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U.S. DEPARTMENT OF ENERGY

Bioenergy Research Centers

2022 Program Update

U.S. DEPARTMENT OF ENERGY | Office of Science

Biological and Environmental Research Program

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DOE Biological and Environmental Research Program
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Great Lakes Bioenergy Research Center (GLBRC)
glbrc.org

Joint BioEnergy Institute (JBEI)
jbei.org

Genomic Science Program
genomicscience.energy.gov

About BER

The Biological and Environmental Research (BER) program supports transformative science and scientific user facilities examining complex biological, Earth, and environmental systems for clean energy and climate innovation. BER research seeks to understand the fundamental biological, biogeochemical, and physical principles needed to predict a continuum of processes occurring across scales, from molecules and genomes at the smallest scales to environmental and Earth system change at the largest scales. This research—conducted at universities, U.S. Department of Energy (DOE) national laboratories, and research institutions across the country—is contributing to a future of reliable, resilient energy sources and evidence-based climate solutions.

Front cover image credits: Sustainability, Center for Advanced Bioenergy and Bioproducts Innovation. Feedstock Development, Lawrence Berkeley National Laboratory/Joint BioEnergy Institute. Deconstruction and Separation, Great Lakes Bioenergy Research Center. Conversion, National Renewable Energy Laboratory/Center for Bioenergy Innovation.

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Biological and Environmental Research Program

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U.S. Department of Energy
Bioenergy Research Centers

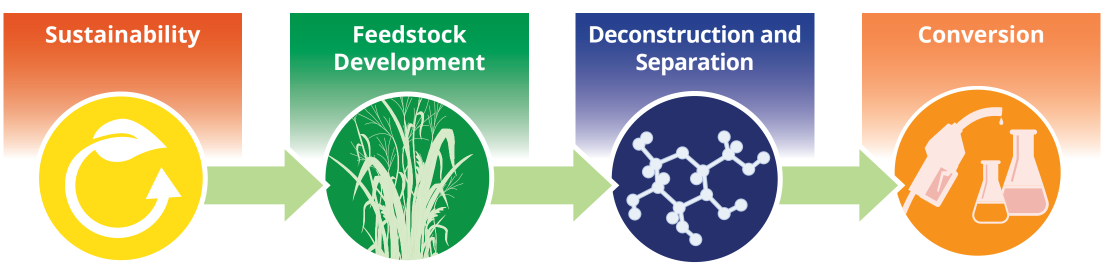
Introduction

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. 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, and creating jobs in rural areas.

Lignocellulose is the most abundant biological material on Earth. Most often contained in plant cell walls, it is composed of two kinds of carbohydrate polymers, cellulose and hemicellulose, and an aromatic-rich polymer called lignin. Although cellulose can be converted into biofuels and bioproducts by microbes, the matrix of cellulose, hemicellulose, and lignin in plant cell walls is highly resistant to degradation. 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 to biofuels and bioproducts (see figure, From Feedstocks to Advanced Biofuels and Bioproducts, this page).

Since 2007, DOE's Biological and Environmental Research (BER) program within the Office of Science has supported Bioenergy Research Centers (BRCs) whose mission is to break down the barriers to actualizing a domestic bioenergy industry (see sidebar, BER Genomic Science Advances DOE Missions, p. 2). As part of BER's Genomic Science program, the BRCs have made significant advances in realizing this new biobased economy. The BRCs have produced multiple breakthroughs in the form of deepened

From Feedstocks to Advanced Biofuels and Bioproducts



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

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

Feedstock Breakdown
Improve enzymes and microbes that break down feedstocks into sugars and lignin.

Biofuel and Bioproduct Synthesis
Engineer metabolic pathways in microbes to produce biofuels and bioproducts.

understanding of sustainable biomass production practices, targeted re-engineering of biomass feedstocks, development of new methods for deconstructing feedstocks, and engineering of microbes for more effective production of a diverse range of biofuels. These breakthroughs have led to:

- Engineering of lignin composition and deposition to reduce plant cell wall recalcitrance without affecting plant viability and to facilitate lignin valorization.
- Development of effective biomass pretreatments that can be adapted commercially to lower costs.
- Discoveries of novel microbes and enzymatic pathways for more efficient deconstruction of lignocellulosic biomass.
- Research for the refinement of consolidated bioprocessing (i.e., simultaneous breakdown and conversion to biofuels and bioproducts by cellulolytic microbes).
- Metabolic engineering of microbes and plants for the biological production of numerous advanced biofuels, bioproducts, or their immediate precursors.
- Identification of new plant genes and an improved understanding of their role in cell wall biosynthesis.
- Refinement of technoeconomic and lifecycle assessments of biofuels and bioproducts production.

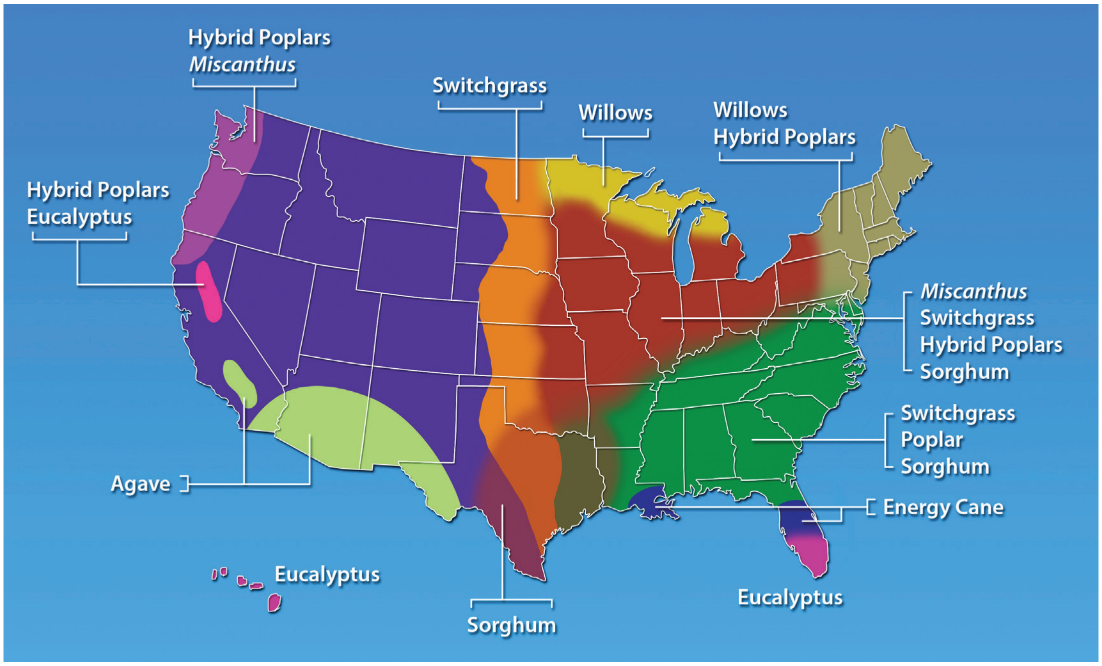
Advanced Biofuels and Bioproducts

These 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. They also may be novel chemicals that cannot be efficiently produced from petroleum.

The four BRCs are based in the geographically diverse Midwest, Southeast, and West Coast regions. BRC locations correspond to the geographic ranges of biomass crops being developed (see figure, Approximate Geographic Distribution of Potential Dedicated Biomass Crops, p. 3). BRC partners include universities, private companies, nonprofit organizations, and

BER Genomic Science Advances DOE Missions

Understanding the instructions for life encoded in the genomes of natural systems offers a wealth of potential for advancing biological solutions to many of today's energy and environmental challenges. The mission of the U.S. Department of Energy's (DOE) Biological and Environmental Research (BER) program is to support transformative science and scientific user facilities to achieve a predictive understanding of complex biological, Earth, and environmental systems for clean energy and climate innovation. BER's Genomic Science program provides the necessary fundamental science to understand, predict, manipulate, and design biological systems that underpin innovations for bioenergy and bioproduct production and to enhance understanding of natural, DOE-relevant environmental processes. This fundamental research explores organisms and ecosystems that can influence the U.S. energy system and deepen insights into the relationships between energy and environment from local to global scales, contributing to stable, reliable, and resilient energy sources and the growing bioeconomy.



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.

DOE and other government national laboratories (see DOE Bioenergy Research Centers and Partners map, back cover). The 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 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.

- **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 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 bioenergy 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 bioenergy 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 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 DOE Bioenergy Research Center Strategies at a Glance, p. 5).

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 need to be engineered for sustainable production and efficient conversion to biofuels and bioproducts. These feedstocks include dedicated crops for biofuels and bioproducts as well as nonfood crops for oils or other nonpharmaceutical *in planta*–produced products.

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.

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 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 spatially explicit economic and environmental analyses for a sustainable bioeconomy	Develop "plants as factories" for sustainable and resilient production of biofuels and bioproducts	Develop industrially relevant process and extraction technologies for feedstock oils and sugars	Establish artificial intelligence/ machine learning–driven biofoundry for biofuels and bioproducts
CBI	Optimize water and nutrient use for high-yielding bioenergy crops with improved soil carbon storage	Create process advantaged bioenergy crops exploiting natural genetic variation found in feedstock plants	Advance integrated and consolidated bioprocessing with co-treatment	Generate drop-in biofuels (i.e., sustainable aviation fuel) and bioproducts from biomass and lignin residues
GLBRC	Conduct long-term studies of growing bioenergy crops on bioenergy lands	Design productive and high-value bioenergy cropping systems	Develop cost-effective biomass deconstruction and separation strategies	Identify and engineer novel biomass conversion microbes
JBEI	Design sustainable and cost-effective bioenergy cropping systems and conversion processes	Engineer bioenergy crops for high yield, environmental resilience, and efficient conversion into biofuels and bioproducts	Develop and demonstrate affordable feedstock-agnostic biomass deconstruction technologies based on ionic liquids	Develop high-throughput biosystems design tools and microbial hosts for scalable, carbon-efficient 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.

- Developing quantitative models informed by experimentation to predict how bioenergy feedstock genotypes perform under different geographic and environmentally relevant conditions.
- Developing new methods for large-scale phenotyping using drones and automated imaging techniques.

Lignocellulosic Deconstruction and Separation. Further research is needed to make deconstruction processes low cost, low energy, and more efficient. Additional process improvements are required to minimize environmental impacts and maximize cellulosic or hemicellulosic sugar production from a range of lignocellulosic biomass types for conversion into biofuels and bioproducts. Also needed are technologies to convert the relatively large fraction of carbon found in the lignin portion of lignocellulosic biomass into biofuels and bioproducts (see 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.
- 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 potential environmental and economic benefits of using biomass to produce chemicals currently derived from petroleum, as well as the potential unbounded diversity of new molecules that could be produced from biomass. The synergies between the methods and approaches for biofuel and bioproduct synthesis create an opportunity to leverage basic research in biofuels development with broader possibilities toward advancing a biobased economy.

BER goals for this scientific focus area include:

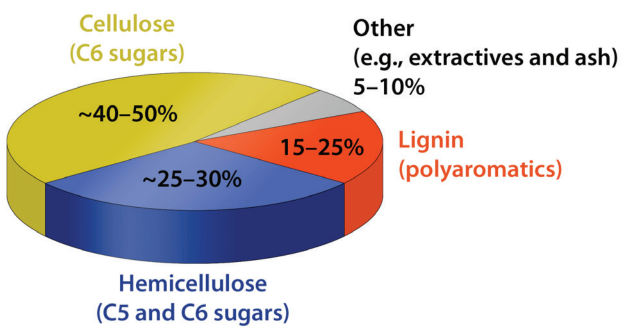
- Developing high-throughput methods to screen or select high-performance strains to improve product formation rates, titers, yields, and selectivity (i.e., the ability to produce only the desired product while minimizing byproducts).
- Establishing a broader set of platform microorganisms suitable for metabolic engineering to produce biofuels and bioproducts, as well as high-throughput methods for experimental validation of gene function.
- Developing new approaches and models using machine learning and artificial intelligence 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 (CBP).
- 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.

Plant Cell Wall Recalcitrance: A Key Scientific Challenge

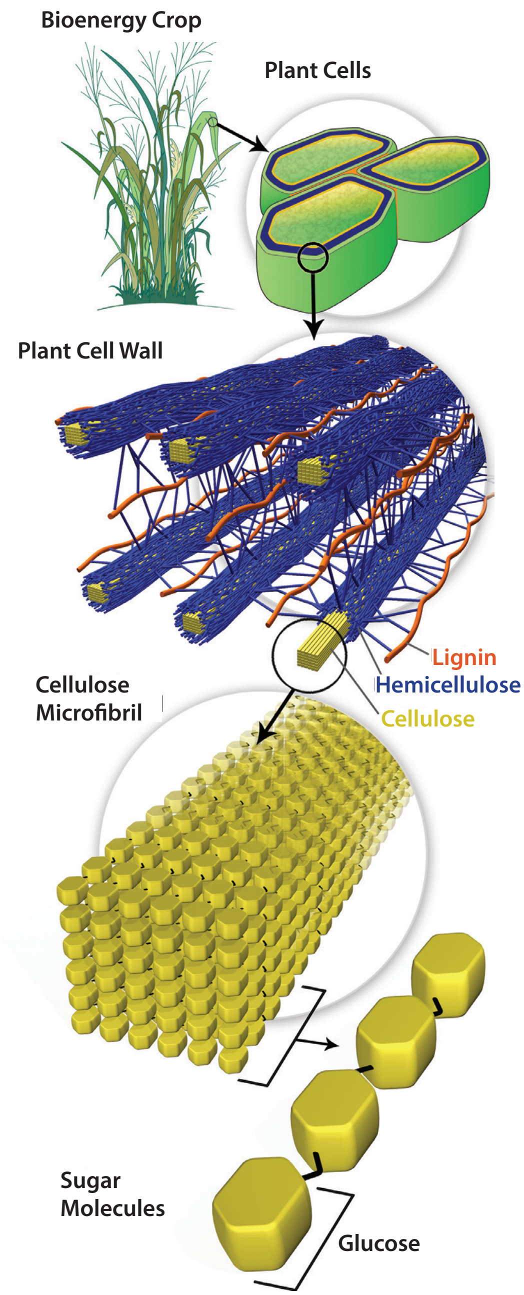
The tough structural materials in plant cell walls form a complex composite exquisitely designed to support plant structure and resist biological and chemical assaults. This natural resistance to degradation is called "recalcitrance" and represents one of the greatest challenges to attaining a viable, 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 (C6) sugar readily converted into biofuels and bioproducts by microbes. However, physically accessing these sugars 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-carbon (C5) and C6 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.



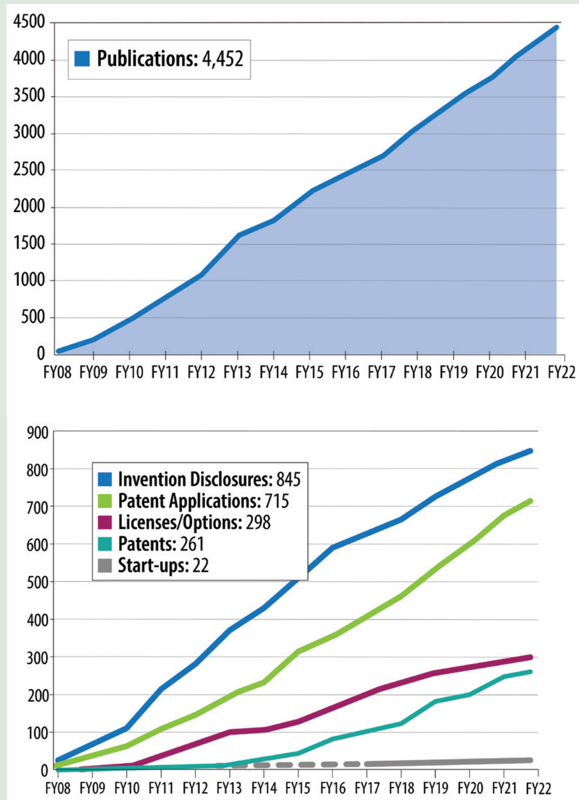
Approximate distribution of the three primary components of herbaceous perennial plant cell walls—cellulose, hemicellulose, and lignin.



Accelerating Deployment of Biofuel and Bioproduct Science and Commercialization

Scientific advances made by the U.S. 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 (IP) 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.

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.



Summary of Bioenergy Research Center Scientific and Technological Output Through June 2022.

- 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 BRCs are addressing these challenges to provide a broad scientific underpinning for producing biofuels and bioproducts from sustainable biomass resources. Their research also is producing the building blocks of new technological advances and speeding the translation of basic research results to industry. As of the end of June 2022, the BRCs have produced 4,452 peer-reviewed publications, 845 invention disclosures, 715 patent applications, 298 licenses or options, 261 patents, and 22 company start-ups. Through this work, they transferred substantial insight and expertise to industry via cooperation with other researchers and both large and small companies (see Accelerating Deployment of Biofuel and Bioproduct Science and Commercialization, this page).

