U.S. Department of Energy Bioenergy Research Centers

Developing the fundamental science, research technologies, and knowledgebase necessary to enable cost-effective, sustainable production of biofuels from plant biomass

Key Advances Update: 2014–2016

Advanced biofuels from renewable lignocellulosic biomass—plant stalks, trunks, stems, and leaves—are expected to achieve multiple societal benefits. These benefits include ensuring future energy security, lowering greenhouse gas production to mitigate climate impacts, diversifying the range of available biobased products, producing less toxic chemicals and byproducts, creating jobs in rural areas, and improving the U.S. trade balance.

The U.S. Department of Energy (DOE) established three Bioenergy Research Centers (BRCs) in 2007 to accelerate transformational breakthroughs in the basic sciences needed to develop cost-effective, sustainable, commercial production of cellulosic biofuels on a national scale. These centers are part of the Genomic Science program within DOE's Office of Biological and Environmental Research (BER), which is managed under DOE's Office of Science. The BRCs coordinate sustainable biofuels research along the entire pathway, from creating new energy crops and new methods for deconstructing lignocellulosic material into chemical building blocks to creating new metabolic pathways inserted into microbial hosts to produce ethanol and other hydrocarbon fuels. This center-scale approach allows technology development specialists to design automated pipelines that streamline workflows and increase research efficiencies, enables the testing of research ideas from proof of concept to field trials, and allows research breakthroughs in one area to immediately inform research direction in other areas.

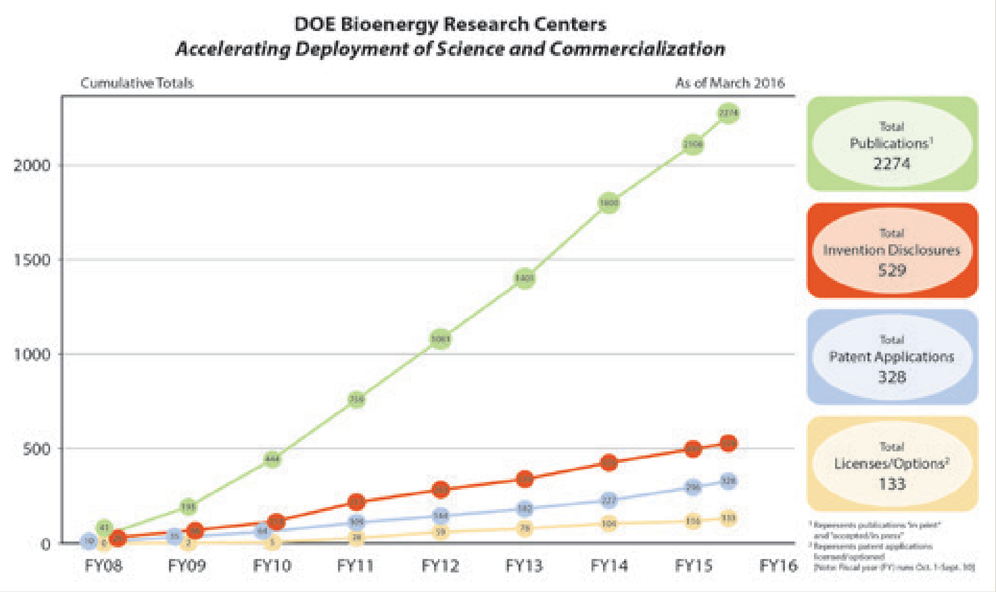
The three BRCs are based in the geographically diverse Southeast, Midwest, and West Coast regions. BRC partners include universities, private companies, nonprofit organizations, and DOE national laboratories.

- **BioEnergy Science Center** (BESC; Oak Ridge, Tennessee) is developing better understanding of how to modify plant cell wall components to facilitate deconstruction and conversion and developing thermophilic cellulolytic microbes for consolidated bioprocessing.
- **Great Lakes Bioenergy Research Center** (GLBRC; Madison, Wisconsin) aims to increase the energy density of grasses by understanding and manipulating the metabolic and genetic circuits that control the accumulation of easily digestible, energy-rich compounds in plant tissues.
- **Joint BioEnergy Institute** (JBEI; Emeryville, California) is applying synthetic biology to engineer microbes that convert sugars into advanced biofuels and plants that overproduce preferred polysaccharides.

Leading the World in Fundamental Biofuels-Relevant Research

These three BRCs have generated a number of important breakthroughs toward developing new dedicated bioenergy feedstocks and new products from renewable biomass. These significant achievements include (1) demonstrating that lignin composition and deposition can be genetically engineered to reduce plant cell wall recalcitrance without impacting plant viability; (2) developing effective, commercially adaptable biomass pretreatments to lower costs; (3) discovering novel microbes and enzymatic pathways for more efficient deconstruction of lignocellulosic biomass; (4) conducting proof-of-concept research for consolidated bioprocessing (i.e., the production of ethanol and other biofuels by naturally cellulolytic microbes directly from nonpretreated biomass); (5) metabolically engineering microorganisms and plants for biological production of numerous advanced biofuels or their immediate precursors; and (6) identifying hundreds of new plant genes and developing an understanding of their role in cell wall biosynthesis.

