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Basic Research Opportunities in Genomic Science to Advance the Production of Biofuels and Bioproducts from Plant Biomass

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Contents

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Background

The Genomic Science program of the Office of Biological and Environmental Research[1] (BER) within the U.S. Department of Energy (DOE) Office of Science focuses on understanding microbes, microbial communities, and plants as integrated systems of relevance to DOE's energy and environmental missions. One aspect of the program seeks to develop the fundamental science, research technologies, and knowledgebase necessary to enable the cost-effective, sustainable production of biofuels and bioproducts from plant biomass. This initial potential of biomass was outlined in the Billion-Ton Study (U.S. DOE 2005; update, U.S. DOE 2011a). Achieving multiple societal benefits underlies DOE's research efforts to support a viable and sustainable domestic lignocellulosic advanced biofuels and bioproducts industry. 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. An earlier DOE workshop sought ways to realize these benefits by accelerating the emergence of a robust, new cellulosic ethanol industry. The resulting report, *Breaking the Biological Barriers to Cellulosic Ethanol* (U.S. DOE 2006), outlined a path toward this future, emphasizing integrated research opportunities, from feedstock development to conversion technologies.

Since then, DOE BER has supported transformational bioenergy research through the DOE Bioenergy Research Centers (BRCs) and development of biomass feedstocks and biofuel-relevant microbes.[2] The BRCs, in particular, have led large multidisciplinary, team-oriented research efforts using integrated systems approaches intended to address key obstacles limiting the cost-effective production of advanced biofuels from renewable plant biomass. A number of important breakthroughs have resulted from this fundamental research, including (1) the demonstration that lignin composition and deposition can be genetically engineered to reduce plant cell wall recalcitrance without impacting plant viability; (2) the development of effective, commercially adaptable biomass pretreatments to lower costs; (3) discoveries of novel microbes and enzymatic pathways for more efficient deconstruction of lignocellulosic biomass; (4) proof-of-concept research for consolidated bioprocessing (CBP; i.e., the production of ethanol and other biofuels by naturally cellulolytic microbes directly from nonpretreated biomass); (5) the metabolic engineering of microorganisms and plants for biological production of numerous advanced biofuels or their immediate precursors; and (6) the identification of hundreds of new plant genes and development of an understanding of their role in cell wall biosynthesis.

Remaining Basic Science Opportunities

To address the remaining basic science needs for establishing an economically viable and sustainable domestic biofuels and bioproducts industry, future BER efforts in bioenergy research will build and expand on the success of the current BRCs, specifically, and the bioenergy research community, in general. BER's understanding of the remaining basic science opportunities in this area has been informed in large part by two BER-sponsored workshops and resulting reports: *Research for Sustainable Bioenergy: Linking Genomic and Ecosystem Sciences* (held in 2013; U.S. DOE 2014b) and *Lignocellulosic Biomass for Advanced Biofuels and Bioproducts* (held in 2014; U.S. DOE 2015a). Other contributing resources include related workshops and reports on metabolic engineering and synthetic biology techniques for more sustainable biofuel and bioproduct manufacturing (U.S. DOE 2011b; U.S. NAS 2013; U.S. NAS 2015) as well as other DOE program planning documents (U.S.

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[1] Office of Biological and Environmental Research (science.energy.gov/ber/).

[2] More detailed descriptions of research accomplishments can be found in U.S. DOE 2014a or on individual BRC websites (bioenergycenter.org, jbei.org, glbrc.org).

DOE 2014c; U.S. DOE 2015b). BER envisions that future basic science opportunities will use an integrated systems approach in the six areas described below.

Sustainability

The design of sustainable biofuel and bioproduct systems requires knowledge about interactions among crops and their environment, impacts of crop choice and management systems, and key plant-microbe-environment interactions, including nutrient uptake, water use efficiency, and maintenance of soil carbon stocks. Linking advances in genome-driven research to breakthroughs in ecosystem science enables the use of fully integrated systems biology approaches for the fundamental design of sustainable biofuel and bioproduct systems.

Examples of basic science opportunities focusing on sustainability include, but are not limited to:

- Mechanistic understanding of how bioenergy crops interact with biotic and abiotic environmental factors in providing key ecological services such as crop yields, carbon and nutrient cycling, soil erosion control, water quality, and pest and disease control.
- Process and technoeconomic evaluation for biomass-to-fuels technologies addressing the economics of biofuel and bioproduct production to guide basic research priorities.
- Multiscale modeling toward a predictive understanding of the biofuel cropping ecosystem.
- Determination of the biomass crops and crop management systems needed to ensure a sustainably produced feedstock.