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 Urbana-Champaign, has substantially developed scientific understanding and technological innovations required to produce economically and ecologically sustainable liquid biofuels and platform chemicals. Production system sustainability is guided and evaluated by models that integrate technoeconomic assessment (TEA) and life-cycle assessment (LCA), which are tools informed by cutting-edge measurements of agroecosystem function and industrially relevant process data. Researchers pursue a vision of "plants as factories," in which biofuels, bioproducts, and foundation molecules are directly synthesized by highly productive, resilient, and sustainable grass feedstocks carrying out efficient C4 photosynthesis. Plant-derived oils and sugars are further upgraded to high-value platform compounds by highly engineered nonmodel yeasts. CABBI's research approach maximizes team expertise in ecosystem ecology, agricultural economics, agronomy, plant and microbial engineering, bioprocessing, genomics, and computational biology.

Over the last 5 years, CABBI has advanced transformative technologies for the economic and sustainable production of biofuels and bioproducts from plants by pursuing the following long-term goals:

- Provide an integrated economic and environmental framework for determining feedstock supply and sustainability.
- Provide a regionally adaptive, yet national-scale, platform for grass-based biorefining based on high-yielding feedstocks with improved environmental resilience.
- Provide a broad set of platform microorganisms, and automated tools to engineer them, to produce value-added products from plant-produced feedstocks or substrates.

CABBI Partners

- **University of Illinois Urbana-Champaign** (Lead Institution)
- **Archbold Biological Station**
- **Boston University**
- **Brookhaven National Laboratory**
- **Colorado State University**
- **HudsonAlpha Institute for Biotechnology**
- **Iowa State University**
- **Lawrence Berkeley National Laboratory**
- **Lawrence Livermore National Laboratory**
- **Mississippi State University**
- **Princeton University**
- **Texas A&M AgriLife Research**
- **The Pennsylvania State University**
- **University of California–Berkeley**
- **University of Florida**
- **University of Idaho**
- **University of Minnesota–Twin Cities**
- **University of Nebraska–Lincoln**
- **University of Wisconsin–Madison**
- **U.S. Department of Agriculture's Agricultural Research Service**
- **West Virginia University**

Quantifying Ecosystem Processes in Feedstock Cropping Systems. Researchers Joanna Ridgeway (left) and Hayden Starcher (right) sample soil respiration in a *Miscanthus* field to measure litter carbon respired by microbial decomposers. [Courtesy CABBI]

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

Research Focus Areas

CABBI's research is organized into three focus areas: Sustainability, Feedstock Production, and Conversion.

Sustainability: Improving the Environmental and Economic Bottom Line. CABBI is providing a holistic and systems-based approach to assess the economic and ecological sustainability of feedstocks, biofuels, and bioproducts developed in the Feedstock Production and Conversion research focus areas, at scales ranging from field to biorefinery to bioeconomy. Over the last 5 years, CABBI has improved fundamental understanding of ecosystem carbon, nitrogen, water, and energy fluxes in sorghum, *Miscanthus*, and *Saccharum* cropping systems and the effects of management practices and plant-microbe interactions on these ecosystem processes (Dracup et al. 2021; Tejera et al. 2019; Studt et al. 2021; Schetter et al. 2021; Hartman et al. 2022; Burnham et al. 2022; Yang, J., et al. 2022). Experimental results have been incorporated into a suite of ecosystem models: FUN-BioCROP, DayCent, and Agro-IBIS (Juice et al. 2022; Kent et al. 2020; Moore, C. E., et al. 2020; Ferin et al. 2021; Edmonds et al. 2021). The resulting improvements in cropping system representation have enabled model simulations that researchers used to generate mechanistic hypotheses for experimental testing (Hartman et al. 2022) and to assess ecosystem services production by CABBI crops across the rainfed United States (see image, Quantifying Ecosystem Processes in Feedstock Cropping Systems, this page).

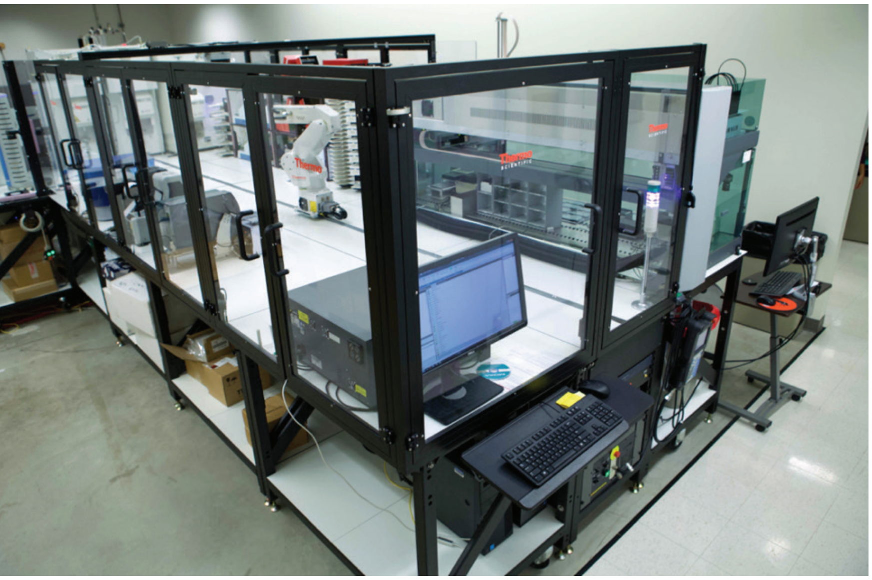
CABBI has developed a robust platform, BioSTEAM, for conducting rapid TEA-LCA under uncertainty (Cortés-Peña et al. 2020a, b; Shi et al. 2020). BioSTEAM has been applied to characterize the viability of biodiesel and ethanol production from CABBI feedstocks and to set research and development targets for both feedstock composition and conversion technologies (Cortés-Peña et al. 2020b; Li, Y., et al. 2021; Bhagwat et al. 2021; McClelland et al. 2021). CABBI has also developed novel approaches to quantifying conventional cropland available for conversion to bioenergy crops, including (1) remote sensing of historical land-use changes (Jiang et al. 2021), (2) assessing confidence in the classification of lands as economically marginal, (3) evaluating the net social benefits of conventional food crops, and (4) adding environmental externalities to the definition of economic marginality (Khanna et al. 2021). Additionally, CABBI has developed an integrated ecosystem-economic modeling framework, called the Biofuel and Environmental Policy Analysis Model (BEPAM), which couples ecosystem models with an economic model (Ferin et al. 2021). BEPAM was used to evaluate optimal locations, feedstock mixes, biofuels and bioproducts, and the economic and environmental consequences (Yang, P., et al.

Oilcane Harvest. CABBI researcher Scott McCoy harvests a *Saccharum* line, engineered to hyper-accumulate oils in its vegetative biomass, for bioprocessing. [Courtesy CABBI]

2022) of large-scale bioenergy production and to characterize the complex interactions among bioenergy policies, feedstock attributes, conversion technology, and market conditions that affect bioeconomy sustainability (Chen et al. 2021a, b).

Feedstock Production: Growing the Right Crops. CABBI is working to increase the value and resiliency of its target crops: annual sorghum (*Sorghum* species), temperate perennial miscanthus (*Miscanthus* species), and subtropical perennial energy cane (*Saccharum* species; see image, Oilcane Harvest, this page). Researchers have engineered the production of oils, specialty fatty acids, and other organic compounds by vegetative (nonseed) tissues in these grasses and increased biomass yield, resource use efficiency, and stress resilience. Proof-of-principle genetic crop designs have demonstrated roughly 50-fold increased oil production compared to wild-type when grown in the field, providing feedstock to the Conversion focus area and field-relevant data, materials, and infrastructure to the Sustainability focus area (Parajuli et al. 2020). CABBI has successfully engineered all three target crops and made genomic discoveries that will speed additional discovery and manipulation of natural and engineered genetic variation (Li, A., et al. 2018; Zhao et al. 2019; Eid et al. 2021; Mitros et al. 2020). These discoveries include key bioenergy traits under genetic control and improved genotypic and phenotypic methods to identify them (Dong et al. 2019, 2021; Clark et al. 2019).

Collaborations with other BRCs and DOE user facilities have produced tools and knowledge that enable targeted expression of engineered traits, paving the way for development of CABBI crops that produce oil in stem storage tissues at the end of the growing season. Methodological foundations from the Sustainability focus area provided mechanistic models, multiscale ground measurements, and remote sensing used to predict and assess hard-to-measure traits of field-grown CABBI crops (Varela et al. 2021, 2022). This knowledge informed the identification, creation, and testing of genetic variation, which increased productivity, thermotolerance, water use efficiency, and pollution resiliency in target crops (Li, S., et al. 2019, 2021, 2022; Kim, S., et al. 2020, 2021; Wang, S., et al. 2021; Jaikumar et al. 2021; Wang, Y., et al. 2021b). Focused collaborations with commercial partners have



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. [Courtesy CABBI]

extended CABBI's knowledge and impact, such as through the collection and use of the first commercial-scale *Miscanthus* yield monitoring data available in the United States.

Conversion: Turning Plants into High-Value Chemicals. CABBI is developing a biofoundry for biosystems design and characterizing and engineering nonmodel yeasts including *Issatchenkia orientalis*, *Rhodosporidium toruloides*, and *Yarrowia lipolytica*. These organisms convert plant-derived sugars and oils developed in the Feedstock Production focus area to biofuels and value-added bioproducts such as fatty alcohols, triacetic acid lactone (TAL), 3-hydroxypropanoic acid (3-HP), and citramalate. Many new tools and workflows for the design-build-test-learn (DBTL) cycle have been developed and implemented on the Illinois Biological Foundry for Advanced Biomanufacturing, an automated biofoundry platform (see image, iBioFAB, this page). Examples include:

- OptRAM, an *in silico* strain design tool (Shen et al. 2019)
- Genetic tools for metabolic engineering of *I. orientalis* and *R. toruloides* (Schultz et al. 2019)
- Artificial enzymes with novel reactivity (Huang et al. 2020; Mirts et al. 2018)
- ECNet, a deep-learning model for protein engineering (Luo, Y., et al. 2021)
- BioAutomata, a machine learning–enabled, fully closed DBTL cycle for automated pathway engineering (HamediRad et al. 2019b)
- PlasmidMaker, a versatile, automated, and high-throughput end-to-end platform for design and construction of plasmids (Enghiad et al. 2022)

Engineering guided by metabolic modeling and metabolomics has generated *I. orientalis* strains capable of producing 19 g/L 3-HP, an acrylic acid precursor, and 6 g/L citramalate, a methacrylate precursor, as well as *R. toruloides* strains capable of producing 28 g/L TAL and 3.7 g/L fatty alcohol (Cao et al. 2022; Schultz et al. 2022). Among the four target bioproducts, TAL production and recovery is close to financial viability, according to an initial TEA-LCA study. Chemical catalysis and biocatalysis are being explored as means to upgrade the target compounds as well as fatty acids derived from oils produced in the Feedstock Production focus area. Finally, various biomass pretreatment and deconstruction methods have been developed or optimized to achieve high-efficiency recovery of sugars and oils from CABBI crops. In particular, researchers established time-domain nuclear magnetic resonance spectroscopy as a dry, inexpensive, and rapid method to quantify total lipid content and free fatty acid composition and to qualitatively determine the fractions of bound and free oil within the biomass matrix (Maitra et al. 2021).

By developing this overarching framework for a closed-loop integration of research and outcomes among the Sustainability, Feedstock Production, and Conversion research focus areas, CABBI experts are engaged in innovative research needed to achieve a sustainable bioeconomy. The framework was tested during two center-wide Feedstocks-to-Fuels pipeline collaborations that generated engineered crops for juice, oil, and

RISE Students at a CABBI Retreat. Participants in the Research Internship in Sustainable Bioenergy (RISE) program presented their projects to researchers at CABBI's annual science planning retreat. [Courtesy CABBI]

bagasse production. *Saccharum* lines engineered to hyperaccumulate oils in their vegetative biomass and grown in Florida, Mississippi, and Illinois field trials produced almost as much oil as soybeans per unit land area, according to preliminary extrapolation of results. Agronomic performance was evaluated and triacylglycerol accumulation was analyzed by Sustainability researchers to compare the microbiome of the engineered oilcane to wild-type *Saccharum*. Approximately 400 kg of biomass were harvested, frozen, and shipped to the Integrated Bioprocessing Research Laboratory (IBRL) where it was bioprocessed at an industrially relevant scale. Conversion researchers used the extracted sugar and oil to generate fermentation target products, and the bagasse was hydrothermally pretreated and hydrolyzed into cellulosic sugars. The cellulosic sugars were then fermented to produce additional lipids using oleaginous yeast and the remaining oil in the bagasse was centrifuged and recovered.

Industry Partnerships

One of CABBI's objectives is to translate its research results to commercial deployment. CABBI and the IBRL utilize an Industrial Affiliates program to engage industry in cutting-edge bioprocessing techniques and de-risk new intellectual property (IP) for transition to commercialization. The 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. IBRL also offers an annual professional biofuels course in which CABBI technologies are extensively discussed. Finally, industry representatives serve on CABBI's strategic advisory board, bringing a corporate perspective to its research directions.

Education and Outreach

CABBI's outreach efforts help grade-school through undergraduate students better understand bioenergy feedstock production; conversion methods to produce valuable fuels and chemicals; and economic and environmental sustainability in the field, the laboratory, and the world. Three such hands-on efforts include (1) development and implementation of the Research Internship in Sustainable Bioenergy (RISE) program for undergraduates from groups currently underrepresented in science, technology, engineering, and mathematics (STEM) research; (2) sponsorship and advising of University of Illinois undergraduates in the annual International Genetically Engineered Machine (iGEM) competition; and (3) support of the Pollen Power camp in conjunction with the Institute for Genomic Biology, an annual weeklong summer camp that provides middle school girls an opportunity to study sustainable agriculture and plant responses to climate change.

RISE participants are mentored by CABBI faculty, postdocs, and graduate students as they gain professional skills and learn about graduate school (see image, RISE Students at a CABBI Retreat, this page). The iGEM undergraduates conduct their own synthetic biology research project under the mentorship of CABBI faculty, postdocs, and graduate students. Members learn cutting-edge research techniques, how to work as a team, and science communication skills. At Pollen Power, small groups of girls gain first-hand experience in a range of research techniques by female graduate student mentors, such as using confocal microscopes to image fossil pollen, a technique used to reconstruct vegetation patterns in past climates.

Center for Bioenergy Innovation

cbi.ornl.gov

CBI Overview

The Center for Bioenergy Innovation (CBI), led by Oak Ridge National Laboratory, is pursuing high-impact scientific and technological innovations to create dedicated bioenergy crops (e.g., poplar and switchgrass) as a cost-efficient substitute for petroleum-based fuels and products (see image, Feedstock Optimization, this page). Through basic science research on dedicated bioenergy crops and a suite of engineered microbes, CBI researchers are developing high-yielding, process-advantaged, sustainable plant biofeedstocks and designing economic and efficient bioprocessing approaches using microbes that produce biofuels and bioproducts, including hydrocarbons for jet fuel and chemical feedstocks for plastics precursors.

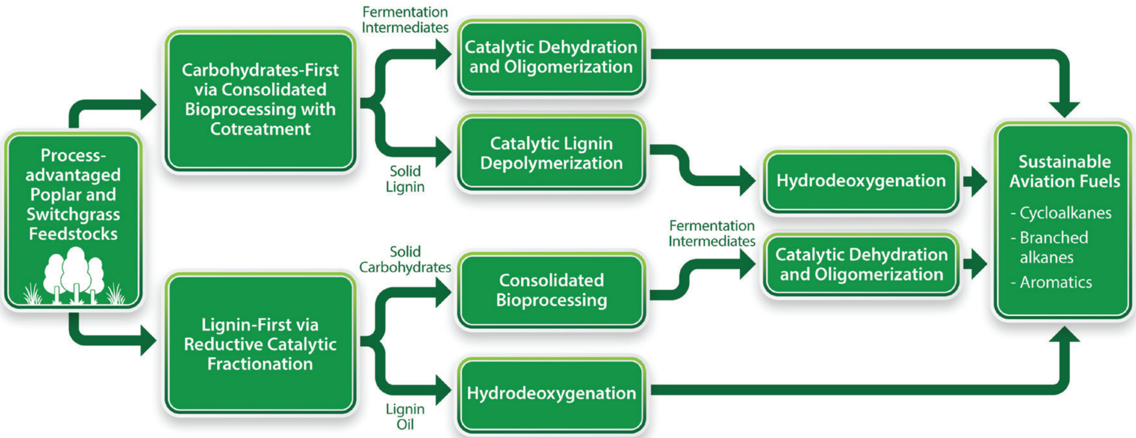
Feedstock Optimization. Udaya Kalluri, a senior staff scientist at Oak Ridge National Laboratory, examines naturally varying greenhouse-grown poplar genotypes. Greenhouse and field trials enable researchers to measure important phenotypes of growth, composition, and sustainability. [Courtesy CBI]

Specifically, the CBI team is accelerating progress toward using natural genetic variants found in plants to improve growth, yield, composition, and sustainability traits to lower feedstock costs and improve year-round feedstock supplies. Additionally, CBI is developing consolidated bioprocessing with cotreatment (C-CBP) as a process to simultaneously digest plant biomass and convert it to select biofuels and bioproducts. Advancements in C-CBP combine multiple approaches and tools to overcome industrially relevant barriers to using microbes in biomass deconstruction and conversion, including cotreatments such as brief milling during deconstruction.

