

# DOE Office of Biological & Environmental Research: Biofuels Strategic Plan

## I. Current Situation

The vast majority of liquid transportation fuel used in the United States is derived from fossil fuels. In addition to the significant problems associated with security and renewability of these resources, their continued use results in massive releases of the greenhouse gas carbon dioxide and other pollutants that drive global climate change. Although the past five years have seen expanded availability of ethanol from corn starch and biodiesel from soybeans, concerns have been raised regarding competition of fuel production with the food supply and long-term sustainability, especially given the energy intensive type of agriculture required for these crops.

Cellulosic plant biomass (i.e. fibrous or woody plant materials such as stems and leaves) can be broken down into its component sugars by a combination of physical, chemical, and enzymatic treatments. These sugars can then be converted into ethanol or other liquid biofuels by fermentative microbes or other chemical processes. Biofuels derived from cellulosic plant biomass have the potential to provide a secure, renewable source of energy that will reduce our dependence on fossil fuels and emission of greenhouse gases. The Renewable Fuel Standard of the United States Energy Independence and Security Act (EISA) of 2007 mandates that 36 billion gallons of biofuels are to be produced annually by 2022, of which 16 billion gallons are expected to come from cellulosic feedstocks.

In addition to agricultural residues (i.e. non-edible parts of crop plants), cellulosic biomass crops such as switchgrass and poplar are being studied and further developed as dedicated feedstocks for biofuels production. Unlike ethanol produced from corn starch or biodiesel from soybeans, biofuels derived from cellulosic feedstocks do not directly compete with food resources and can be grown on lands unsuitable for traditional food agriculture. Dedicated biomass feedstocks also have the potential for much greater yields of cellulosic material, decreased requirements for water or fertilizer, and greater tolerance to pests and disease.

Despite these advantages, cellulosic biofuels are not yet widely available. Compared with the relatively simple sugar chain of starch, cellulosic biomass is a complex, heterogenous material that is much more difficult to degrade into its component sugars. As such, cellulosic biofuels are currently not cost-competitive with either fossil fuels or ethanol produced from corn starch. In addition, ethanol has considerably lower energy density and is more difficult to distribute via existing infrastructure than gasoline. Significant technical barriers need to be addressed before cellulosic biofuels can be more broadly adopted for use. These include:

- Limited understanding of the structural properties of plant cell walls that impart strength and resistance to degradation, hindering development of better biomass deconstruction strategies

- Relative inefficiency and high cost of currently available enzymatic, chemical, and physical treatments for breakdown of cellulosic biomass and conversion of resulting sugars to biofuel compounds
- Gaps in fundamental understanding of enzymes and metabolic pathways of microorganisms mediating deconstruction of complex plant biomass and synthesis of ethanol or other potential biofuel compounds

Also, although dedicated cellulosic biomass feedstocks have great potential for more sustainable production, sustainability issues surrounding this relatively new form of agriculture remain poorly understood and warrant further study.

## **II. Inputs & Resources**

The U.S. Department of Energy's Office of Biological & Environmental Research (BER) is uniquely well suited to provide the transformational breakthroughs necessary to overcome the barriers listed above. BER supports fundamental research and technology development aimed at achieving predictive systems-level understanding of complex biological systems with a strong emphasis on integration of results across multiple research approaches and scientific disciplines. This approach will provide the foundational understanding of the genomic, metabolic, and structural systems of plants and microbes that will be needed to advance development of next generation cellulosic biofuels.

BER supports fundamental research enabling the development of next generation biofuels through a variety of different mechanisms:

- DOE Bioenergy Research Centers (BRCs)

The three BRCs are large scale, highly integrated multi-institutional research efforts which will provide fundamental knowledge needed to meet the goals outlined in the previous section. The BRCs will address inherently interdisciplinary scientific problems requiring scientific expertise and technological capabilities that span the physical and biological sciences, including genomics, microbial and plant biology, analytical chemistry, computational biology and bioinformatics, and engineering.

- Fundamental Genomics Science and Systems Biology Research

BER will continue to support biofuels research by individual investigators or collaborative teams at academic institutions and by researchers working in Science Focus Areas (SFAs) at DOE National Laboratories that take advantage of experimental resources and combinations of expertise complementary to those available within the BRCs. In addition to addressing the goals stated above, fundamental biofuels research supported by this mechanism will be used to explore emerging opportunities, develop new areas of study, and provide foundational knowledge that underpins multiple BER missions.

