamic Insights into Medium-Chain Fatty Acid Production Using an Anaerobic Microbiome," *mSystems* **3**, e00221–18. DOI: 10.1128/mSystems.00221-18.

Schultz, J. C., et al. 2019. "Development of a CRISPR/Cas9 System for High-Efficiency Multiplexed Gene Deletion in *Rhodosporidium toruloides*," *Biotechnology and Bioengineering* **116**(8), 2103–09. DOI:10.1002/bit.27001.

Sechet, J., et al. 2018. "Suppression of *Arabidopsis* GGLT1 Affects Growth by Reducing the L-Galactose Content and Borate Cross-Linking of Rhamnogalacturonan-II," *The Plant Journal* **96**(5), 1036–50. DOI:10.111/tpj.14088.

Shen, F., et al. 2019. "OptRAM: *In-Silico* Strain Design via Integrative Regulatory-Metabolic Network Modeling," *PLOS Computational Biology* **15**(3), e1006835. DOI:10.1371/journal.pcbi.1006835.

Sun. Y., et al. 2019. "Vitamin A Production by Engineered *Saccharomyces cerevisiae* from Xylose via Two-Phase *In Situ* Extraction," *ACS Synthetic Biology* **8**(9), 2131–40. DOI:10.1021/acssynbio.9b00217.

Tejera, M., et al. 2019. "Multi-Year and Multi-Site Establishment of the Perennial Biomass Crop *Miscanthus* × *giganteus* Using a Staggered Start Design to Elucidate N Response," *BioEnergy Research* **12**(3), 471–83. DOI:10.1007/s12155-019-09985-6.

Tran, V. G., et al. 2019. "Development of a CRISPR/Cas9-Based Tool for Gene Deletion in *Issatchenkia orientalis*," *mSphere* **4**(3), e00345-19. DOI:10.1128/mSphere.00345-19.

Wang, Z., et al. 2019. "Improving Ethanol Yields with Deacetylated and Two-Stage Pretreated Corn Stover and Sugarcane Bagasse by Blending Commercial Xylose-Fermenting and Wild Type *Saccharomyces* Yeast," *Bioresource Technology* **282**, 103–09. DOI:10.1016/j.biortech.2019.02.123.

Wehrs, M., et al. 2019. "Sustainable Bioproduction of the Blue Pigment Indigoidine: Expanding the Range of Heterologous Products in *R. toruloides* to Include Non-Ribosomal Peptides," *Green Chemistry* **21**(12), 3394–406. DOI:10.1039/c9gc00920e.

Xie, M., et al. 2018. "A 5-Enolpyruvylshikimate 3-Phosphate Synthase Functions as a Transcriptional Repressor in *Populus*," *The Plant Cell* **30**, 1645–60. DOI:10.1105/tpc.18.00168.

Yarbrough, et al. "Unraveling the Nature of the Interaction Zone During Biomass Deconstruction by *C. thermocellum*." In preparation.

Yu, X., et al. 2019. "Expression of a Lychee *Phosphatidylcholine: Diacylglycerol Cholinephosphotransferase* with an *Escherichia coli Cyclopropane Synthase* Enhances Cyclopropane Fatty Acid Accumulation in *Camelina* Seed," *Plant Physiology* **180**(3), 1351–61. DOI:10.1104/pp.19.00396.

Zhang, S., et al. 2019. "pH Selectively Regulates Citric Acid and Lipid Production in *Yarrowia lipolytica* W29 During Nitrogen-Limited Growth on Glucose," *Journal of Biotechnology* **290**, 10–15. DOI:10.1016/j.jbiotec.2018.10.012.

Zhang, Y., et al. 2019. "Xylose Utilization Stimulates Mitochondrial Production of Isobutanol and 2-Methyl-1-Butanol in *Saccharomyces cerevisiae*," *Biotechnology for Biofuels* **12**, 223. DOI:10.1186/s13068-019-1560-2.

Zhao, Y., et al. 2019. "Generation of a Selectable Marker Free, Highly Expressed Single Copy Locus as Landing Pad for Transgene Stacking in Sugarcane," *Plant Molecular Biology* **100**(3), 247–63. DOI:10.1007/s11103-019-00856-4.