Finally, CBI is committed to translating its research results into applications and potential commercial deployment to meet DOE bioenergy objectives. Through economic and sustainability analyses, CBI is assessing how its research on new supply chains and process configurations can reduce environmental impacts, costs, and scale-up risks from biomass planting and harvest through conversion to fuels and products.

Ultimately, CBI aims to:

- Create high-yielding bioenergy crops that display uniform productivity and increased sustainability by harnessing natural diversity via genomic selection in two perennial feedstocks—poplar and switchgrass.
- Reduce the cost of CBP technologies that convert carbohydrates to fermentation intermediates that can be catalytically upgraded to sustainable aviation fuels (SAFs).
- Utilize plant cell wall components more completely, specifically lignin, to improve production of fuels, coproduct chemicals, and novel materials.



Feedstocks to Fuels. CBI's process follows either one of two conversion paths from sustainable, process-advantaged feedstocks to sustainable aviation fuels (SAFs). In one path (top), carbon-efficient conversion occurs via consolidated bioprocessing (CBP) with cotreatment in which carbohydrates are converted first. Fermentation intermediates are then catalytically upgraded, and lignin-enriched residuals are deconstructed and deoxygenated into SAF blendstocks. In an alternate path (bottom), carbon-efficient conversion occurs via a reductive catalytic fractionation (RCF) process in which lignin in whole biomass is converted to RCF oil that is then deoxygenated to SAF blendstocks. Carbohydrate-enriched residuals are subsequently deconstructed and converted to fermentation intermediates via CBP and then catalytically upgraded into SAF blendstocks. [Courtesy CBI]

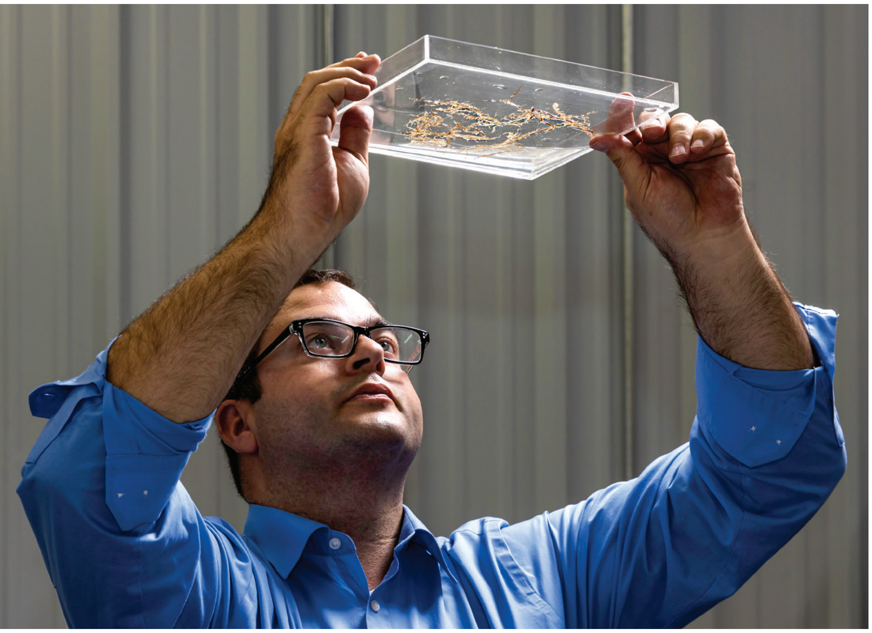
Research Focus Areas

CBI's feedstocks-to-fuels process (see figure, Feedstocks to Fuels, this page) encompasses several innovation targets that will enable the future bioeconomy. During feedstock and process development, CBI will address fundamental and enabling science questions using systems and synthetic biology and advanced computational approaches to improve process-advantaged plant traits, microbial biorefining, and robust catalysts.

Sustainable Process-Advantaged Bioenergy Crops. In the last few years, CBI has exploited genomics-based plant domestication to accelerate the identification of traits and plant-microbe interactions that improve sustainability and biomass yield (see image, Imaging Feedstock Root Growth, p. 16). Such characteristics improve year-round feedstock supplies and lower feedstock costs. A focus on native perennial plants, like poplar and switchgrass, provides immediate advantages in terms of sustainability, including reduced chemical inputs and

CBI Partners

- **Oak Ridge National Laboratory** (Lead Institution)
- **Brookhaven National Laboratory**
- **Dartmouth College**
- **Institut National de Recherche Agronomique** (Montpellier, France)
- **Massachusetts Institute of Technology**
- **National Renewable Energy Laboratory**
- **Poplar Innovations, Inc.**
- **The Pennsylvania State University**
- **University of California–Davis**
- **University of California–San Diego**
- **University of Georgia**
- **University of Maryland Eastern Shore**
- **University of Tennessee–Knoxville**
- **University of Virginia**
- **University of Wisconsin–Madison**
- **Washington State University**



Imaging Feedstock Root Growth. Larry York, a CBI plant physiologist, examines switchgrass root architecture prior to collecting a digital image that is later analyzed to extract and measure important root traits that impact plant biomass yield and sustainability. [Courtesy CBI]

improved soil conservation. CBI researchers are using genetic mapping and advanced plant phenotyping to study the vast natural diversity in these dedicated bioenergy crops and to uncover discrete genetic determinants for complex traits including cell wall chemistry, disease resistance, favorable microbial colonization, and drought tolerance. These genetic traits associated with favorable plant traits are further evaluated using genomic editing and artificial intelligence–based genomic selection to create process-advantaged, high-yielding, high-quality, and resilient perennial feedstocks. Sustainability will improve with the development of poplar and switchgrass plants with superior water use efficiency, nitrogen use efficiency, and pathogen resistance. Process-advantaged feedstocks will be developed with enhanced lignin and carbohydrate compositions to maximize biofuel and bioproduct yields.

C-CBP to Fermentation Intermediates. C-CBP eliminates the need for added enzymes and pretreatment, the two largest processing cost components in biofuel production. CBI scientists are focused on developing robust anaerobic microbial platforms that achieve one-step saccharification and fermentation at high rates, fermentation titers, and product yields (see image, Microbial Engineering, p. 17). This feedstock-to-fuels process generates fermentation intermediates for subsequent catalytic upgrading to SAF. CBI technoeconomic assessments estimate that process intensification associated with combining saccharification and fermentation will reduce capital and operating costs during technology scale-up.

Fundamental and broadly enabling science questions that arise during development of nonmodel CBP microbes are also being addressed. CBI researchers use adaptive laboratory evolution and CRISPR-based genomic editing tools to rapidly domesticate these microorganisms and to describe the foundational science of microbial biomass deconstruction processes that are applicable to a wide range of biofeedstocks and relevant to the new bioeconomy. C-CBP microbes have recently achieved near-total carbohydrate solubilization of poplar and switchgrass biomass. Industrially relevant C-CBP will be developed for the feedstock-to-fuels process by characterizing, designing, and optimizing metabolic performance to improve production of targeted intermediates.

Carbon-Efficient Lignin Conversion to Biofuels and Bioproducts. CBI is developing new processes and technologies to transform lignin-rich residues into biofuels and bioproducts, including chemical feedstocks such as propanol guaiacol and propanol catechol. Researchers use cutting-edge tools like nuclear magnetic resonance to study natural variation in plant cell wall biopolymers, like lignin, and identify target structural modifications that enhance lignin extraction for conversion to specialty biofuels including SAFs. Lignin is being selectively removed from plant cell walls using reductive catalytic fractionation (RCF), a process that solubilizes and partially depolymerizes lignin by targeting its carbon-oxygen bonds. CBI researchers aim to use the resulting lignin oils as upgradable intermediates for SAF.

In addition, researchers are employing biological funneling to produce chemical precursors for value-added biomaterials. In this process,

Microbial Engineering. CBI researcher Leah Burdick uses genomic editing tools to rapidly domesticate microorganisms for improved production of biofuels and bioproducts while also providing a roadmap for gene-to-trait discovery in nonmodel organisms. [Courtesy CBI]

microbial biocatalysts are designed to exhibit ligninolytic aromatic-catabolic activities, funnel heterogeneous aromatic monomers to central aromatic intermediates, and 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.

Catalytic Upgrading to Advanced Biofuels. In the last few years, CBI has developed a combination of biological and catalytic processes that have accelerated conversion of lignocellulosic biomass to biofuels. Researchers are using robust and selective catalysts to upgrade C-CBP fermentation products, such as ethanol, and RCF-derived lignin intermediates to produce high yields of branched alkanes for SAF. Scalable and selective catalyst development will enable economically viable, integrated processes for 100% biobased SAF and bioproducts for a carbon-negative bioeconomy.

Industry Partnerships

CBI seeks to form important industrial relationships, disseminate research results, gain feedback on industrial bottlenecks and concerns, and generate information about commercial opportunities including collaborations. Significant interactions have occurred between CBI and various companies including DSM, Forage Genetics International, Gevo, Commercial Aviation Alternative Fuels Initiative, Chevron, LanzaJet, and Phenotype Screening Corporation. For example, together with DOE's Joint Genome Institute and LanzaTech, CBI released the genome sequences of more than 200 industrial Clostridia species.

Intellectual property (IP) is disclosed to CBI's Commercialization Council, which consists of technology transfer representatives from each CBI partner institution. The council reviews the technical merit and commercial potential of CBI-funded inventions and shares licensing leads. Each owner institution protects its CBI inventions according to that institution's standard practices and coordinates any joint IP. CBI and the other BRCs also jointly market industrial interactions and key meetings. In fiscal year 2021, CBI partners produced 21 invention disclosures, 14 patent applications, and 4 issued patents.

Education, Outreach, and DEIA

CBI offers interdisciplinary research opportunities for graduate students, postdoctoral researchers, and visiting scientists and seeks to broaden public understanding of bioenergy and the pipeline of future bioeconomy workers. For example, the "Farming for Fuels" hands-on and distance-learning programs with hubs at science centers in 14 states, built in collaboration with the Creative Discovery Museum in Chattanooga, Tenn., have reached more than 330,000 students, parents, and teachers in 13 years. The programs incorporate Next Generation Science Standards (K-12 science content standards) and are available at learnbioenergy.org. In 2021, lessons were downloaded by approximately 20,000 users. The ongoing program is self-sustaining with direct costs only in training, distance learning, and curricula development.

In addition, CBI collaborated with various partners to establish a diversity, equity, inclusion, and accessibility (DEIA) task force. The task force produced a comprehensive DEIA plan consisting of actionable items to improve CBI's diversity, incorporate training opportunities and enable career advancement, make meaningful impacts in the community through outreach, and ultimately enable and support CBI's scientific endeavors. It also developed, distributed, and analyzed baseline results from a demographic and cultural climate survey with input from several CBI partner institutions to identify specific objectives and measure CBI's DEIA efforts and perception for the next 5 years. CBI has added the historically black University of Maryland Eastern Shore as a research partner in feedstock sustainability.

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 provide climate benefits without diverting land from food production and will create new economic opportunities for biorefineries, farmers, and rural communities that have been traditionally underserved by the current fuels and chemicals industry. To fulfill this vision, the center is addressing key knowledge gaps that currently limit the industrial-scale production of specialty biofuels and bioproducts from such purpose-grown energy crops.

Research Focus Areas

The technoeconomic success of lignocellulosic biorefineries hinges on maximizing conversion of biomass into a profitable mix of biofuels and bioproducts. GLBRC's research teams span multiple scientific domains to develop innovative solutions within three crosscutting research themes: sustainable bioenergy cropping systems, sustainable biomass conversion, and sustainable field-to-product optimization.

Sustainable Bioenergy Cropping Systems. GLBRC is improving systems for growing dedicated energy crops on lands not currently used for agricultural purposes. This approach of using nonagricultural land for nonfood crops, such as poplar, switchgrass, energy sorghum, and mixed perennial species, reserves arable U.S. farmland for food production and simultaneously provides potential environmental benefits including climate change mitigation and increased biodiversity. The center's goals are to generate a diversified portfolio of economically valuable bioenergy crops suitable for growth on bioenergy lands that also confer environmental benefits to enable sustainable conversion of crops to specialty biofuels and bioproducts (see image, Bioenergy

GLBRC Partners

- **University of Wisconsin–Madison** (Lead Institution)
- **Michigan State University**
- **Michigan Technological University**
- **Princeton University**
- **Texas A&M University**
- **University of British Columbia**

Bioenergy Crop Research. Federica Brandizzi (right), a Michigan State University (MSU) Foundation professor and GLBRC science director, and Sang-Jin Kim, an MSU research assistant professor and GLBRC researcher, examine research crops. [Courtesy MSU]

Crop Research, this page). To achieve these goals, GLBRC research teams are:

- Engineering plants with modified lignin and highly digestible polysaccharides to improve biomass digestibility and conversion into specialty biofuels and bioproducts.
- Identifying and developing plant and microbiome traits that improve energy crop productivity and tolerance to environmental stress.
- Investigating micro- and landscape-scale controls on soil carbon sequestration, nitrous oxide emissions, and nitrogen fixation in energy cropping systems.

Sustainable 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 generate the knowledge and microbial strains needed to sustainably convert as high a proportion of a plant's biomass as economically feasible into biofuels and bioproducts (see image, Improved Biomass Conversion, p. 21). To achieve these goals, GLBRC research teams are:

- Identifying and overcoming metabolic burdens and lignocellulosic hydrolysate stresses that pose barriers to efficient production of the specialty biofuel isobutanol by industrially accepted microbes.
- Generating and implementing new designs for industry-ready platform microbes capable of producing optimal bioproducts and commodity chemicals from lignin and carbohydrate fractions not used for biofuel production.
- Generating and applying new high-throughput methods or approaches enabled by artificial intelligence and machine learning for protein, microbe, and plant biodesign.

Sustainable Field-to-Product Optimization. The path from farm field to bioproducts consists of several interdependent phases, including crop production, biomass deconstruction, and conversion into targeted bioproducts valuable to industry. GLBRC's multidisciplinary research teams will define and mitigate the impacts of feedstock and landscape variability on biomass conversion to biofuels and bioproducts and model sustainable operations of integrated lignocellulosic biorefineries (see figure, Research Integration, this page). To achieve these goals, GLBRC research teams are:

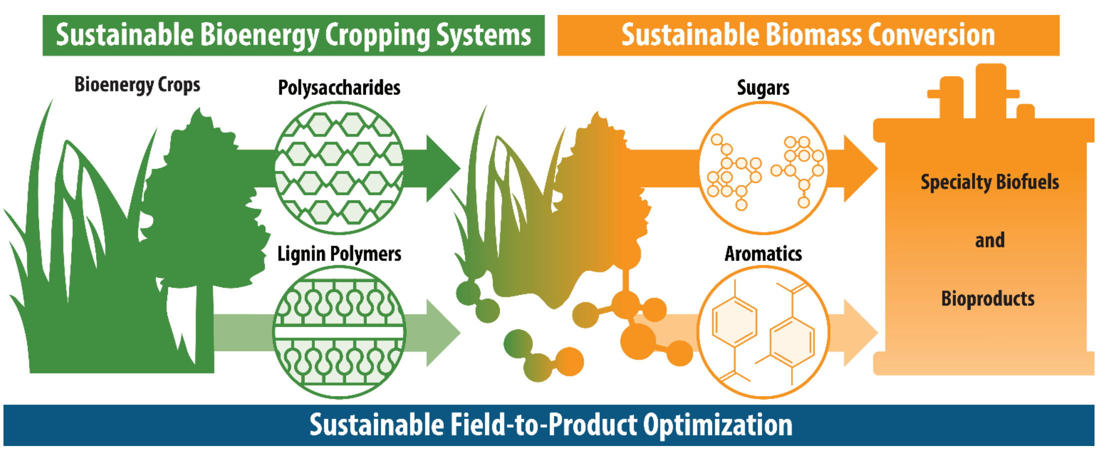
- Improving methods for feedstock-agnostic biomass deconstruction and separation by depolymerization and extraction of lignin-derived aromatics before cellulose and hemicellulose deconstruction.
- Understanding how the performance of conversion microbes is affected by seasonal or environmental changes in energy crops and different deconstruction methods.
- Creating novel and robust plant, landscape, and biorefinery models to predict the sustainability, life cycle, and economic outcomes of optimal field-to-product pipelines.

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. Industry representatives on GLBRC's scientific advisory board provide valuable perspective and guidance on research directions.

Improved Biomass Conversion. GLBRC researcher Trey Sato monitors yeast cultures in his lab at the University of Wisconsin–Madison. Sato and colleagues have engineered yeast to feast on a previously unpalatable sugar, potentially improving the microorganism's ability to convert sugars to specialty biofuels. [Courtesy GLBRC]

GLBRC intellectual property (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 to GLBRC inventions and copyrights to drive commercialization and create new economic opportunities for biorefineries, farmers, and rural communities.



Research Integration. The Great Lakes Bioenergy Research Center is developing economically viable and environmentally sustainable biofuels and bioproducts. [Courtesy GLBRC]

Community Outreach. Allison Bender, a GLBRC outreach coordinator, helps a child explore a memory game with tiles featuring information about petroleum- versus plant-based products at a Juneteenth celebration in Madison, Wis. [Courtesy UW–Madison]

Additionally, GLBRC works closely with a scientific advisory board comprised of leaders from industry, academia, and other groups engaged in BRC-relevant research. Board members provide objective strategic and technical advice on how to align GLBRC priorities with those of the BRC program and the evolving needs of the industry.