- DOE/USDA Bioenergy Feedstocks Genomics Program

In collaboration with the U.S. Department of Agriculture (USDA) Cooperative State Research, Education, and Extension Service, BER supports fundamental research on the structure, function, and organization of plant genomes leading to improved biomass characterization. The goal of this program is to accelerate plant breeding programs and enable improvement of biomass feedstocks by characterizing the genes, proteins, and molecular interactions that influence biomass production.

- Metabolic Engineering Working Group (MEWG)

As part of interagency effort with the National Science Foundation (NSF), the Department of Defense (DOD), the Environmental Protection Agency (EPA), the USDA, and other agency partners, BER supports research aimed at understanding metabolic and regulatory pathways of plants and microbes with the goal of enabling engineering of cellular pathways relevant to biofuels production.

- Small Business Innovation Research (SBIR)

This program supports research at small businesses, including the development of new technologies and instrumentation for characterization and manipulation of plant and microbial systems relevant to biofuels production.

In addition to direct support of biofuels relevant research, BER also oversees a number of facilities and information resources that will provide enabling capabilities for biofuels research:

- DOE Joint Genome Institute (JGI)

JGI provides high throughput sequencing of plant and microbial genomes, annotation of functional genes, and bioinformatics resources. The Bioenergy Research Centers and other BER-supported investigators make use of JGI's sequencing capacity for large scale sequencing of plant genomes, microbial community genomes (i.e. metagenomes) to bioprospect for new genes and enzymes of interest, and re-sequencing of experimentally evolved strains of microbes with altered biomass deconstruction or biofuels synthesis properties.

- Systems Biology Knowledgebase

BER is currently developing a knowledgebase that will serve as an integrated experimental framework for accessing, comparing, analyzing, modeling, and testing systems biology data. This resource will serve as a data repository, a collection of tools for data analysis, and a predictive framework for creating, testing, and improving models

enabling manipulation and engineering of biological systems. The knowledgebase will allow free access to data and encourage information exchange for the BER research community.

- Environmental Molecular Science Laboratory (EMSL)

EMSL is a national scientific user facility that provides integrated experimental and computational resources for systems biology research. Instrumentation and capabilities particularly relevant to next generation biofuels research include mass and nuclear magnetic-resonance spectrometers for high throughput proteomics and metabolite measurement and imaging facilities for determination of protein structures.

In addition to these efforts, BER will coordinate and share information with other DOE programs involved in biofuels research. In particular, BER will continue to work closely with the Office of Energy Efficiency and Renewable Energy's Office of Biomass Program (OBP) to leverage efforts between fundamental and applied research programs within DOE. These efforts will enable better integration and deployment of new research findings and enabling technologies and provide important opportunity for evaluation and improvement of ongoing fundamental research. BER will also continue to coordinate efforts with DOE's Offices of Basic Energy Sciences (BES) and Advanced Scientific Computing Research (ASCR) on relevant programs.

BER's research activities are coordinated with the larger federal biofuels research endeavor via participation in the Biomass Research and Development Board, which was created by the Biomass Research and Development Act of 2000 and was further amended by the Food, Conservation and Energy Act of 2008. The Board's mission is to coordinate federal research and development activities relating to biobased fuels, power, and products. In addition to the Department of Energy, membership includes the following agencies:

- U.S. Department of Agriculture
- National Science Foundation
- Environmental Protection Agency
- Department of the Interior
- Office of Science and Technology Policy
- Office of the Federal Environmental Executive
- Department of Transportation
- Department of Commerce
- Department of the Treasury
- Department of Defense

### **III. Near Term Goals (1-3 Years):**

- Genome sequencing and genetic analysis of potential biomass feedstock plants

Genome sequencing and comparative genome analysis will provide the fundamental blueprint of biomass feedstock plants structural and functional properties. This foundational level of information is necessary to build further understanding of metabolic and regulatory pathways that control overall biomass yield, recalcitrance to degradation, and effective utilization of resources in the field.

- Physical, chemical, and molecular characterization of the architecture of plant cell walls and metabolic and regulatory processes involved in cell wall synthesis and breakdown

To fully understand the properties that render lignocellulose resistant to degradation and enable development of plants with altered properties, more research is needed on the structural characteristics of plant cell walls. This must be accomplished in both experimentally tractable model plants and potential biomass feedstocks such as switch grass and poplar. Understanding the processes by which plants build and degrade their cell walls will provide new insights into the properties imparting strength and durability to plant biomass. Given the very large diversity of biomass samples that will need to be screened, it is also critical to develop higher throughput methods for characterization and analysis.