Feedstock Development[3]

Creation of new bioenergy feedstocks designed for sustainable production and efficient conversion to biofuels or bioproducts will require a systems-level understanding of plant growth and an ability to manipulate crops to achieve predicted beneficial traits.

Examples of basic science opportunities in feedstock development include, but are not limited to:

- Fundamental understanding of plant biology to develop a broader set of biomass crops that are economically viable and environmentally sustainable over a range of geographically distinct field conditions.
- Enhanced, regionally adapted bioenergy feedstocks with improved traits for yield, water use, nutrient uptake and recycling, resilience to biotic and abiotic stress, and efficient conversion to biofuels and bioproducts.
- Genetic tools and biosystems design approaches to advance bioenergy feedstock crop creation and production.
- High-throughput analytical tools to facilitate bioenergy feedstock crop development, evaluation, and production.
- Field testing of potential bioenergy feedstock crops under environmentally relevant conditions across multiple geographic regions and over time to assess viability and robustness.
- Quantitative models informed by experimentation to predict how bioenergy feedstock genotypes perform under various geographic and environmentally relevant conditions.

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[3]Feedstocks include dedicated crops for conversion to biofuels and bioproducts as well as crops that produce biofuels and bioproducts *in planta*.

- Knowledge of the role of microbial interactions with plants in conferring resistance, tolerance, and adaptability to abiotic and biotic stress and in controlling nutrient availability.

Although model plants serve an important role as tools, BER believes that the sophistication of genomic techniques has advanced sufficiently to enable focused research on current or potential dedicated bioenergy feedstock crops such as switchgrass, poplar, *Miscanthus*, and energy cane.

Lignocellulose Deconstruction

New fundamental knowledge is needed to facilitate the development of deconstruction processes that are lower cost, more efficient, and capable of converting a broader range of lignocellulosic biomass types into hydrolysates that are more readily convertible into biofuels and bioproducts. Current deconstruction processes simply separate out the lignin portion of biomass, resulting in the production of a substantial byproduct of limited value. Better understanding of lignin's chemical and physical properties can facilitate ways to convert a significantly greater proportion of biomass into biofuels and other bioproducts. Additionally, stronger integration between advances in biomass development and biofuel and bioproduct production will strengthen these deconstruction efforts.

Basic science opportunities in biomass deconstruction and separation include, but are not limited to:

- Detailed understanding of plant cell wall biosynthesis, composition, structure, and properties during deconstruction.
- Improved enzymes and approaches for biomass breakdown and cellulose, hemicellulose, and lignin processing.
- Quantitative understanding and multiscale modeling of plant cell wall deconstruction for improving process efficiency.
- Robust feedstock-agnostic pretreatment and separation systems to more efficiently deconstruct and separate plant biomass into its various components for more efficient downstream biofuel and bioproduct production with minimal inhibitor formation.

Specialty Fuels Production from Biomass[4]

Advances in metabolic engineering and synthetic biology have resulted in bioconversion of biomass into an expanded suite of potential biofuel molecules beyond ethanol. Many of these compounds have energy densities and handling properties more similar to gasoline, diesel fuel, jet fuel, and other current liquid fuel sources. To engineer economically viable biobased production of specialty fuels, greater understanding is needed of interconnecting metabolic systems, factors influencing pathway efficiency, fuel yield, production rate, energy balance, and tolerance of platform organisms to industrial production conditions.

Examples of basic science opportunities in specialty fuels production from biomass include, but are not limited to:

- A predictive systems-level understanding of the cellular and molecular processes to improve fermentation yields, expand diversity of production pathways, and increase tolerance for major chemical classes of inhibitors.

[4]Specialty fuels are defined as nonfood crop–based liquid biofuels other than ethanol.

- Development of a broader set of platform microorganisms suitable for metabolic engineering in biofuel production and CBP.
- High-throughput methods to screen or select high-performance strains or constructs for improving product formation rates, titers, yields, and selectivity (i.e., the ability to produce only the desired product without byproducts).
- Development of new, broad-based genetic and metabolic engineering and synthetic biology techniques to access a greater diversity of microorganisms and plants for bioenergy purposes and enhance production efficiency of advanced biofuels.
- New approaches and models that can 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 in scaled-up applications.