Education and Outreach

The mission of GLBRC's outreach team is to inform various audiences—including the general public, K-12 students, undergraduate students, and educators—about bioenergy research, energy concerns, and sustainability issues affecting the planet (see image, Community Outreach, this page). Engaging the community with fun, informative, hands-on activities, GLBRC's outreach efforts are designed to pique curiosity and promote discussion through a wide variety of in-person and virtual events. 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 program and sponsorship in the International Genetically Engineered Machine competition. These world-class interdisciplinary training programs are leveraged to reach a broad audience, especially students from historically underrepresented groups and institutions with limited research resources. These efforts are critical to preparing a diverse community of future scientists for the bioenergy industry.

Joint BioEnergy Institute

jbei.org

JBEI Overview

The Joint BioEnergy Institute (JBEI) 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 are not efficiently produced from petroleum.

Ultimately, JBEI aims to:

- Develop robust and sustainable bioenergy crops for different regions.
- Advance basic understanding of plant cell walls, environmental interactions, biomass recalcitrance, and microbial physiology and metabolism.
- Establish predictive biosystems design tools for plants, microbes, and enzymes.
- Develop technologies for feedstock-agnostic biomass deconstruction that liberate high yields of sugars and lignin-derived intermediates suitable for bioconversion to biofuels and bioproducts.
- Enable production of drop-in biofuels and novel bioproducts.
- Promote an inclusive bioenergy enterprise based on diversity and equity.

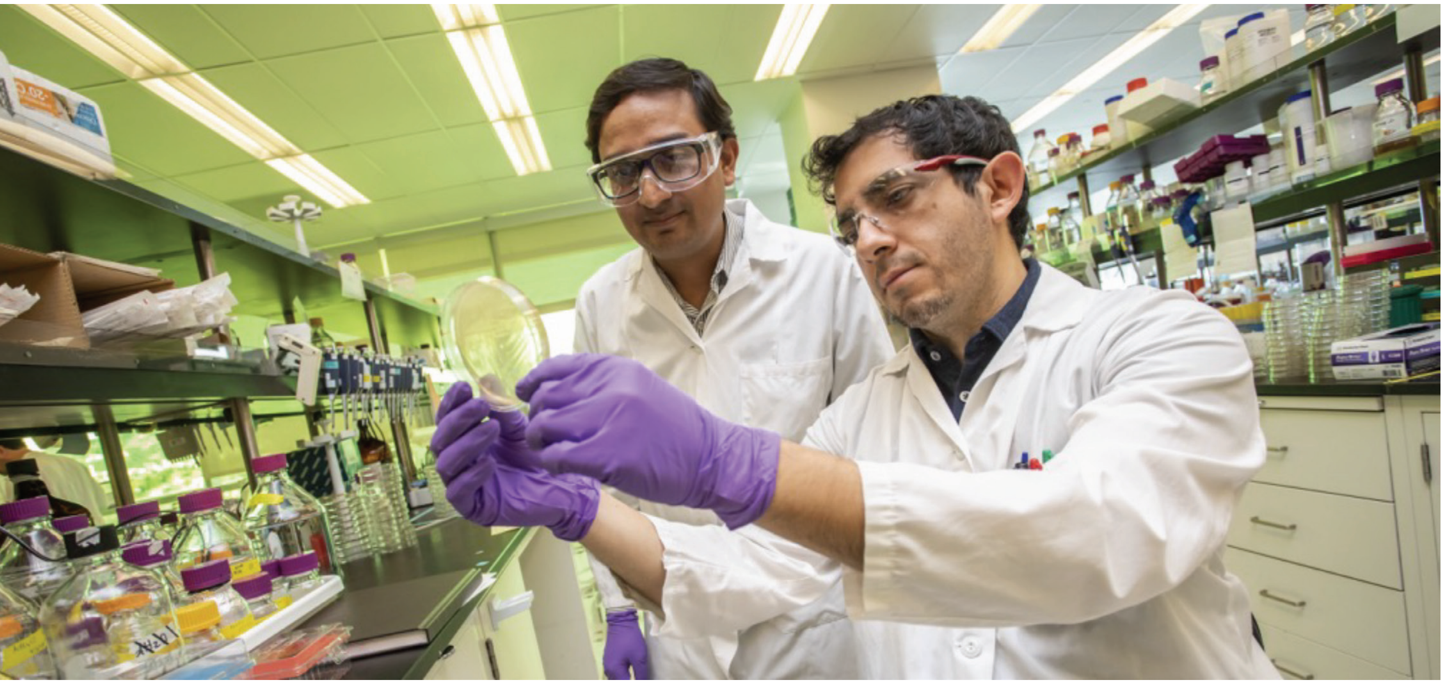
Research Focus Areas

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 affordable and sustainable replacements for petroleum-derived gasoline, diesel, jet fuel, and bioproducts. Additionally, JBEI is developing new approaches, technologies, and software to increase sample throughput, decrease reagent use via microfluidics, increase measurement fidelity, and reduce assay time in biofuels research. These analytical technologies provide the high-quality data needed to feed machine-learning algorithms that can systematically direct metabolic engineering processes.

Sustainability. JBEI seeks to ensure that new bioenergy crops and conversion processes are low-cost, low-input, scalable, and resilient

JBEI Partners

- **Lawrence Berkeley National Laboratory** (Lead Institution)
- **Brookhaven National Laboratory**
- **Georgia Institute of Technology**
- **Iowa State University**
- **Lawrence Livermore National Laboratory**
- **Northwestern University**
- **Pacific Northwest National Laboratory**
- **Sandia National Laboratories**
- **The University of Adelaide** (Australia)
- **University of California–Berkeley**
- **University of California–Davis**
- **University of California–San Diego**
- **University of California–Santa Barbara**



Designing Sustainable Aviation Fuels. Biojet fuel production can be scaled up *in silico* using technoeconomic and life-cycle assessment models to identify key engineering and scientific challenges that must be overcome. Research by Nawa Baral (left) and Daniel Mendez-Perez (right) and others at JBEI has shown that energy-dense molecules improve biojet fuel efficiency and product competitiveness and extend aircraft range. [© The Regents of the University of California, Lawrence Berkeley National Laboratory]

(see image, Designing Sustainable Aviation Fuels, this page). By combining systems-level economic and environmental models with experimental results, JBEI develops performance goals and prioritizations for biofuels and bioproducts produced from sugars, lignin-derived intermediates, and *in planta* intermediates. Techno-economic and life-cycle assessment models integrated with high-throughput experimental pipelines enable rapid iteration and improvement of production processes. Multisite field tests of bioenergy crops, along with agroecosystem models, provide insights into plant-microbe interactions, crop yields, and emissions impacts across a variety of soil and climate conditions.

Feedstock Development. JBEI engineers field test sorghum bioenergy crops, with some additional research on poplar, to validate high biomass yield and low susceptibility to disease and drought (see image, Developing Better Plants for Biofuels, p. 25). To support engineering efforts, JBEI is developing a suite of plant biosystem design tools and improved methods for sorghum transformation. JBEI is also improving the fundamental understanding of plant biology, with a focus on biomass composition, cell wall structure, and plant-microbe interactions. Bioenergy crops developed by JBEI are tailored for facile biomass deconstruction into sugars and lignin-derived intermediates and subsequent microbial conversion into biofuels and bioproducts. Bioenergy crops are also engineered to produce bioproducts, such as precursors of plastic polymers, directly in plant biomass.

Deconstruction and Separation. JBEI is developing an integrated, feedstock-agnostic biomass deconstruction process that pretreats biomass with renewable and biocompatible ionic liquids and deep eutectic solvents, as well as optimized enzyme mixtures, which then depolymerize plant polysaccharides and lignins into bioavailable intermediates, such as glucose, xylose, and p-coumarate. This integrated "one-pot" deconstruction process liberates high yields (as much as 90% or more) of the sugars and lignin-derived intermediates contained in bioenergy crops. JBEI's sorghum and poplar lines are suitable for conversion into biofuels

Developing Better Plants for Biofuels. Building a successful lignocellulosic biofuels industry depends, in part, on developing specialized, high-yielding biofuel crops that incorporate traits for optimized deconstruction, conversion, and sustainability. Pictured here, the bioenergy crop sorghum is being grown at the University of California–Davis, a JBEI partner. [© The Regents of the University of California, Lawrence Berkeley National Laboratory]

and bioproducts using these technologies and feeding the intermediates to JBEI-engineered microbes. The work includes exploration of ecosystems and microbial communities that naturally produce deconstruction enzymes targeted for optimization (see image, Engineered Cellulases, this page). In addition, JBEI researchers are developing predictive biomass deconstruction tools that will enable efficient design of affordable and scalable deconstruction processes.

Conversion. JBEI is engineering microbes, such as *Pseudomonas putida* and *Rhodosporidium toruloides*, that fully metabolize the sugars and aromatics generated during the deconstruction process to produce a variety of targeted biofuels and bioproducts at industrially relevant titers, rates, and yields (see image, An Advanced Biofuel, p. 26). These are products that otherwise would be made from petroleum using traditional chemistry. JBEI has developed advanced tools to design new biological routes and engineer microbial metabolic pathways and genomes guided by genome-scale models, functional genomics, and data-driven approaches.

Engineered Cellulases. Cellulases are important components of enzyme cocktails used to convert biomass to sugars. JBEI research facilitated the engineering of a GH family 9 endoglucanase J30, shown here as a model based on X-ray crystallography, to improve its stability at high temperatures. [Courtesy JBEI]

To produce drop-in biofuels and bioproducts that directly replace existing products, as well as novel bioproducts with differentiated properties, JBEI has developed a versatile repertoire of biological conversion routes to

An Advanced Biofuel. Scientists created a fuel type called POP-FAMEs (polycyclopropanated fatty acid methyl esters) using engineered polyketide synthases expressed in bacteria. The new fuel could eventually power jet planes and rockets. [© The Regents of the University of California, Lawrence Berkeley National Laboratory]

major biosynthesis products such as terpenes, polyketides, and fatty acids. A prioritized JBEI biofuel target is isoprenol (3-methyl-3-butene-1-ol). This valuable gasoline blendstock is convertible to the sustainable aviation fuel dimethylcyclooctane (DMCO) and is an important precursor of several commodity chemicals such as isoprene. JBEI has also pioneered the development of novel advanced biofuels such as fuelimycin A, a high-energy-density compound and potential rocket fuel derived from polyketide synthase.

Industry Partnerships

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

Lawrence Berkeley National Laboratory 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 goal of JBEI's education and outreach program is to share JBEI's research efforts and successes with the public and the bioenergy research sector, advocate for biofuels and bioproducts research, and highlight the benefits of publicly funded basic science research. The program also serves as an educational resource for next-generation work forces in science, technology, engineering, mathematics (STEM); biomanufacturing; and clean energy. JBEI is committed to creating a culture of inclusion that focuses on equity and valuing diversity in the workplace and surrounding community. This approach is key to attracting and engaging the brightest minds and advancing scientific excellence and groundbreaking innovations.

JBEI's current efforts include outreach and training opportunities for the future bioeconomy workforce, including educational programs and internships for K-12, undergraduate, and graduate students. JBEI's key initiative, the Introductory College Level Experience in Microbiology (iCLEM) program, provides under-resourced San Francisco Bay Area high school students with paid summer internships conducting scientific research. The iCLEM program is nationally recognized, with nearly all alumni attending college and more than 80% majoring in STEM. Each year, iCLEM also extends its reach by hosting teachers and teachers-in-training who incorporate iCLEM lessons into their curricula and develop new curriculum modules.

BRC RESEARCH **ACCOMPLISHMENTS**

Over the past 5 years, BRC research has generated multiple breakthroughs in advancing the fundamental science underlying commercial production of biofuels and bioproducts. The following sections highlight featured accomplishments from each BRC in sustainability, plant feedstock development, biomass deconstruction and separation, and conversion, followed by brief summaries of additional achievements in these research areas.

Sustainability p. 28

Feedstock Development p. 32

Deconstruction and Separation p. 36

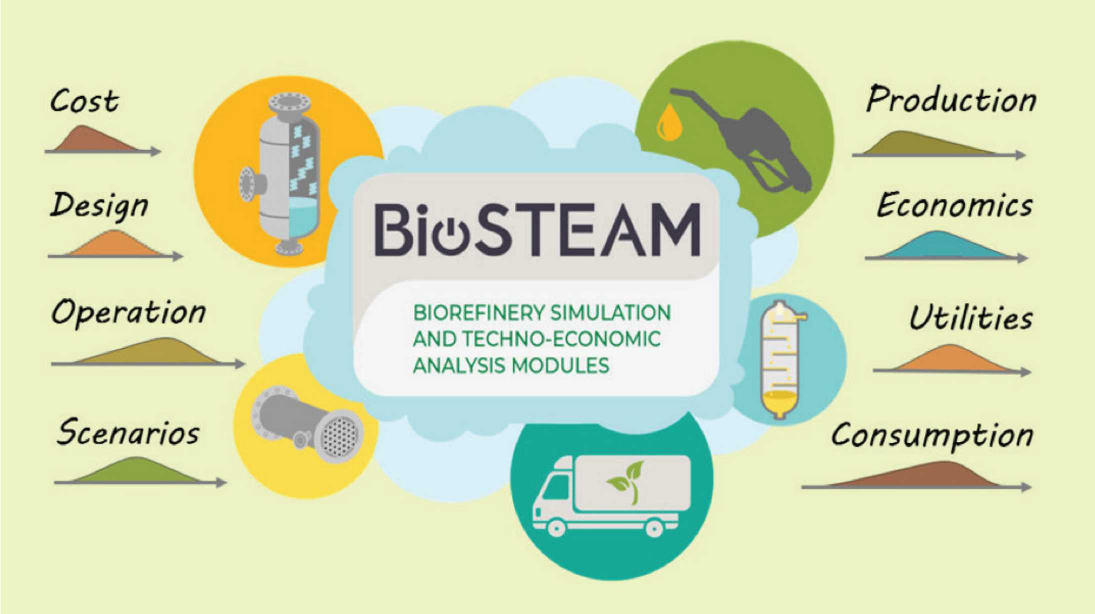
Conversion p. 40

Software Analyzes Technoeconomics of Biorefineries

Perennial grasses can be converted into everything from ethanol to bioplastics, but which bioproducts hold the greatest potential is unclear. CABBI researchers developed BioSTEAM, an open-source simulation software package in the Python programming language that gives scientists, engineers, biotechnology companies, and funding agencies a fast, flexible tool to analyze the economics of producing different biofuels and bioproducts (Cortés-Peña et al. 2020b). Using algorithms to automate biorefinery design, BioSTEAM, which stands for Biorefinery Simulation and Techno-Economic Analysis Modules, enables researchers to compare and prioritize strategies for converting plant biomass to fuels and products in a matter of seconds. BioSTEAM–Life Cycle Assessment provides additional capabilities to characterize the environmental impact of biorefineries with different designs, technology performance assumptions, contexts, and uncertainties (Shi et al. 2020). Overall, the suite offers an efficient, agile way to evaluate biorefinery processes; the production of potential biofuels and bioproducts; and trade-offs among productivity, economics, and environmental impacts. BioSTEAM is accessible to anyone wanting to design and simulate a new biorefinery, and the code is publicly available so users can change scenarios and explore the data themselves. The goal is to make technoeconomic assessment capabilities more available to bioenergy researchers in academia, government, and private industry to help identify the most promising ideas or technologies to pursue. BioSTEAM has identified critical parameters governing the industrial feasibility of producing biodiesel and ethanol from oilcane and other feedstocks as well as the conversion of broader sources of plant biomass into lactic acid (Li, Y., et al. 2021), linear alpha olefins (McClelland et al. 2021), and other vital components of valuable bioproducts.

Related Publications

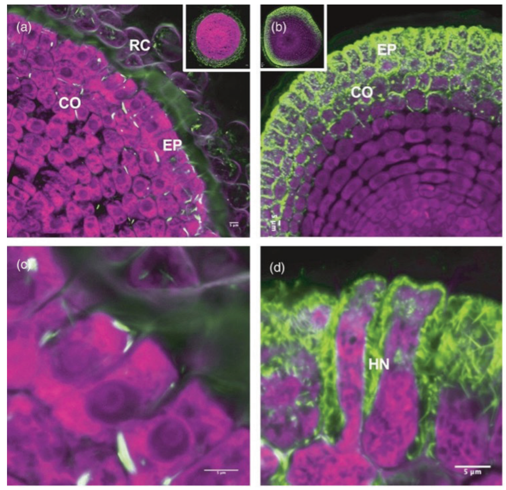
Bhagwat et al. 2021; Cortés-Peña et al. 2020a, b; Li, Y., et al. 2021; McClelland et al. 2021; Shi et al. 2020



A schematic for BioSTEAM, an open-source simulation software package for biorefinery design. [Reprinted with permission from Cortés-Peña, Y., et al. 2020. "BioSTEAM: A Fast and Flexible Platform for the Design, Simulation, and Techno-Economic Analysis of Biorefineries Under Uncertainty," *ACS Sustainable Chemistry & Engineering* **8**(8), 3302–10. © 2020 American Chemical Society (ACS); permission related to the use of this material should be directed to ACS.]