- Characterization of microbes and microbial communities from environments with high rates of lignocellulose degradation

There is a relatively limited set of microorganisms and microbial enzymes currently available that are capable of breaking down plant biomass to component sugars, especially in industrial settings. By using a combination of advanced cultivation techniques and cultivation independent approaches to explore environments known for high rates of biomass degradation (tropical forests, insect guts, terrestrial hot springs, etc.), new organisms and enzyme systems will be identified that can be further developed for more efficient deconstruction of cellulosic biomass under a wider range of conditions.

### **IV. Mid Term Goals (4-6 Years):**

- Development of systems biology approaches and integrated, predictive modeling capabilities for metabolic and regulatory networks of biomass degrading microorganisms

Understanding and accurately modeling the cellular networks that control physiological properties of microorganisms will enable more effective metabolic engineering and synthetic biology approaches. Systems oriented research on model microbes capable of degrading complex biomass will lead to development of organisms with increased rates degradation, the ability to utilize an expanded range of biomass components (i.e. hemicellulose, lignin monomers, etc.), and decreased susceptibility to end product

inhibition. These strains could be further developed with increased tolerance to physicochemical stresses associated with industrial scale biofuels production (elevated temperature, altered pH levels, or toxicity of fuels) or to incorporate novel enzyme systems isolated from environmental microbes.

- Understand impacts of biomass crop production on structure and function of microbial communities in underlying soil

Microbes inhabiting the soil rhizosphere (i.e. the region surrounding plant roots) play important roles in enhancing plant productivity, processing of nutrients such as nitrogen, catalyzing formation of soil structure, controlling emission of greenhouse gasses, and mediating a variety of other biogeochemical processes. Monitoring shifts in microbial community composition and associated functional properties that may occur during cultivation of biomass feedstock plants will provide an early indication of potential impacts on soil processes and characteristics relevant to the long-term sustainability of biomass production.

## **V. Long Term Goals (7-10 Years)**

- Development of systems biology approaches and integrated, predictive modeling capabilities for cellulosic biomass feedstock plants

Poplar, switchgrass, and other potential biomass feedstock plant species possess considerably larger genomes and more complex metabolic and regulatory systems than microbes. Much more effort is needed to develop systems biology and computational modeling approaches for plant systems. Given that most bioenergy feedstock plants remain relatively undeveloped for agriculture, achieving advances in this area of research will allow rapid improvement of bioenergy feedstock crop characteristics such as enhanced biomass yield and increased tolerance to drought and disease. These approaches could also lead to feed stock plants with modified cell walls that retain their structural integrity in the field but could be more easily broken down into their component sugars after harvest.

- Development of systems biology approaches and integrated, predictive modeling capabilities for metabolic and regulatory networks of biofuel- synthesizing microorganisms

Understanding and accurately modeling the cellular networks that control physiological properties of microorganisms will enable more effective metabolic engineering and synthetic biology approaches. Systems oriented research on phototrophic or fermentative microbes will allow directed improvement or alteration of metabolic pathways to increase yield of biofuel compounds and enable synthesis of a broader range of molecules that can be used as next generation biofuels. In addition, coupling these results with systems biology work on biomass degrading microbes provides the potential for development of new consolidated biomass processing approaches, in which a single organism (or

assemblage of organisms) performs coupled deconstruction of biomass and synthesis of biofuel compounds.

- Understand impacts of biomass crop production on stability of soil carbon pools and emission of greenhouse gases from agricultural soils.

Understanding impacts of biomass crop production on soil microbial community structure and function will allow expansion of a broader research agenda assessing overall storage of organic carbon in underlying soils, potential alterations to stability of carbon pools, and microbially-mediated emissions of carbon dioxide, methane, nitrous oxide, and other greenhouse gasses from underlying soils. Results of these studies will enable comparative analyses of impacts of different biomass feedstocks crops or agricultural practices on soil characteristics relevant to long-term sustainability of biomass production.

## **VI. Impact: Positive Outcomes for Science and Society**

Increased production of biofuels from cellulosic plant biomass will have numerous benefits to the US energy economy. These include:

- Increased knowledge of systems properties of microbes and plants, providing new biofuels production capabilities as well as enhanced foundational understanding of biological systems relevant to environmental, agricultural, and industrial processes
- Critical interdisciplinary training opportunities for the next generation of bioenergy researchers
- Greater portion of domestic transportation fuel needs being met with a more secure and renewable resource
- Decreased emissions of carbon dioxide and other pollutants associated with combustion of fossil fuels
- Generation of new jobs in biotechnology, agriculture, and industry associated with a new domestic biofuels economy