These and other BRC breakthroughs are highlighted on the following pages. Through intellectual property licensing agreements, partnerships, and targeted collaborative affiliations, DOE's BRCs are working to speed the translation of basic research results to industry for contributions to clean energy (see graph, DOE Bioenergy Research Centers, this page).



Bioenergy Research Centers: Key Advances for Lignocellulosic Biofuels Production

Improved Biomass

Overcome the natural resistance of plant biomass to microbial and chemical hydrolysis through the development and optimization of high-yield, sustainable feedstocks that are easily converted into biofuels.

Advances and Results

- Discovered that the major plant cell wall polymers (i.e., cellulose, lignin, hemicellulose, and pectin) all contribute to recalcitrance, information that is assisting in fine-tuning cell wall properties to render biomass more amenable to biofuel conversion (Kalluri, Yin, and Davison 2014; Baxter et al. 2014; Biswal et al. 2015).
- Conducted a comprehensive systems biology study of *Populus* and switchgrass to identify natural variants with modified composition; findings advance opportunities for rapid discovery of specific genes best suited for producing biofuel from biomass planted in different environments (Serba et al. 2015; Muchero et al. 2015; Evans et al. 2014).
- Revealed robust, reduced-recalcitrance phenotypes and better growth with field trials of high-performing *Populus* and switchgrass lines, demonstrating that transgenic feedstocks can be maintained in the field (Baxter et al. 2014).
- Manipulated transcription factors to reduce cell wall recalcitrance and enhance polysaccharide accumulation, with no significant impacts on plant growth and development (Vega-Sánchez et al. 2015).
- Identified and characterized several novel plant nucleotide sugar transporters that are powerful tools for cell wall engineering (Ebert et al. 2015).
- Coupled metabolomics analysis with genetic information to identify metabolite-gene associations, yielding critical insights into biomass formation in *Populus* (Payyavula et al. 2014).
- Modified the lignin biosynthetic pathway to enable design of plant cell walls that are easier and cheaper to convert into fuels and chemicals (Wilkerson et al. 2014).
- Altered lignin content and composition *in planta* to enhance biomass deconstructibility, significantly reducing the amount of energy required for higher sugar yields from pretreatment (Eudes et al. 2016; Scullin et al. 2015).

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*[**Note**: Full references appear on the back page of this document. For easier access, direct links to most of the abstracts are provided in the online version of this document at: genomicscience.energy.gov/centers/brcbrochure/.]*

Deconstruction

Increase the energy efficiency and cost-effectiveness of processes for deconstructing lignocellulosic biomass into its constituent sugars, while minimizing the formation of inhibitors and using as few chemicals as possible.

Advances and Results

- Developed and demonstrated an affordable, scalable biomass deconstruction technology based on ionic liquids for a wide range of single and blended feedstocks (Sun et al. 2015; Papa et al. 2015; Li et al. 2015).
- Discovered a renewable solvent (gamma-valerolactone) derived from biomass that can produce sugar and lignin streams from multiple biomass sources without expensive biomass-degrading enzymes (Alonso, Wettstein, and Dumesic 2013; Luterbacher et al. 2014; Luterbacher et al. 2015).
- Developed a novel pretreatment method (e.g., cosolvent-enhanced lignocellulosic fractionation) that greatly reduces enzyme loadings compared to traditional dilute acid pretreatment and significantly increases biofuel yields (Ngyuyen et al. 2015a; Nguyen et al. 2015b; Smith et al. 2015).
- Optimized biocatalyst-feedstock combinations to achieve high solubilization, demonstrating the potential for biomass processing with little or no thermochemical pretreatment (Paye et al. 2016).
- Identified enzymes that can enhance the hydrolyzation of multiple polysaccharides derived from plant cell walls into fermentable sugars (Deng et al. 2014; Takasuka et al. 2014; Deng et al. 2015a; Deng et al. 2015b; Walker et al. 2015).
- Conducted targeted bioprospecting in unique environments for novel enzymes, revealing diverse microbial communities that potentially could efficiently deconstruct plant polysaccharides (Vishnivetskaya et al. 2014; Yu et al. 2015; Simmons et al. 2014).
- Discovered and optimized a cellulase mixture that tolerates ionic liquids, which could provide a new, more efficient and tunable biomass pretreatment method (Gladden et al. 2014; Shi et al. 2013).
- Discovered that *Clostridium thermocellum* (anaerobic, thermophilic microorganism) performs demonstrably better than industry-standard fungal cellulases; findings are being used to develop optimal systems for breaking down lignocellulosic biomass (Paye et al. 2016; Xu et al. 2016).
- With Mascoma Inc., engineered yeasts that more efficiently convert cellulose to sugar than previous strains; these yeasts are used in about 20% of corn ethanol production, with C5-utilizing strains now available for cellulosic ethanol production (Guilliams et al. 2016; Henningsen et al. 2015).