Single Gene Enables Plant-Fungi Symbiosis

Microorganisms are critical drivers of biological processes, such as nutrient acquisition, that contribute significantly to plant sustainability and productivity. However, the mechanisms that shape plant-microbe interactions are complex and difficult to discern because they can be regulated by genetics and the environment. To confront this scientific challenge, CBI researchers use a powerful genetic mapping approach that screens over 1,000 individual poplar trees to identify the genetic basis for traits like beneficial plant-microbe interactions. This technique was used to discover that a single gene, called *PtLecRLK1*, regulates symbiosis between poplar roots and the beneficial fungal organism *Laccaria bicolor* (Labbé et al. 2019). CBI scientists have shown that *PtLecRLK1* can be inserted into other nonhost plants, such as *Arabidopsis* and switchgrass, to add this symbiosis (Qiao et al. 2021). The ability to engineer advantageous plant-microbe interactions could alleviate challenges related to plants growing in unfavorable environments or conditions, such as drought. For example, CBI researchers recently demonstrated that poplar trees more tolerant to water deficiencies recruit drought-tolerant microbes that could be exploited to enhance plant yield and biomass quality in hotter, drier environments (Kristy et al. 2022).



At left, wild-type switchgrass root cells (purple) are nonmycorrhizal and not effectively colonized by *Laccaria bicolor* (green). However, after transformation with the poplar gene *PtLecRLK1*, the fungus penetrates the root to form a symbiotic relationship (right). [Reprinted under a CC BY-NC-ND 4.0 license from Qiao, Z., et al. 2021. "Towards Engineering Ectomycorrhization into Switchgrass Bioenergy Crops via a Lectin Receptor-Like Kinase," *Plant Biotechnology Journal* **19**(12), 2454–68. DOI:10.1111/pbi.13671. © 2021 John Wiley & Sons Ltd.]

Related Publications

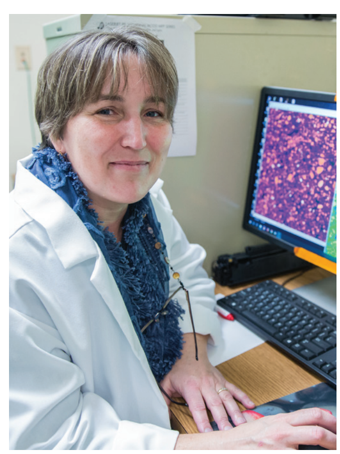
Kristy et al. 2022; Labbé et al. 2019; Qiao et al. 2021

Bioenergy Crop Pairings Promote Soil Carbon Storage

Plants pull carbon dioxide from the air and deposit it as organic compounds into the soil. Diverse plant communities do this most effectively, keeping agricultural lands healthy and productive while reducing contributions to climate change. Now, research at GLBRC has revealed that root interactions among Midwest prairie perennials, including the bioenergy crop switchgrass, increase soil carbon when grown in specific pairings. Beneficial plant combinations create large soil pores that support fungi associated with soil carbon accrual. These results inform sustainable agriculture practices on bioenergy lands.

Related Publication

Kravchenko et al. 2021



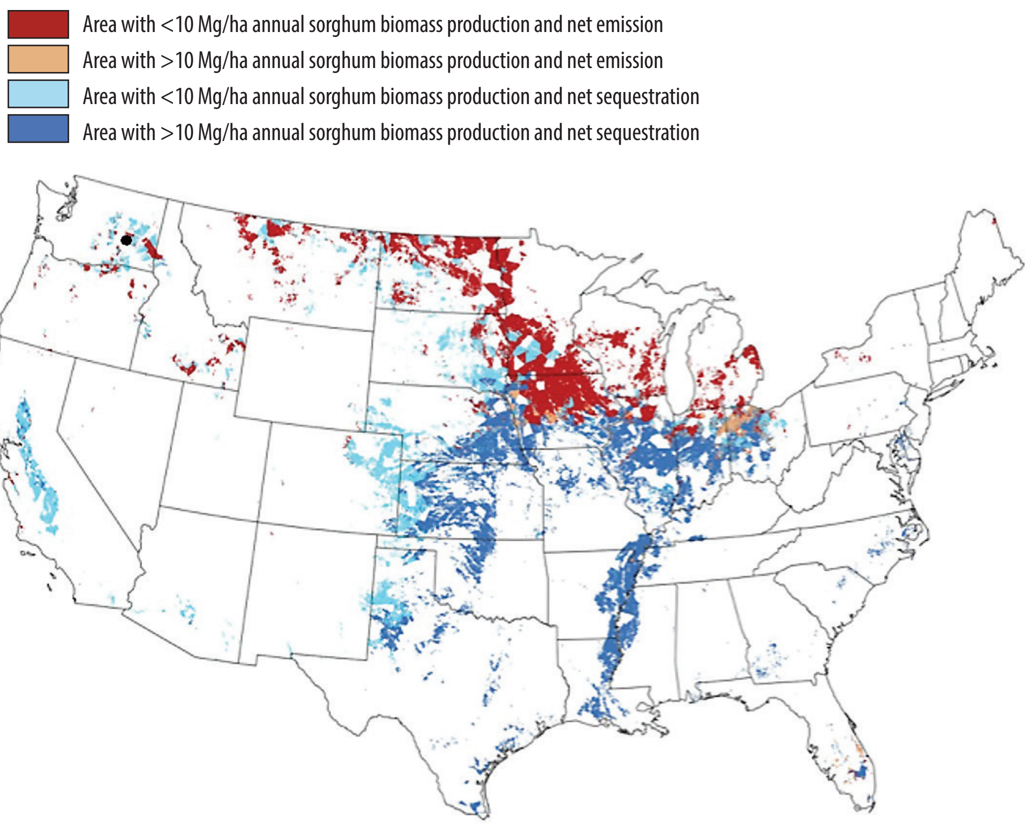
Sasha Kravchenko, GLBRC researcher and professor of plant, soil, and microbial sciences at Michigan State University, examines data from ongoing research on soil carbon and nitrogen cycling. [Courtesy GLBRC]

Sorghum Shows Potential to Mitigate Soil Carbon Loss

Climate change is accelerating soil carbon loss in certain U.S. regions, but deep-rooted sorghum may slow the trend. National-scale projections of bioenergy crop yields and their environmental impacts are essential to identifying appropriate planting locations and ensuring sustainable land-use strategies. JBEI researchers used the process-based Daily Century (DayCENT) biogeochemical model with site-specific environmental data to simulate sorghum (*Sorghum bicolor* L. Moench) biomass yield, soil organic carbon change, and nitrous oxide emissions across cultivated lands in the continental United States. Simulated rainfed dry biomass productivity ranged from 0.8 to 19.2 megagrams per hectare per year. In subsequent work, the team used machine learning to identify regions prone to soil carbon loss due to climate change and regions with the potential to mitigate those losses, such as the South Central and Southeastern United States.

Related Publications

Gautam, S., et al. 2020, 2022; Huntington et al. 2020; Mishra et al. 2021



U.S. regions with high sorghum yields and carbon sequestration potential. [Reprinted under a CC BY 4.0 license from Gautam et al. 2020. "Sorghum Biomass Production in the Continental United States and its Potential Impacts on Soil Organic Carbon and Nitrous Oxide Emissions," *GCB Bioenergy* **12**(10), 878–890. DOI: 10.1111/gcbb.12736. © 2021 John Wiley & Sons Ltd.]

ADDITIONAL ACCOMPLISHMENTS IN **SUSTAINABILITY**

Improved Bioenergy Crop Models Can Inform Policy Decisions

Expertise from both agronomists and economists is key to building sustainable federal biofuel policies that consider impacts on the environment, food production, consumers, and the broader economy. CABBI researchers integrated ecosystem and economic modeling tools, including Agro-IBIS, BEPAM, and DayCENT, to assess the locations, crop mixes, land types, and extent of land converted to bioenergy crops necessary to achieve policy goals for greenhouse gas emissions, water quality, and bioenergy and bioproduct production in a cost-effective manner.

Chen et al. 2021a, b; Ferin et al. 2021

New Approach Identifies Land Suitable for Bioenergy Crops

CABBI researchers used historical high-resolution satellite data to track land-use changes and identify economically marginal cropland suitable for conversion to bioenergy crops at low cost and without diverting resources from food production. The approach also expands the definition of marginal land to incorporate the environmental impacts of land use, providing a new social marginality framework that enables researchers to better pinpoint croplands that are economically beneficial but socially marginal.

Jiang et al. 2021; Khanna et al. 2021

Atlas Informs Sustainable Biofuel and Product Development

As climate change threatens global crop productivity and, consequently, food and fuel security, it is imperative to identify lands suitable for growing biomass crops designed for biofuels or bioproducts that do not compete with food crops. Based on abandoned cropland data from the U.S. Department of Agriculture, researchers developed the GLBRC Atlas of U.S. Bioenergy Lands, which informs ecosystem ensemble modeling, optimization of refinery depot locations, and biodiversity modeling for sustainably producing biofuels and bioproducts.

GLBRC Atlas of U.S. Bioenergy Lands, atlas.glbrc.org

Microbial Communities Maximize Bioenergy Crop Yields

Sustainable bioenergy agriculture relies on extensive understanding of complex switchgrass-microbiome relationships that influence crop growth, immunity, and stress responses. GLBRC researchers characterized the diversity, dynamics, and host-specificity of switchgrass microbial communities above- and belowground to establish functional correlations between switchgrass genotypes, phenology, and growth conditions and microbial impacts on plant development.

Dirks and Jackson 2020; Grady et al. 2019; Lovell et al. 2021; Smercina et al. 2021a, b; Ulbrich et al. 2021; VanWallendael et al. 2020

In Planta Bioproducts Improve Biorefinery Economics

Bioenergy crops can be engineered to accumulate a range of high-value bioproducts *in planta* that can be fractionated and recovered for sale from biorefineries, thereby improving the cost-competitiveness of biofuels. JBEI research showed that accumulating bioproducts in bioenergy crops at levels as low as less than 1% can improve biorefinery economics across a wide range of commodity prices and that *in planta* accumulation can be competitive with microbial production routes.

Yang, M., et al. 2020, 2022

Path Charted to Low-Cost, Low-Carbon Biojet Fuels

Decarbonizing the air transportation sector remains a hurdle to mitigating climate change, but lignocellulosic biomass–derived jet fuel blendstocks can potentially outperform petroleum jet fuel and achieve cost-competitive carbon mitigation. JBEI scientists identified several viable chemical conversion pathways to improving aircraft fuel efficiency and achieving greenhouse gas emission mitigation costs of less than $100 per tonne of carbon dioxide equivalents for a range of biojet fuel molecules, including dimethylcyclooctane.

Baral et al. 2019, 2021; lead.jbei.org

Genomic Toolkit Enables Miscanthus *Optimization*

CABBI collaborated with researchers at the DOE Joint Genome Institute and other institutions in the United States, Europe, and Asia to sequence and analyze the first *Miscanthus* genome, *M. sinensis*, to provide scientists with a roadmap to systematically optimize desirable traits (Mitros et al. 2020). *Miscanthus* is a highly productive and sustainable perennial biomass crop better suited to temperate environments than its close relative, sugarcane. It is extremely adaptable, is easy to grow on marginal lands, requires limited fertilization, is highly tolerant to drought and cool temperatures, and uses the more efficient C4 form of photosynthesis. The effort produced an atlas of when and in which tissues genes are expressed in *M. sinensis* during its seasonal life cycle. The atlas reveals, for example, new regulators of perenniality, a desirable trait for biofuel and other crops, and insights into genetic modifications that improve nutrient remobilization and reduce fertilizer inputs. CABBI researchers also developed the first gene-editing procedures for *Miscanthus* using CRISPR/Cas9, which enables the mutation of specific endogenous genes to knock out function (Trieu et al. 2022 preprint). The ability to target specific genetic loci represents a groundbreaking new capability for improvement of this important biomass crop with a specificity and speed unattainable with previous technologies.

Mohammad Belaffif, a CABBI postdoc at the HudsonAlpha Institute for Biotechnology in Huntsville, Ala., walks in a *Miscanthus* field. [Courtesy CABBI and HudsonAlpha]

Related Publications

Clark et al. 2019; Dong et al. 2019; Mitros et al. 2020; Trieu et al. 2022

Engineered Poplar More Easily Converted to Bioproducts

A key obstacle to extracting sugars from plant biomass is the presence of the complex polymer lignin, a major component of plant cell walls that provides structural integrity and is most resistant to deconstruction. GLBRC researchers have mobilized the flexible nature of lignin formation in plants to produce a transgenic poplar tree that diverts carbon away from lignin and toward the synthesis of naringenin or p-hydroxybenzoate, which are platform chemicals and precursors to high-value products currently derived from petroleum. Without adversely affecting overall yield, the resulting poplar wood is easier to deconstruct and therefore produces higher sugar yields.

A poplar seedling emerges from a research plot at the Kellogg Biological Station in Hickory Corners, Mich. [Courtesy GLBRC]

Related Publications

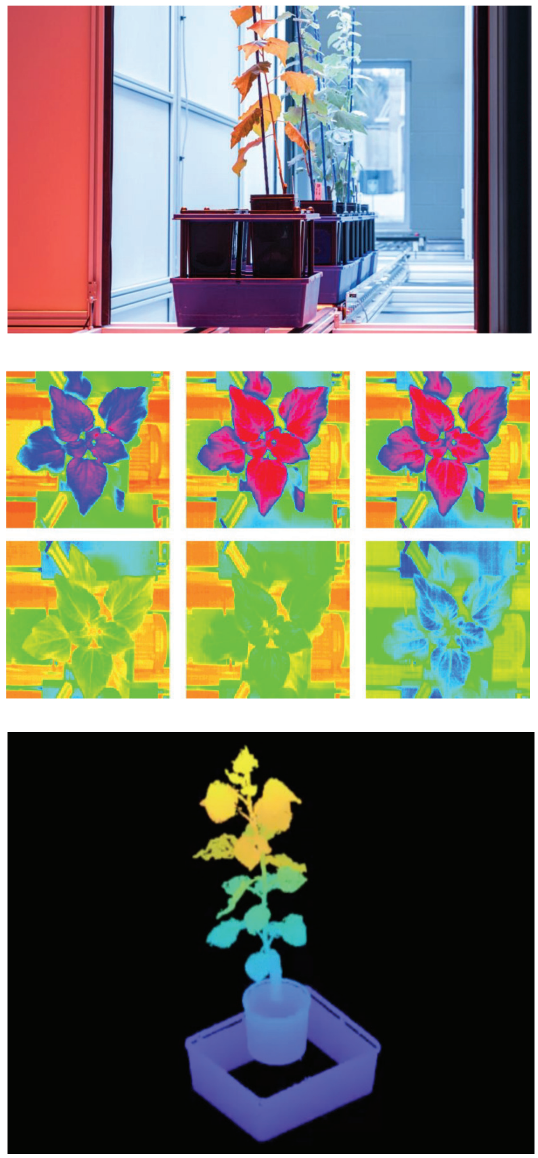
Mahon et al. 2022; Unda et al. 2022

AI Phenotyping Reveals Beneficial Bioenergy Crop Traits

Advanced imaging strategies are generating large datasets of favorable above- and belowground bioenergy crop traits. Creating sustainable process-advantaged bioenergy crops requires an extensive understanding of the traits most favorable for increasing biomass yield, composition, convertibility, and resilience. CBI researchers are advancing plant imaging strategies to identify morphological, physiological, and phenological traits that confer a fitness advantage in particular environments. Image capturing via unmanned aerial vehicles or Oak Ridge National Laboratory's Advanced Plant Phenotyping Laboratory enables CBI scientists to bring important leaf and root traits into focus and use explainable artificial intelligence (AI) to extract known and cryptic phenotypic traits. One automated AI-based phenotyping strategy can extract more than 100 traits from a leaf image within a single minute. Plant root system architecture is equally important to assess but is far more challenging to image due to its placement belowground. Deep rooting traits benefit plant productivity by increasing access to water during prolonged dry periods. CBI researchers recently developed a new semi-automated tool called RhizoVision Explorer to improve the accuracy and reliability of image-based analysis of root trait measurements (Seethepalli et al. 2021). This tool enabled CBI scientists to detail root architecture for switchgrass and show that greater root length and depth leads to improved nutrient acquisition (Griffiths et al. 2022).