Acronyms and Abbreviations

BER	DOE Office of Biological and Environmental Research
BRC	Bioenergy Research Center
CABBI	Center for Advanced Bioenergy and Bioproducts Innovation
CBI	Center for Bioenergy Innovation
CBP	consolidated bioprocessing
C3H	coumarate 3-hydroxylase
CRISPR	clustered regularly interspaced short palindromic repeats
CRISPR/Cas	targeted genome editing system using engineered nucleases (e.g., Cas9)
DOE	U.S. Department of Energy
EPSP	5-enolpyruvylshikimate 3-phosphate
ETC	electron transport chain
GAUT	galacturonosyltransferase
GLBRC	Great Lakes Bioenergy Research Center
GWAS	genome-wide association study
HG	homogalacturonan
IBA	isobutanol
iCLEM	Introductory College Level Experience in Microbiology program
iGEM	International Genetically Engineered Machine competition
IP	intellectual property
JBEI	Joint BioEnergy Institute
LBNL	Lawrence Berkeley National Laboratory
LME	lignin-modifying enzyme
MCFA	medium-chain fatty acid
MEP	methyl erythritol-4-phosphate
MLG	mixed-linkage glucan
MSU	Michigan State University
NIMS	nanostructure-initiator mass spectrometry
NRP	nonribosomal peptide
OMV	outer membrane vesicle
OptSSeq	Optimization by Selection and Sequencing
ORNL	Oak Ridge National Laboratory
PDC	2-pyrone-4,6-dicarboxylic acid
RCF	reductive catalytic fractionation
REU	Research Experience for Undergraduates program
sgRNA	single-guide RNA
STEM	science, technology, engineering, and mathematics
TCS	total carbohydrate solubilization
UW	University of Wisconsin

DOE Bioenergy Research Centers and Partners

genomicscience.energy.gov/biofuels/

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(Argonne, Illinois)

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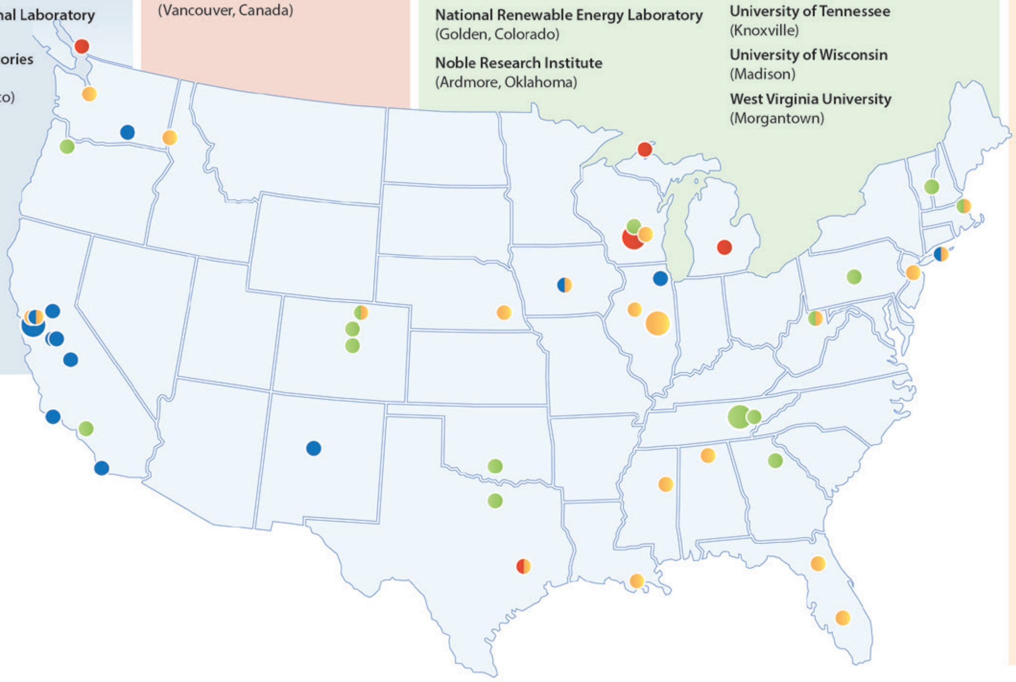

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