The U.S. Department of Energy's three Bioenergy Research Centers (BRCs) have made numerous advances toward overcoming critical challenges to cost-effective production of renewable fuels from biomass. Select BRC advances (from 2014 to present) listed below are key to improved biomass feedstocks, deconstruction, and conversion, as well as the cross-cutting areas of enabling technologies and sustainability. Results from BRC research are grouped under their respective challenges and goals.

Conversion

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

Advances and Results

- Used genomic knowledge of how various plant-derived compounds negatively impact the performance of biofuel microbes to engineer strains with increased tolerance to individual inhibitors, thereby increasing biomass conversion rates and ethanol yields (Piotrowski et al. 2014; Piotrowski et al. 2015a; Piotrowski et al. 2015b; Dickinson et al. 2016; Pisithkul et al. 2015; Keating et al. 2014; Parreiras et al. 2014).
- Elucidated the mechanisms of ionic liquid tolerance in microbes, which could advance the development of gene expression systems for tolerance mechanisms of other toxic compounds (Ruegg et al. 2014; Frederix et al. 2014; Dickinson et al. 2016).
- Used targeted proteomics for biofuel metabolic pathway and protein monitoring, proving its use as an efficient quantitative analysis method (Alonso-Gutierrez et al. 2015; Dickinson et al. 2016).
- Optimized production of select advanced biofuels using model hydrolysates (Kirby et al. 2015; Kang et al. 2015; Foo et al. 2014).
- Developed a consolidated process for pretreatment, saccharification, and fermentation using ionic liquids, with potential to significantly reduce biofuel production costs (Xu et al. 2016; Liszka et al. 2016; Frederix et al. 2016).
- Developed the capability to genetically engineer both *C. thermocellum* and *Caldicellulosiruptor bescii* (thermophilic, cellulolytic anaerobes) to produce more desired biofuels and bioproducts (Chung et al. 2013; Olson, Sparling, and Lynd 2015).
- Efficiently converted mixed lignocellulosic feedstocks into isopentenol and other advanced biofuels using ionic liquids, offering a solution for overcoming biomass supply challenges (Shi et al. 2015).

Lignin as a Valuable Resource Instead of a Waste Product

- Converted lignin into "bionic liquids," which could replace ionic liquids derived from nonrenewable sources such as petroleum or natural gas (Socha et al. 2014).
- Developed methods for removal or bioconversion of aromatic compounds from lignin, potentially leading to higher-value products crucial to the economic viability of integrated biorefineries (Luterbacher et al. 2015; Rahimi et al. 2014; Austin et al. 2015).
- Characterized the structure and biochemistry of lignin-degrading enzymes, providing new insight into their catalysis mechanisms and informing future enzyme engineering efforts (Pereira et al. 2016; Helmich et al. 2016).
- Developed a novel assay for lignin-degrading enzymes, potentially enabling rapid assessment of catalytic, enzymatic, and microbial degradation of lignin (Kent et al. 2015).
- Identified novel lignin-degradation genes in multiple natural environments (Hudson et al. 2015; Woo et al. 2014).

Cross-Cutting Achievements

Enabling Technologies

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

- Developed rapid methods for analyzing cell wall structure and integrity in plants and during processing, providing a foundation for high-throughput profiling and correlating pretreatment conditions with biomass digestibility (Lu and Ralph 2014; Kim and Ralph 2014; Vismeh et al. 2013; Tobimatsu 2013; Chylla et al. 2013).
- Developed glycome profiling to analyze plant cell wall polysaccharides; immunological approach is aimed at understanding the spatial distribution of secondary cell wall components affecting recalcitrance (Pattathil et al. 2015).
- Developed nanostructure-initiator mass spectrometry (NIMzyme) technology to rapidly screen glycoside hydrolases, which are key plant cell wall–degrading enzymes (Deng et al. 2015b; Deng et al. 2014).
- Developed and deployed new synthetic biology software tools (i.e., DIVA and j5) for more efficient genetic design, transformation, and manipulation (Hillson 2014; Galdzicki et al. 2014; Hillson, Rosengarten, and Keasling 2012).
- Created a new suite of bioinformatics tools for metagenomics and plants (Wu, Simmons, and Singer 2015; Lee et al. 2015; Mann et al. 2013).
- Developed and deployed synthetic biology on a chip; automated microfluidic platform has the potential to significantly reduce the design-build-test cycle (Linshiz et al. 2014; Gach et al. 2016; Shih et al. 2015).

Sustainability and Economic Analysis

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

- Determined that marginal lands can provide significant cellulosic biomass, along with substantial climate change mitigation and other environmental benefits, allowing fertile lands to be reserved for food production (Gelfand and Robertson 2015).
- Incorporated state-of-the-art technologies into an open, wiki-based technoeconomic model that simulates critical factors in the biorefinery process (e.g., production costs and energy balances) under different scenarios, enabling researchers to focus on the most promising strategies for cost-efficient operations (Klein-Marcuschamer and Blanch 2015; Konda et al. 2014).

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