Related Publications

Griffiths et al. 2022; Seethepalli et al. 2021



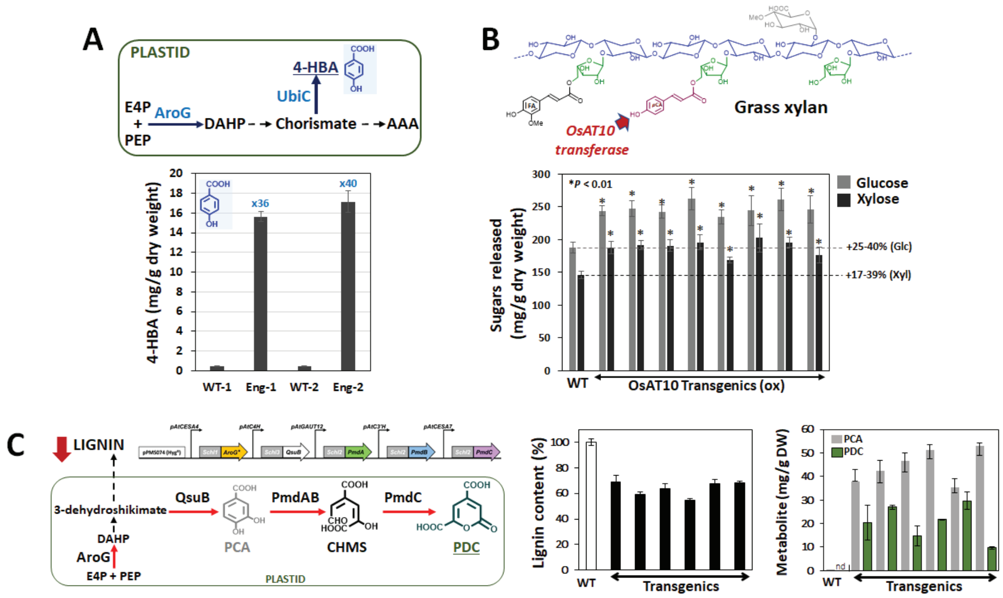
The Advanced Plant Phenotyping Laboratory will gather multiple plant phenotypes at high throughput generating terabytes of data. (Top) The poplar plants are grown on a conveyor belt in a greenhouse and sent for regular automated imaging. (Center) Hyper-spectral images indicate leaf water, nitrogen, and biochemistry. (Bottom) A computer reconstruction of the plant. [Courtesy CBI]

Sorghum Gains Improved Feedstock Characteristics

Sorghum plants were engineered by JBEI researchers to display reduced recalcitrance to conversion, accumulation of valuable bioproducts *in planta*, high yields, and drought tolerance. Reducing lignin recalcitrance and altering lignin composition demonstrate the key strategies of lignin valorization in which biofuels production costs are reduced by maximizing conversion of lignin to biofuels and bioproducts. Based on a tissue-specific engineering approach previously demonstrated in model plants (Liang et al. 2019) and switchgrass (Li, G., et al. 2018), researchers decreased lignin content in sorghum by expressing a rice transferase that participates in the synthesis of xylan-bound aromatics (Tian et al. 2021). Another strategy for increasing the value of bioenergy crops and improving the economics of advanced bioproducts is to accumulate co-products in the feedstock biomass (Yang, M., et al. 2020; Lin et al. 2020). JBEI researchers introduced multigene DNA constructs for biosynthetic pathways and bioproducts into sorghum and poplar and validated the engineered metabolic pathways for production of valuable platform chemicals like protocatechuate (Hao et al. 2021; Tian et al. 2022), muconate (Eudes et al. 2018), 2-pyrone-4,6-dicarboxylate (Lin et al. 2021), and 4-hydroxybenzoate (Lin et al. 2022; Wang, Y., et al. 2021a). An appealing approach to developing closed-loop biorefineries is the conversion of lignin-derived compounds into ionic liquids and deep eutectic solvents (Wang, Y., et al. 2021a; Kim, K. H., et al. 2019).

Related Publications

Eudes et al. 2018; Hao et al. 2021; Kim, K. H., et al. 2019; Li, G., et al. 2018; Liang et al. 2019; Lin et al. 2020, 2021, 2022; Tian et al. 2021; Wang, Y., et al. 2021a; Yang, M., et al. 2020



(A) An engineered metabolic pathway overproduces the platform chemical 4-hydroxybenzoate (4-HBA) in transgenic sorghum expressing *aroG* and *ubiC* genes from *Escherichia coli*. The engineered sorghum lines (Eng-1 and Eng-2) accumulated 36 and 40 times more 4-HBA (up to 1.7% of biomass on a dry weight basis) compared to their wild-type controls (WT-1 and WT-2). [AAA, aromatic amino acids; DAHP, 3-deoxy-D-arabinoheptulosonate 7-phosphate; E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate.] **(B)** Sorghum engineered with low recalcitrance was obtained by heterologous expression of the rice p-coumarate-xylan transferase protein OsAT10. The biomass of OsAT10 transgenics releases more simple sugars (glucose and xylose) than wild type upon pretreatment with ionic liquid followed by enzymatic saccharification. **(C)** The schematic represents metabolic engineering of *Arabidopsis* to accumulate the valuable co-product 2-pyrone-4,6-dicarboxylate (PDC) at the expense of lignin. [All figures reprinted under a CC BY 4.0 license. (A) Lin, C.-Y., et al. 2022. "Engineering Sorghum for Higher 4-Hydroxybenzoic Acid Content," *Metabolic Engineering Communications* **15** (e00207). © 2022 Elsevier Inc. (B) Tian, Y., et al. 2021. "Overexpression of the Rice BAHD Acyltransferase AT10 Increases Xylan-Bound p-Coumarate and Reduces Lignin in *Sorghum bicolor*," *Biotechnology for Biofuels* **14**, 217. © 2021 Springer Nature. (C) Lin, C.-Y., et al. 2021. "*In-planta* Production of the Biodegradable Polyester Precursor 2-Pyrone-4,6-Dicarboxylic Acid (PDC): Stacking Reduced Biomass Recalcitrance with Value-Added Co-Product," *Metabolic Engineering* **66**, 148–56. © 2021 Elsevier Inc.]

ADDITIONAL ACCOMPLISHMENTS IN **FEEDSTOCK DEVELOPMENT**

First Precision Breeding of Sugarcane with CRISPR/Cas9

CABBI studies successfully demonstrated, for the first time, the use of CRISPR/Cas9 for targeted gene editing of sugarcane, an important bioenergy crop with a highly complex genome that traditionally requires more than a decade to develop an improved cultivar. Such tools enable specific genetic improvements, such as reduced fertilizer requirements and high herbicide resistance, at orders of magnitude faster than conventional breeding methods.

Eid et al. 2021; Oz et al. 2021; Zhao et al. 2019

Improved Bioenergy Crops Hyperaccumulate Oils

Biosynthesis and accumulation of oils in the vegetative tissues of high-biomass crops can potentially reduce U.S. reliance on traditional oilseed crops for vegetable oil production. Researchers at CABBI successfully engineered lines of sugarcane and sorghum that hyperaccumulate triacylglycerol in vegetative tissues relative to wild-type controls, validating CABBI's "plants as factories" approach and creating a foundation for production of specialized high-value fatty acid bioproducts with superior performance as lubricants, sustainable aviation fuels, and polymers.

Parajuli et al. 2020; Park, K., et al. 2021

New Technique Links Lignin Diversity to Genetics

CBI researchers working to actively optimize reductive catalytic fractionation (RCF) for selective removal of lignin from plant cell walls for conversion to fuel-range aromatics discovered that RCF is also a powerful analytical method for determining the lignin phenotypes of feedstocks, including poplar and switchgrass. The high-throughput phenotyping has enabled identification of novel candidate genes that influence lignin traits, providing additional guidance for selecting genetic targets for *in planta* lignin engineering or breeding.

Anderson et al. 2019; Bartling et al. 2021; Yoo et al. 2018

Poplar Yield and Composition Impact Biofuel Economics

Biomass feedstock yield and quality are important considerations for selecting cost-effective perennial bioenergy crops. CBI researchers used feedstock cost models and technoeconomic assessment to determine that poplar tree size has the strongest impact on the economics of feedstock conversion to biofuels. Results show that additional economic gains can be achieved by selecting for cell wall composition as a measure of feedstock quality.

Happs et al. 2021

Genes Required for Root Symbiosis Identified

Bioenergy crop performance can be improved by enhancing root interactions with symbiotic bacteria and fungi. When modified by a specific glycan, glycosyl inositol phosphoryl ceramide (GIPC) in plant roots enables symbiosis with nitrogen-fixing rhizobia bacteria and arbuscular mycorrhizal fungi. JBEI researchers identified and characterized key genes required for glycosylation of GIPCs and identified critical roles for these lipids in mediating pathogenic and symbiotic plant-microbe interactions, regulation of plasmodesmata function, and cellulose biosynthesis.

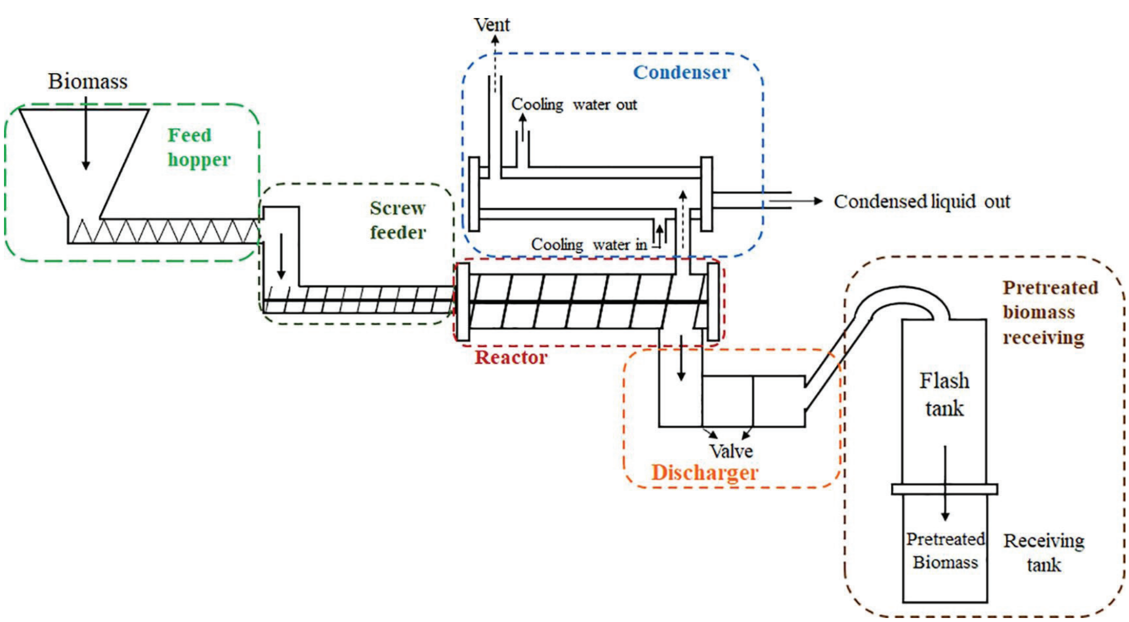
Cassim et al. 2021; Ebert et al. 2018; Ishikawa et al. 2018; Jing et al. 2021; Lenarčič et al. 2017; Moore, W. M., et al. 2021; Mortimer et al. 2020; Yan, D., et al. 2019

New Biomass Treatments Maximize Oil and Sugar Recovery

CABBI researchers are engineering bioenergy feedstocks using a "plants as factories" approach aimed at producing valuable molecules within the plants themselves. However, this makes bioenergy crops more challenging to process than traditional varieties. Conversion researchers have recently developed biomass treatment methods that cost-effectively recover oil and sugar streams from oil-rich sugarcane varieties and process them into useful chemicals. Technoeconomic assessment of deacetylation followed by hot water pretreatment and disk refining demonstrated that improved steam recovery and reduced enzyme costs can render production of cellulosic ethanol from sugarcane bagasse economically feasible (Cheng et al. 2019a). Another sugarcane bagasse study demonstrated that combining novel pretreatment steps with engineered yeast strains incrementally improved ethanol yields (Wang, Z., et al. 2019). Pretreating sorghum with chemical-free, continuous hydrothermal methods minimizes inhibitor formation and successfully scales up from laboratory bench to pilot plant, suggesting a promising industrial application (Cheng et al. 2019b). More recently, researchers optimized a chemical-free hydrothermal pretreatment of transgenic oilcane bagasse to maximize recovery of lipids for biodiesel production and fermentable sugars for ethanol production while limiting inhibitor generation (Maitra et al. 2022). Oilcane is metabolically engineered sugarcane designed to hyperaccumulate oils. The pretreatment prevented decomposition of *in situ* lipids during the recovery process and successfully demonstrated that nuclear magnetic resonance spectroscopy can rapidly quantify total lipids in cellulosic biomass, characterize *in situ* lipids into bound and free fractions, and determine the fatty acid composition of cellulosic biomass.

Related Publications

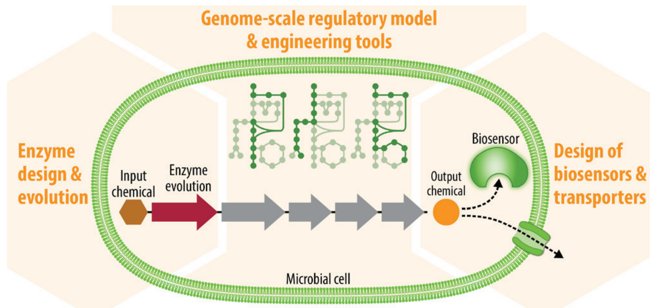
Cheng et al. 2019a, b; Maitra et al. 2022; Wang, Z., et al. 2019



Model for a continuous pretreatment reactor used in a CABBI study that combined pilot-scale continuous hydrothermal pretreatment with disk refining to maximize sugar production from bioenergy sorghum. [Reprinted from *Bioresource Technology*, **289**, Cheng, M.-H., et al., "Sugar Production from Bioenergy Sorghum by Using Pilot Scale Continuous Hydrothermal Pretreatment Combined with Disk Refining," 121663, ©2019, with permission from Elsevier.]

Computer-Aided Design Improves Bioenergy Enzymes

Efficient and robust enzymes, as catalysts for polysaccharide depolymerization, can greatly accelerate sugar production for microbial conversion to lignocellulosic biofuels and bioproducts. GLBRC has developed a new pipeline for bioenergy-relevant biodesign and enzyme engineering that combines computational protein design and biosensor-guided screening in microfluidic droplets for massively multiplexed assay of enzyme variants. This ultra-high-throughput screening method, combined with computational predictions of enzyme properties, enables GLBRC to produce the next generation of biocatalysts with dramatically improved properties for sustainable lignocellulosic biofuels and bioproducts production.



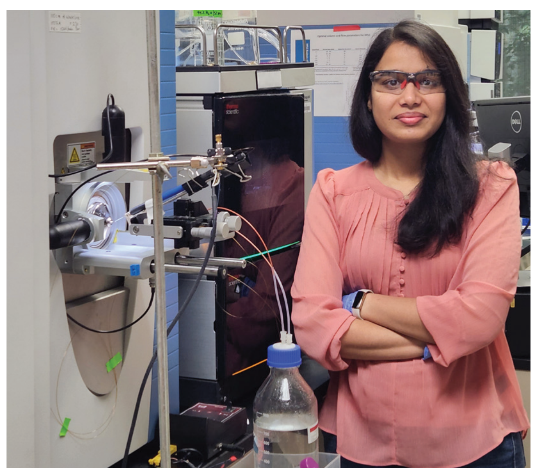
GLBRC's biodesign and enzyme engineering pipeline combines computational protein design and biosensor-guided ultra-high-throughput screening to enable researchers to produce the next generation of enzymes for biofuels and bioproducts. [Courtesy GLBRC]

Related Publications

Dolberg et al. 2021; Liu et al. 2019

Microbiome Overcomes High Solids Roadblock

Metaproteomics studies are revealing the enzymatic strategies deployed by anaerobic microbiomes to deconstruct lignocellulose at high solids concentrations. Economically viable production of cellulosic biofuels requires operation at high solids loadings, on the order of 15% by weight. However, *Clostridium thermocellum* performance, and its conversion of carbohydrates to target intermediates for catalytic upgrading, stalls at high solids loadings. CBI researchers used metaproteomics to discover that anaerobic biomass-degrading microbiomes maintain biomass deconstruction even at increasing switchgrass biomass loadings (Chirania et al. 2022). The enhanced performance can likely be explained by an increased abundance of specific carbohydrate-active enzyme classes in the microbiome accompanied by notable stress-response proteins at higher solids, all of which indicate that removal of deconstruction inhibitors is important for observed undiminished solubilization. For one of these strategies, CBI researchers showed that *C. thermocellum* is unable to effectively degrade the Rhamnogalacturonan (RG-I) component of pectin in poplar biomass (Biswal et al. 2022). The accumulation of RG-1 may slow solubilization of other polymers as well. This makes RG-I a potential target for improving the enzymatic ability of *C. thermocellum* to effectively solubilize woody biomass.