Bioproduct Development from Biomass[5]

Advances in biofuel production made possible by basic research clearly have revealed that developed technologies can be applied directly to the production of numerous other commodity chemicals from renewable biomass. In fact, basic research to diversify the range of fuels and products that can be produced from renewable biomass can increase overall cost efficiencies and provide market flexibility to a burgeoning bioenergy and bioproduct industry. Shifting the production of commodity chemicals to those originating from biomass instead of petroleum recognizes the potential for environmental and economic benefits as well as for the unbounded diversity of new molecules that could be produced via biological conversion pathways. The synergies between methods and approaches for fuel and bioproduct syntheses create an opportunity to leverage basic research in biofuels development with potentially advancing a broader biobased economy (OSTP 2012).

Basic science opportunities in bioproduct development from biomass include, but are not limited to:

- Identification and optimization of mass- and energy-conserving microbial pathways to obtain promising intermediates and final bioproducts from biomass that are less toxic and more environmentally benign compared to current products produced from petroleum or natural gas.
- High-throughput, real-time, *in situ* analytical techniques for understanding and characterizing the pre- and postbioproduct separation streams in detail.
- Methodologies for efficiently identifying viable target molecules, identification of high-value bioproducts in existing biomass streams, and utilization of current byproduct streams.
- Identification and improvement of plant feedstocks with higher extractible levels of desired bioproducts or bioproduct precursors, including lignin streams that are homogeneous and consistent.

Research Technologies

Success in scientific discovery is intimately connected to the development of new technologies for research. New approaches in biotechnology, such as miniaturization and automation of systems, serve both to accelerate discovery and to reduce its costs. These developments certainly have been seen in the advancement of

[5]Bioproducts are those nonpharmaceutical chemicals that can directly replace or substitute for chemicals currently derived from petroleum or natural gas.

DNA sequencing technologies over the past couple of decades. Therefore, BER sees the development and application of relevant new technologies as critical to research efforts in biofuels and bioproducts.

These new research technology efforts can be leveraged with capabilities available at the DOE Office of Science national scientific user facilities. DOE operates several major user facilities including the Synchrotron Light and Neutron Sources, Joint Genome Institute (JGI), Environmental Molecular Sciences Laboratory (EMSL), and Nanoscale Science Research Centers. These facilities offer multiple state-of-the-art instrumentation capabilities applicable to gaining a detailed understanding of the molecular and systems biology properties of bioenergy-relevant processes.[6]

Additionally, these research efforts are anticipated to be complemented by robust efforts in computational biology and bioinformatics. There is an increasing need to incorporate high-performance computing into systems biology research to enable efficient analysis of large and diverse genomic and experimental datasets. DOE assets in high-performance computing frameworks will provide capabilities not only to work with large datasets, but also to share, collaborate on, and reproduce analyses more easily. These assets are complemented by the development of the DOE Systems Biology Knowledgebase (KBase), an integrated platform for large-scale analyses and combining multiple lines of evidence to model plant and microbial physiology and community dynamics. These capabilities will greatly facilitate a systems approach to bioenergy research, allowing researchers to assess the impact of their results in the broader bioenergy production context. Computational biology tools and models are expected to help accelerate the understanding of complex plant and microbial datasets to generate effective solutions for bioenergy challenges.

BER Vision Forward

Integrated systems approaches are needed to tackle the inherently fundamental and interdisciplinary challenges impeding cost-efficient production of biofuels and bioproducts from renewable biomass. This objective requires integrating basic research skills and expertise in areas such as agronomy, genomics, biochemistry and analytical chemistry, microbial and plant genetics, physiology, structural and computational biology, bioinformatics, systems and synthetic biology, and chemical and process engineering. BER looks to the scientific community to develop novel insights into biological processes and envisions innovative, integrated, multidisciplinary approaches and strategies for addressing the challenges identified in this white paper. Data sharing and integration of research efforts in the focus areas are critical for enabling new knowledge from one discipline to directly inform research in the other areas. BER will continue to focus research at the interface between basic and applied science on applications for biofuels and bioproducts, providing basic knowledge as well as insight into new technical solutions. Basic research must proceed with an in-depth understanding of the current industrial-level roadblocks and bottlenecks that must be overcome to develop research directions that aim to resolve those obstacles. A measure of success of this basic research effort to underpin more applied research will be new developments and advances in areas such as knowledge, processes, biotechnology, and enabling technologies that can be adopted by industry for production of biofuels and bioproducts.

[6]Information about all DOE Office of Science national scientific user facilities is available at science.energy.gov/user-facilities/user-facilities-at-a-glance/.

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