Payal Chirania and other CBI researchers use mass spectrometry as a cutting-edge tool to characterize microbiomes and their functional roles in biomass deconstruction. [Courtesy CBI]

Related Publications

Biswal et al. 2022; Chirania et al. 2022

Successful Scale-Up of One-Pot Biomass Conversion

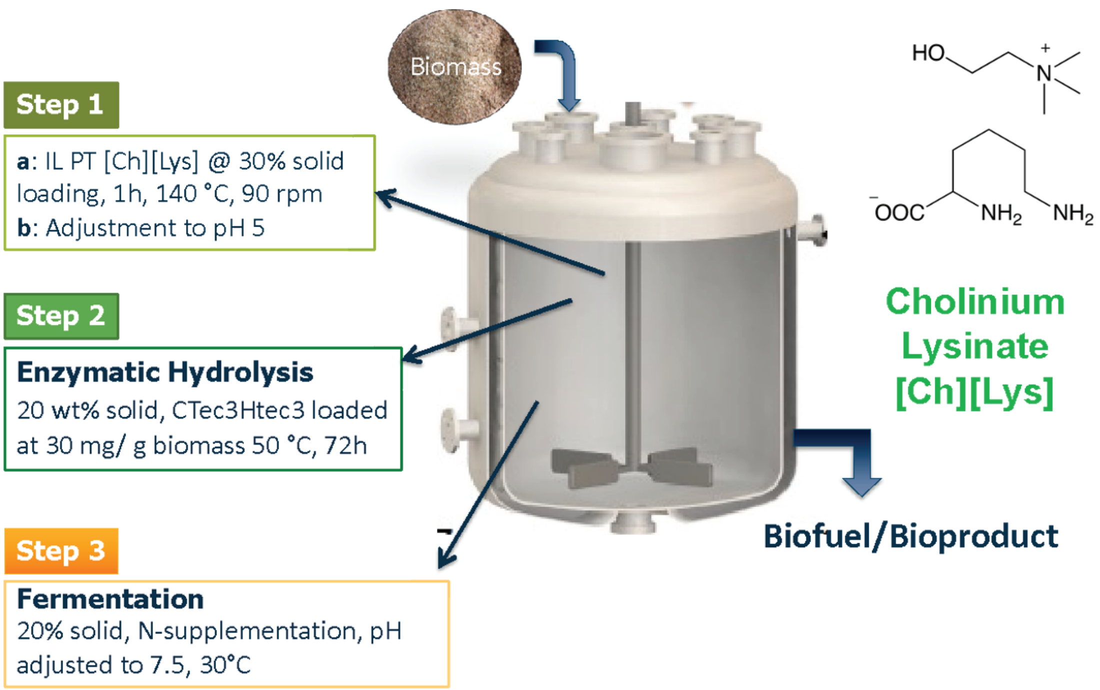
JBEI has successfully developed, optimized, and scaled up an ionic liquid pretreatment technology to convert wood waste biomass to fermentable sugars in hydrolysate at 83% yield and further achieve an overall biomass-to-fuel carbon conversion efficiency of nearly 80%. The conversion process—developed in collaboration with Aemetis, Inc., and Lawrence Berkeley National Laboratory's Advanced Biofuels and Bioproducts Process Development Unit—requires no solid-liquid separations, which reduces complexity and eliminates the loss of intermediate materials. The project demonstrated scale-up from a 2-liter lab-scale fermentation to a 1,500-liter industrial-level fermentation, validating commercial feasibility.

JBEI
Joint BioEnergy Institute

To build on this accomplishment and establish a foundation for producing a broad variety of advanced biofuels using similar woody biomass feedstock and processing technologies, the project team engineered a yeast strain that produces advanced automotive and aviation biofuels such as isoprenol. The team also identified paths for future development of biomass deconstruction and conversion approaches, such as building a pilot plant that commercializes advanced biofuels production from California's wood waste biomass generated through forest-thinning efforts to reduce wildfire risks.

Related Publications

Barcelos et al. 2021; Papa et al. 2022; Perez-Pimienta et al. 2021; Simmons et al. 2021; Sun et al. 2017; Xu et al. 2018; Yan, J., et al. 2019



Schematic depiction of an industrial-level one-pot process for biomass deconstruction and conversion to biofuels and bioproducts developed at JBEI. [Courtesy JBEI]

ADDITIONAL ACCOMPLISHMENTS IN **DECONSTRUCTION AND SEPARATION**

Oilcane Pilot Projects Demonstrate Scalability

CABBI's feedstocks-to-fuels demonstrations serve to stress-test and improve the process pipeline for sustainable conversion of biomass, such as oilcane, to bioproducts at an industrially relevant scale, from propagating, field testing, and harvesting feedstocks to processing, product recovery, and cost and life-cycle assessments. These efforts are developing pilot-scale protocols for economically feasible and environmentally sustainable biofuels production and a system for distributing results and samples throughout the BRCs.

Cotreatment Overcomes Biomass Recalcitrance

CBI researchers examined the effectiveness of cotreatments, pretreatments, and biocatalysts on total carbohydrate solubilization in poplar and switchgrass feedstocks of varying recalcitrance, a key barrier to biological processing of biomass to fuels and chemicals. Results showed that consolidated bioprocessing, especially when augmented by intermittent mechanical grinding cotreatment, can overcome most variation in feedstock recalcitrance to achieve high biomass carbohydrate solubilization without expensive pretreatment or added degrading enzymes.

Holwerda et al. 2019

Fluorescence Microscopy Demystifies Biomass Deconstruction

CBI researchers used super-high-resolution fluorescence microscopy to image the locations of biomass-deconstructing proteins produced by *Clostridium thermocellum*, the most efficient microorganism known to solubilize lignocellulosic biomass. Ongoing experiments show that these cellulosomes migrate on the bacterial surface to bind and attack specific biomass regions, suggesting that a mechanistic understanding of cellulosome formation during microbial growth on biomass feedstocks could improve biomass solubilization rates in consolidated bioprocessing.

Yarbrough et al. Submitted

Method Quantifies Impacts of Feedstock Growth Conditions

A successful sustainable bioenergy refinery relies on predictable yields of biofuels and products, which are susceptible to changing environmental conditions that impact biomass yield and quality. GLBRC researchers established a high-throughput field-to-fuel pipeline to identify the effects of environmental factors, such as drought stress, on biorefinery output from diverse feedstocks to help producers manage risk.

Chandrasekar et al. 2021; Ong et al. 2016

Aromatics-First Deconstruction Advances Biofuel Economics

Recovery of clean and soluble lignin and sugar streams from recalcitrant lignocellulosic biomass presents a central challenge to achieving economically viable biorefineries. GLBRC researchers, in partnerships with other BRCs, developed aromatics-first deconstruction, an alternative to traditional biomass deconstruction in which soluble aromatics are extracted first, yielding clean and soluble lignin streams for efficient conversion to valuable bioproducts; plant sugars can then be converted separately.

Alherech et al. 2021; Luo, H., et al. 2021; Perez et al. 2022

Novel Lignin-Degrading Enzymes Engineered

Few commercially available enzymes break down lignin into bioavailable intermediates, an essential step in its conversion to biofuels and bioproducts. JBEI researchers demonstrated that lignin peroxidase (LiP) and laccase enzymes from anaerobic fungi can break chemical bonds in lignin, but they do not perform optimally in a biorefinery. To address this limitation, they engineered a variant of LiP isozyme H8 with greater stability under acidic conditions and improved cleavage of β-O-4 ether and C-C bonds in lignin.

Park, M., et al. 2020; Pham et al. 2021; Podolsky, et al. 2019; Tom et al. 2022

Automated Biofoundry Rapidly Engineers Microbes

Scientists are increasingly exploring biological systems, such as proteins, metabolic pathways, and whole cells, for a variety of biotechnology applications, including CABBI's work to engineer yeast strains that efficiently produce diverse, high-value bioproducts like biodiesel, organic acids, lubricants, and jet fuel. However, the complexity of biological systems requires many rounds of the design-build-test-learn (DBTL) cycle, which is expensive, time-consuming, and labor-intensive. To address this problem, researchers developed a fully integrated automated biofoundry called the Illinois Biological Foundry for Advanced Bioengineering (iBioFAB). CABBI combined a machine-learning (ML) algorithm with a fully automated workflow for pathway engineering and turned iBioFAB into a self-driving biofoundry that can rapidly engineer microbial strains. CABBI Conversion researchers have developed several tools and high-throughput workflows for the DBTL cycle and implemented them on iBioFAB. These capabilities include synthesis of transcription activator-like effector nucleases (TALENs) using programmable genome-editing tools for genome-engineering applications (Chao et al. 2017) and BioAutomata, a ML-enabled, fully closed DBTL cycle for automated pathway engineering that can be applied to other challenges (HamediRad et al. 2019). In addition, the *in silico* strain design tool OptRAM (Optimization of Regulatory and Metabolic Networks) facilitates yeast engineering by identifying optimization strategies including overexpression, knockdown, or knockout of metabolic genes and transcription factors (Shen et al. 2019). New screening methods for small molecules based on matrix-assisted laser desorption/ionization (MALDI) imaging has expanded analytical capacity for high-throughput metabolic engineering studies, including rapid profiling of medium-chain fatty acid production in yeast (Xue et al. 2020). And PlasmidMaker, a user-friendly automated end-to-end platform for construction of plasmids, includes design tools and automated build processes to increase research efficiency at CABBI and other BRCs (Enghiad et al. 2022).

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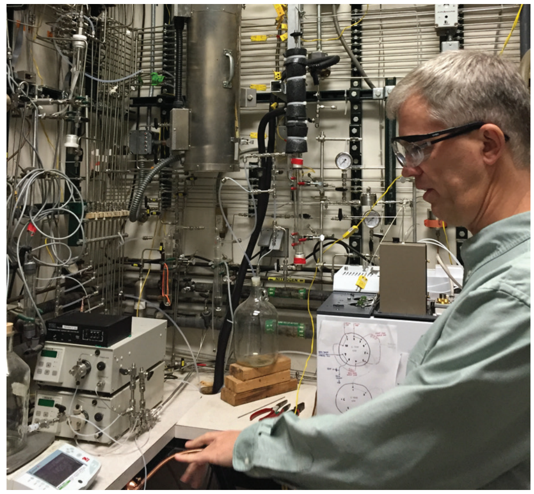
The central robotic arm of the Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB), located at the Carl R. Woese Institute for Genomic Biology, places a 96-well plate into a Tecan Fluent liquid handler. [Courtesy University of Illinois Urbana–Champaign]

Related Publications

Chao et al. 2017; Choe et al. 2021; Enghiad, et al. 2022; HamediRad et al. 2019b; Shen et al. 2019; Xue et al. 2020

One-Step Process Converts Bioethanol to Fungible Fuels

A one-step catalytic upgrading process can economically convert fermentation ethanol into hydrocarbon fuel blendstocks. CBI researchers and collaborators from Vertimass, LLC, performed technoeconomic and life-cycle assessments on a novel consolidated dehydration and oligomerization (CADO) technology that can convert ethanol into gasoline, diesel, and jet fuel blendstocks without adding hydrogen (Hannon et al. 2019). The CADO approach can produce hydrocarbon fuels at about the same cost as the final step in the current process in which water is removed from ethanol. Ethanol produced from perennial energy crops reduces chemical inputs, lowers soil compaction, and increases soil carbon storage relative to annual crops. Therefore, upgrading alcohols into fungible fuels preserves environmental sustainability and economic value. Vertimass is scaling up this BRC-initiated invention with funding from the DOE Office of Energy Efficiency and Renewable Energy and the private sector.



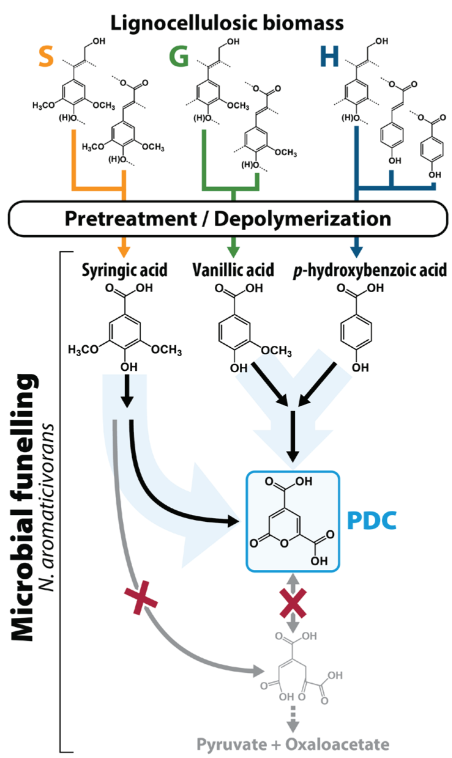
A collaboration between CBI and Vertimass, LLC, has yielded a one-step catalytic ethanol-to-fuel process that the company is now working to scale up. [Courtesy Vertimass/Technip Stone and Webster]

Related Publication

Hannon et al. 2019

Engineered Bacterium Converts Lignin to Plastic Precursors

Efficient and economic production of plant-based fuels and chemicals requires using as much of a plant's total biomass as possible. After plant sugars are extracted and converted to biofuels, the bulk of the remaining biomass consists of the complex polymer lignin, whose breakdown invariably produces heterogeneous mixtures of aromatic compounds. The economic viability of lignocellulosic biorefineries depends on the ability to convert lignin into valuable bioproducts efficiently and cheaply. To this end, GLBRC researchers have engineered the bacterium *Novosphingobium aromaticivorans* to funnel mixtures of lignin-derived aromatics into high yields of a target chemical, 2-pyrone-4,6-dicarboxylic acid (PDC), which possesses high potential value as a platform chemical precursor to adhesives, plastics, and other biopolymers. This advancement could enable sustainable lignocellulosic biorefineries to co-produce valuable commodity chemicals derived from lignin in addition to the biofuels and other chemicals already derived from biomass sugars.



GLBRC researchers engineered *Novosphingobium aromaticivorans* to funnel the major syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) aromatics found in biomass to produce high yields of PDC, a platform chemical and potential precursor to adhesives, plastics, and other biopolymers. [Courtesy GLBRC]

Related Publications

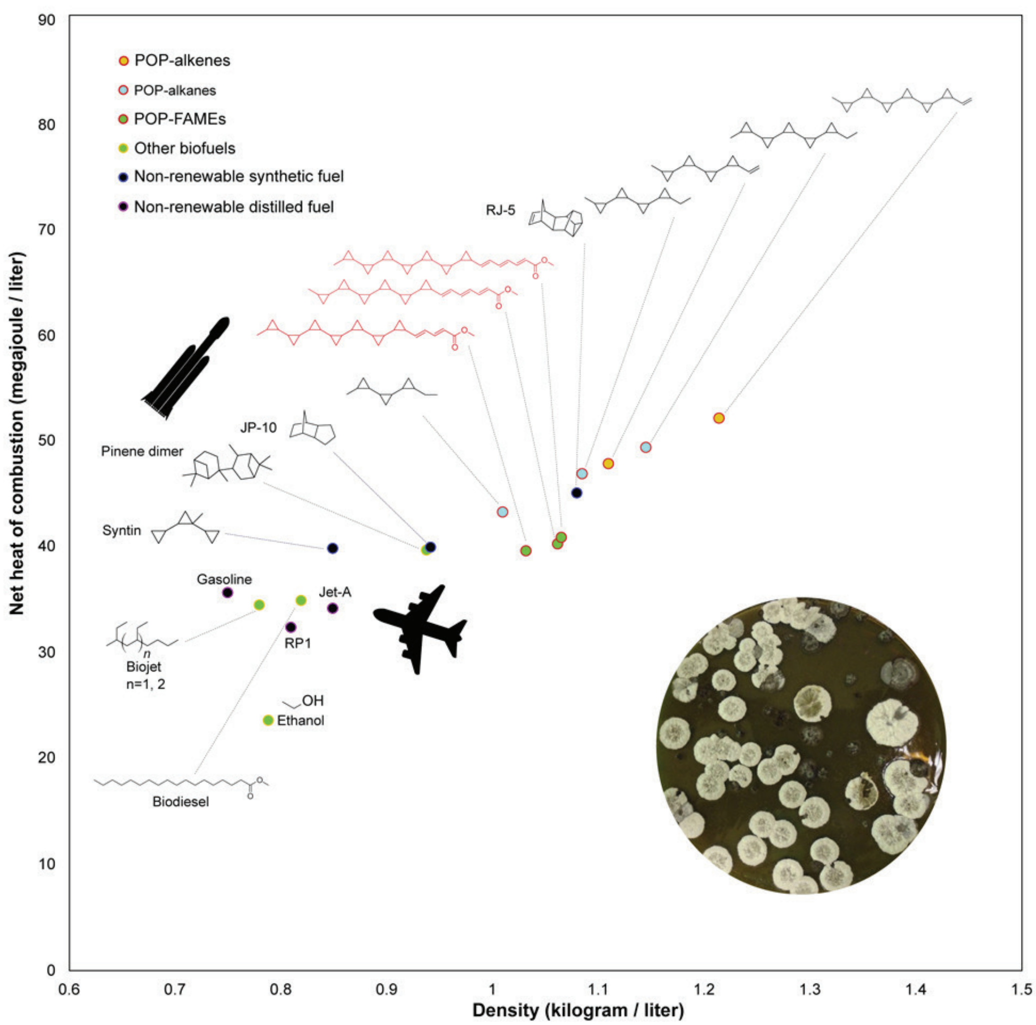
Perez et al. 2019, 2021, 2022

Sustainable Rocket Fuel Candidate in Development

Activities like rocketry, aviation, and shipping require energy-dense fuels typically derived from petroleum hydrocarbons. Fuel blends are often rich in cyclic molecules containing strained bond angles that enable higher energy storage relative to noncyclic molecules. Cyclopropanes are a highly energy-dense class of cyclic molecule, but they are challenging to produce using traditional organic synthesis methods. Now, JBEI researchers have produced polycyclopropanated fatty acid methyl ester (POP-FAME) fuels in a bacterial host via a polyketide synthase. Polyketides are a valuable class of compounds for which JBEI has developed software and automation tools to increase development of new pathways in this biosynthetic space. POP-FAMEs possess energy densities greater than 50 MJ/L, which is larger than the energy of the most widely used rocket and aviation fuels. Although this process is currently a lab-scale demonstration, the availability of a bioproduction route opens the possibility to replace petrochemical-based fuels in a fuel sector with few such paradigms.

Related Publications

Cruz-Morales et al. 2022; Eng et al. 2018; Yuzawa et al. 2018



The energy density of new polycyclopropanated compounds derived from polyketide-based pathways compare well with known high-energy-density compounds. [Reprinted under a CC BY-ND 4.0 license from Cruz-Morales, P., et al. 2022. "Biosynthesis of Polycyclopropanated High Energy Biofuels," *Joule* **6**(7), 1590–605. DOI:10.1016/j.joule.2022.05.011. © 2022 Elsevier Inc.]

ADDITIONAL ACCOMPLISHMENTS IN **CONVERSION**

New-to-Nature Enzymes Engineered with Novel Reactivity

CABBI researchers used photocatalysis to repurpose enzymes found in nature for novel photoenzymatic reactions, creating highly selective, sustainable chiral compounds with applications for producing high-value chemicals. Building on this development, researchers used light to excite an engineered ketoreductase enzyme, enabling a new-to-nature biocatalytic reaction. Known as an asymmetric radical conjugate addition, this reaction is extremely difficult to achieve by chemical catalysis and thus demonstrates a simple yet powerful strategy for creating new enzymes.

Huang et al. 2020, 2022; Litman et al. 2018

Editing Tools Developed for Nonmodel Yeasts

CABBI researchers are developing novel yeasts tuned to metabolize specialty compounds produced within plants or from leftover plant material after high-value products have been extracted from biomass. To further develop these nonmodel organisms, the researchers created toolkits enabling them to engineer *Yarrowia lipolytica*, *Rhodosporidium toruloides*, and *Issatchenkia orientalis* to produce chemical compounds used in biofuels and a range of other products such as detergents, cosmetics, food additives, and phone screens.

HamediRad et al. 2019a; Jagtap et al. 2019; Schultz et al. 2019; Suthers and Maranas 2022; Tran et al. 2019; Zhang et al. 2019

DNA Methylation Enables Engineering of Nonmodel Microbes

A new method developed by CBI researchers uses *Escherichia coli* to generate DNA with methylation patterns recognized and accepted by microbes targeted for engineering, facilitating rapid customization of nonmodel microbes for biofuels production. Proper methylation enabled genetic modification of the important consolidated bioprocessing bacterium *Clostridium thermocellum*, which scientists had previously been unable to engineer. This technique provides a new tool for fundamental biological research in bioenergy and beyond.

Riley et al. 2019; Riley and Guss 2021

Metabolic Flux Analysis Debottlenecks Cellulolytic Conversion

Quantitative metabolomics combined with metabolic flux analysis by CBI researchers revealed that the glycolytic pathway of the cellulolytic anaerobe *Clostridium thermocellum* operates near equilibrium, making it highly susceptible to product feedback inhibition during conversion of cellulosic sugars to biofuels. The insight will facilitate future engineering of high-performance strains capable of transforming cellulosic biomass to biofuels at high yields and titers.

Foster et al. 2022; Jacobson et al. 2020

Biological Funneling Advances Lignin Valorization

Lignin is an abundant source for renewable biobased chemical products but is underutilized due to its resistance to depolymerization. CBI researchers are engineering the soil bacterium *Pseudomonas putida* KT2440 to rapidly catabolize a variety of high–molecular weight lignin and lignin-derived aromatics into a single product to produce performance-advantaged chemicals at economically viable industrial scales.

Erickson et al. 2022; Kuatsjah et al. 2022; Salvachua et al. 2020

Microbiomes Valorize Lignocellulosic Conversion Residues

The complex mixtures of organic matter remaining after biofuel production are rich potential feedstock sources of additional high-value bioproducts. GLBRC researchers are using mixed microbial communities to transform the conversion residue remaining after lignocellulosic biofuel production into valuable molecules like medium-chain fatty acids, which can be used to make a variety of industrial chemicals and pharmaceuticals.

Scarborough et al. 2018; 2020a, b

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Bioenergy Pathway Thermodynamics Determined Via Flux Analysis

Thermodynamics and kinetics determine the amount of energy from biomass feedstocks that is conserved in microbial biofuel products and the rates at which they are produced. GLBRC researchers have developed a new approach to determining the overall energetics of multistep biofuel synthesis pathways that uses forward and reverse flux measurements to assess thermodynamics, revealing novel features of *Zymomonas mobilis* metabolism, an important bioenergy microbe, that can be engineered for more efficient biofuel production in this and other microbes.

Jacobson et al. 2019; Martien et al. 2019, 2021; Tatli et al. 2019

Model-Driven Approaches Improve Bioproduction

JBEI researchers leveraged the availability of a genome-scale metabolic model for *Pseudomonas putida*, a soil bacterium that catabolizes a wide range of carbon substrates, to implement a computational approach called constrained minimal cut set (cMCS) that enables rewiring of microbial metabolism to couple carbon substrate use with bioproduction. The approach, which combines computational models, functional genomics, and strain engineering, provides a basis for additional engineering to enhance titer rates and yields during bioproduction.

Banerjee et al. 2020; Eng et al. 2021; Gauttam, R., et al. 2021; Lim et al. 2021; Thompson et al. 2020

Microfluidics Platform Enables High-Throughput Strain Engineering

JBEI researchers have developed a high-throughput microfluidic system that enables CRISPR-based gene editing and screening on a chip for metabolic engineering of microbes used for bioproduct development. The device performs up to 100 genetic modification reactions in parallel, providing a scalable platform for generating the large number of engineered strains required for combinatorial optimization of genetic pathways and predictable bioengineering, and generating critical data for artificial-intelligence and machine-learning approaches to bioconversion.

Iwai et al. 2022; Lawson et al. 2021; Radivojević et al. 2020

Glossary

5-carbon sugar
a sugar with five carbon atoms

6-carbon sugar
a sugar with six carbon atoms

atom-economical/atom-efficient
processes that minimize atom loss from feedstocks during conversion to attain maximum yield

bioconversion
conversion of organic materials to usable products or energy sources by biological processes or agents

bioeconomy
economic activity driven by research and innovation in the life sciences and biotechnology that is enabled by technological advances in engineering, computing, and information sciences

biofoundry
an integrated facility for designing, building, and testing genetic constructs for biofuels and bioproduct production and other applications

biomass conversion
conversion of the major carbohydrate components (sucrose, starch, cellulose, hemicellulose) in plant material into valuable chemical components or fuel

biomass deconstruction
depolymerization of the polysaccharide content in biomass into its component chemicals

bioproducts
nonpharmaceutical chemicals that directly replace or substitute for chemicals currently derived from petroleum or natural gas

biorefinery
a facility that processes and converts biomass into value-added products ranging from biomaterials to fuels to chemicals

biosystems design
field of study that aims to build novel biological systems designed to carry out particular functions by combining different biological "parts" or molecular assemblies

blendstock
material blended in a refinery to create a final product, such as fuel

C4 photosynthesis
an efficient form of photosynthesis carried out by certain plants (e.g., corn, sorghum, sugarcane, switchgrass) rendering them more productive in high heat and light and low soil moisture conditions than C3 plants

catalytic upgrading
a process for increasing the energy density of biomass-derived liquids

carbon sequestration
a biological or physical process that captures carbon dioxide and converts it into inert, long-lived, carbon-containing materials

cellulase
enzyme involved in the conversion of cellulose to simple glucose molecules

cellulose
one of three primary components of herbaceous perennial plant cell walls along with hemicellulose and lignin; the predominant polysaccharide in plant cell walls, consisting of tightly bound sugar chains organized into strong cable-like microfibril structures

consolidated bioprocessing
simultaneous breakdown and conversion of feedstocks by cellulolytic microbes to biofuels and bioproducts

coproduct
a substance or material that accompanies the production of a fuel product

CRISPR/Cas
a targeted genome editing system using engineered nucleases (e.g., Cas9)

deep eutectic solvents
a class of solvents with properties similar to ionic liquids but with low environmental toxicity and a range of tunable physiochemical properties that enable a wide variety of catalytic, separation, and electrochemical applications

depolymerization
the process of converting a polymer into its component monomers

drop-in biofuels
biofuels with similar molecular composition to petrochemical-derived fuels, enabling them to seamlessly integrate into current fuel infrastructure and replace traditional fuels

feedstock
material used directly as a fuel or converted to an energy product

fungible fuels
fuels in common use, with common specifications, distributed in a comingled manner, and with sufficient specifications and quality control that they, within a given type, can be substituted for each other without concern of source or end use

genomics
the study of genes and their function

genotype
an organism's genetic constitution, as distinguished from its physical characteristics (phenotype)

hemicellulose
one of three primary components of herbaceous perennial plant cell walls along with cellulose and lignin; a mix of short, branched chains of five different sugars

hydrolysate
partially digested plant material that results from the enzymatic hydrolysis of biomass and contains fermentable sugars, inhibitors, and other compounds

hydrolysis
a type of chemical reaction that uses water to cleave chemical bonds and break large molecules into smaller components

in planta
within a living, intact plant

in silico
using computers to simulate and investigate natural processes

in situ
in a natural environment

ionic liquids
environmentally benign organic salts, often used as substitutes for volatile organic solvents, that possess a unique capacity for dissolving lignocellulosic biomass and helping hydrolyze the resulting slurry into sugars

inhibitor
a substance that slows down or prevents a particular chemical reaction or other process or reduces the activity of a particular reactant, catalyst, or enzyme

life-cycle assessment
addresses the environmental impacts throughout a product's life cycle from raw material acquisition through production, use, end-of-life treatment, recycling, and final disposal. Also known as life-cycle analysis, ecobalance, and cradle-to-grave analysis

lignin
one of three primary components of herbaceous perennial plant cell walls along with cellulose and hemicellulose; a complex, insoluble polymer whose structure gives strength and rigidity to cellulose fibers in the cell walls of woody plants; comprises a significant portion of the mass of dry wood and, after cellulose, is the second most abundant form of organic carbon in the biosphere

lignin valorization
conversion of lignin to higher-value compounds

lignocellulose
plant materials made up primarily of lignin, cellulose, and hemicellulose

microbial biorefining
using microorganisms to break down plant biomass into the precursors of biodiesel or other commodity chemicals used to produce petroleum alternatives

microfibril
strong, cable-like structures made of cellulose or proteins; in plants, cellulose microfibrils reinforce plant cell walls

mycorrhizal
relating to fungi that establish symbiotic relationships with plant roots

nitrogen fixation
process carried out by certain species of bacteria and archaea in which atmospheric nitrogen is converted to organic nitrogen-containing compounds that can be used by other organisms

oilcane
metabolically engineered sugarcane designed to hyperaccumulate oils

phenotype
physical characteristics of an organism

polysaccharide
a carbohydrate, such as cellulose or starch, that consists of numerous linked simple sugar units (monosaccharides)

platform chemicals (or compounds)
a chemical that can serve as a substrate for producing various other higher value-added products

pretreatment
any mechanical, chemical, or biological process that converts lignocellulosic biomass from its native form to a form susceptible to enzymatic hydrolysis

recalcitrance
a plant structure's natural resistance to degradation

reductive catalytic fractionation
a lignin-first biorefining strategy that combines biomass fractionation with lignin depolymerization

saccharification
a conversion process using acids, bases, or enzymes in which long-chain carbohydrates are broken down into their component fermentable sugars

substrate
a substance transformed by enzyma tic activity

technoeconomic assessment
analysis of the economic performance of an industrial process

weight percent
the mass of a solute divided by the mass of the solution (solute plus solvent together) times 100

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Acronyms and Abbreviations

3-HP	3-hydroxypropanoic acid
Agro-IBIS	Integrated Biosphere Simulator–Agricultural Version
AI	artificial intelligence
BEPAM	Biofuel and Environmental Policy Analysis Model
BER	DOE Biological and Environmental Research program
BioSTEAM	Biorefinery Simulation and Techno-Economic Analysis Modules
BRC	Bioenergy Research Center
C5 sugar	5-carbon sugar
C6 sugar	6-carbon sugar
CABBI	Center for Advanced Bioenergy and Bioproducts Innovation
CADO	consolidated dehydration and oligomerization technology
CBI	Center for Bioenergy Innovation
CBP	consolidated bioprocessing
C-CBP	consolidated bioprocessing with cotreatment
CRISPR	clustered regularly interspaced short palindromic repeats
DayCent	Daily Century biogeochemical model
DBTL	design-build-test-learn cycle
DEIA	diversity, equity, inclusion, and accessibility
DMCO	dimethylcyclooctane, a sustainable aviation fuel
DOE	U.S. Department of Energy
ECNet	evolutionary context-integrated neural network
FUN-BioCROP	Fixation and Uptake of Nitrogen–Bioenergy, Carbon, Rhizosphere, Organisms, and Protection
GIPC	glycosyl inositol phosphoryl ceramide
GLBRC	Great Lakes Bioenergy Research Center
iBioFAB	Illinois Biological Foundry for Advanced Biomanufacturing
IBRL	Integrated Bioprocessing Research Laboratory
iCLEM	Introductory College Level Experience in Microbiology program
iGEM	International Genetically Engineered Machine competition
IP	intellectual property
JBEI	Joint BioEnergy Institute
LCA	life-cycle assessment
ML	machine learning
MSU	Michigan State University
OptRAM	Optimization of Regulatory and Metabolic Networks strain design tool
PDC	2-pyrone-4,6-dicarboxylic acid
POP-FAMEs	polycyclopropanated fatty acid methyl esters
RCF	reductive catalytic fractionation
RISE	Research Internship in Sustainable Bioenergy
SAF	sustainable aviation fuel
STEM	science, technology, engineering, and mathematics
TAL	triacetic acid lactone
TALENs	transcription activator-like effector nucleases
TEA	technoeconomic assessment
UW	University of Wisconsin

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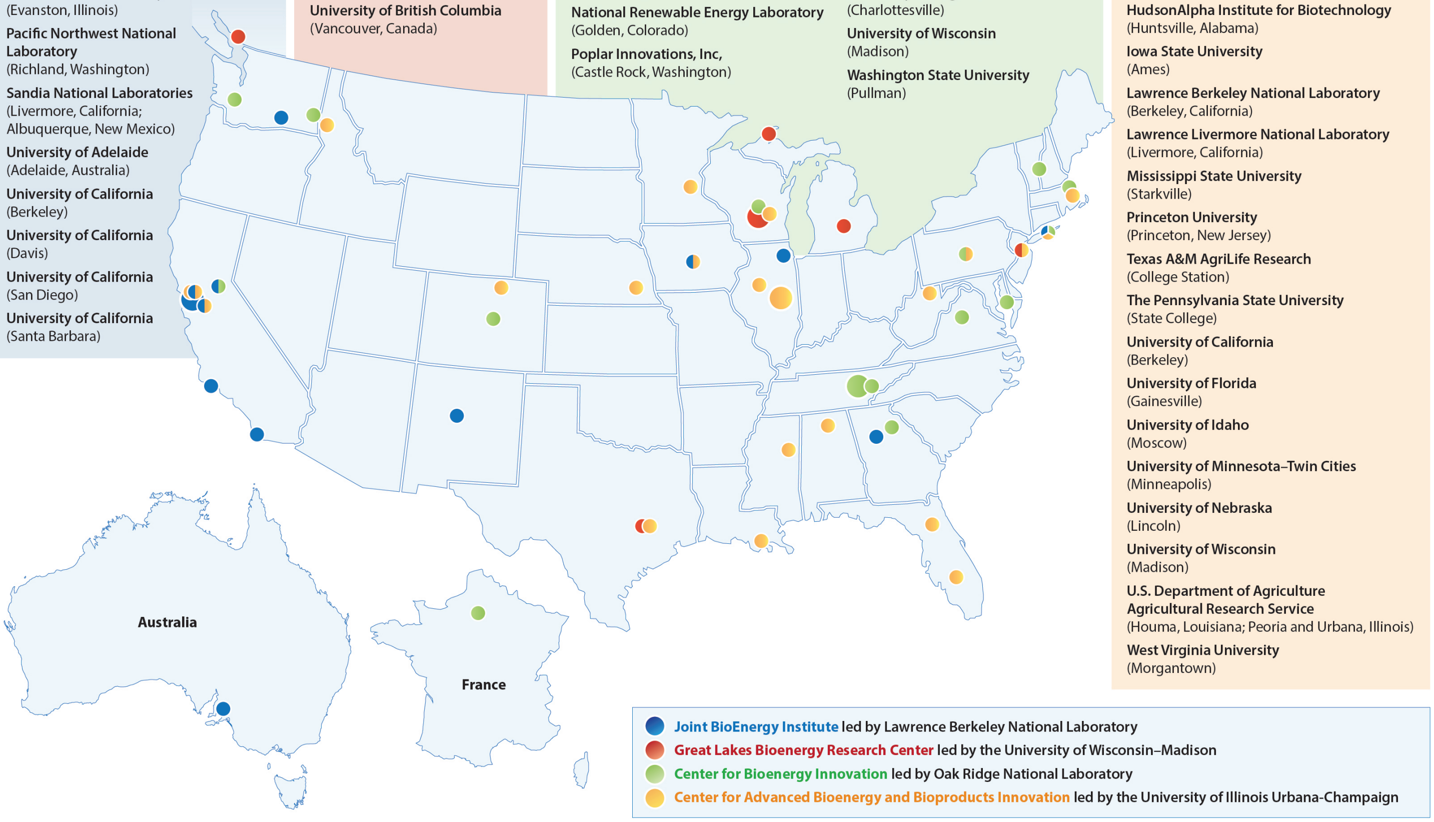

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