Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda

A Research Roadmap Resulting from the Biomass to Biofuels Workshop Sponsored by the U.S. Department of Energy

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John Houghton
Office of Science
Office of Biological and Environmental Research
301.903.8288
John.Houghton@science.doe.gov

Sharlene Weatherwax
Office of Science
Office of Biological and Environmental Research
301.903.6165
Sharlene.Weatherwax@science.doe.gov

John Ferrell
Office of Energy Efficiency and Renewable Energy
Office of the Biomass Program
202.586.6745
John.Ferrell@hq.doe.gov

Technical Strategy: Development of a Viable Cellulosic Biomass to Biofuel Industry

Innovative energy crops—plants specifically designed for industrial processing to biofuels—can be developed concurrently with new biorefinery treatment and conversion processes. Recent advances in science and capabilities, especially those from the nascent discipline of systems biology, promise to accelerate and enhance this paradigm. Resulting technologies will allow the fusion of agriculture, industrial biotechnology, and energy value chains to enable an efficient and economically viable industry for conversion of plant biomass to liquid fuels. Displacing up to 30% of the nation's current transportation fuel by 2030 will require significant policy support and technical advancement over the next 5 to 15 years. Research and technology development described in this roadmap will occur in three phases to enable industry to meet the 2025 and 2030 goals (see Fig. 1. Phased Development of Bioenergy Systems, p. 32). In the Research Phase (this page), application of genome-based systems biology will provide the basis of knowledge, concepts, and tools for concerted research and deployment of technology modules in the Technology Deployment Phase (p. 32). In the Systems Integration Phase (p. 34), both fundamental and applied research and technology development will support multiple bioenergy systems through concurrent development of crops and biorefinery processes for various U.S. agroecosystems. Drivers for each phase and the research targets and goals for Feedstocks, Feedstock Deconstruction, and Fermentation to Ethanol and Recovery are outlined in Table 1. Technical Strategy Timeline, p. 33.

Research Phase (within 5 years)

Optimizing cellulose processing by refining biomass pretreatment and converting crop residues, first-generation energy crops, and other sources to liquid fuels will be the near-term focus. This will entail reducing cost, enhancing feedstock deconstruction, improving enzyme action and stability, and developing fermentation technologies to more efficiently use sugars resulting from cellulose breakdown. One goal is to decrease industrial risk from a first-of-a-kind technology, allowing more rapid deployment of improved methods.

Feedstock Use and Optimization

A range of plant materials (e.g., corn stover and hard woods) with widely varying physical and chemical characteristics could be made available as feedstocks for conversion to ethanol in biorefineries. These legacy feedstocks are expected to satisfy one-fourth to one-third of the nation's anticipated transportation biofuel needs. To achieve higher production goals, new energy crops with greater yield per acre and improved processability are needed. Advanced genome-based capabilities will help determine how soil microbial communities function and how much carbon from crop residues and dedicated energy crops, as well as other nutrients, is needed to sustain soil ecosystem function and productivity.

To establish a new generation of plants as energy crops and develop stable agroecosystems, biological and chemical tools are needed to provide detailed understanding of plant cell walls, their roles in plant function, and factors controlling recalcitrance and optimization of processes for fermentation of sugars. Genome-based capabilities will identify genes involved in the synthesis of cell-wall polymers and higher structures; reactions performed by the multitude of enzymes involved; design principles of cell walls; and factors controlling the amounts, composition, and structure of polymers and polymer matrices. The complex structures of plant cell walls perform numerous critical functions in the plant's growth and maintenance. Only some of these functions are now understood. Plant engineering's end goal is to use rational design for preserving critical plant functions to maximize yield and agroeconomic factors, while optimizing plant biomass makeup and structure for creating biofuels and other products. Once desirable cell-wall traits are established for energy crops, modified varieties must be domesticated for robustness and yield, and bioprocessing steps must be adapted to the superior properties of these varieties.

Plant design, bioprocess engineering, and biomass-processing strategies are intimately linked. Plants have evolved complex mechanisms for resisting assault on their structural sugars (wall polymers) from the microbial and animal kingdoms. Cell-wall polymer organization and interactions are formidable barriers to access by depolymerizing enzymes and must be deconstructed in the pretreatment step to obtain adequate rates of release and sugar yields.

Deconstruction

Understanding factors governing plant cell-wall recalcitrance to processing and deconstruction-enzyme interactions with the cell-wall matrix is critical to achieving the integrated biorefinery concept. Current technologies for biomass pretreatment (breaking down lignin and hemicelluloses and freeing crystalline cellulose) rely on thermochemical processing, which degrades the resulting biomass and results in accumulation of inhibitors that are toxic to ensuing biorefining processes. These harsh and energy-intensive pretreatments must be replaced by more benign procedures, including those that will enhance existing hydrothermal and mechanical methods and free cellulose microfibrils. Economical enzymatic procedures that would allow greater potential for integrated biorefinery strategies also will be incorporated.

Genomics and the application of such relatively new tools as proteomics, metabolomics, and imaging will aid investigators in mining burgeoning genomic databases to understand how microorganisms attack biomass and take advantage of natural enzyme diversity. Available enzymes have relatively low specific activities, so enzyme interactions, mechanisms of action, and fundamental limits must be explored. We need to determine whether low activities reflect a fundamental limit to the hydrolysis rate of certain substrates or if rates can be improved by rational design through experimentation and modeling. New classes of ligninases and hemicellulases will be identified, their mechanisms of action understood, and their performance refined to allow introduction of enzymatic pretreatment that will free cellulose microfibrils for enzymatic saccharification (breakdown to sugars).

Better methods will produce inexpensive and more robust cellulases with higher activity and longer lifetimes for breakdown of cellulose microfibrils to sugars. Mechanistic principles of cellulose-degrading enzymes will be evaluated using a range of genes and proteins found in the biosphere to understand basic design principles and allow rational redesign for enhancing properties. The diversity of cellulase and cellulosome functional schemes will be modeled and optimized for specific biomass substrates (feedstocks). The microbial cellulosome is a unique type of molecular machine that can efficiently solubilize native and pretreated lignocellulose substrates. Cellulosomes can contain the full repertoire of plant cell-wall polysaccharide-degrading enzymes, and a single cellulose-binding module presents the entire complement of enzymes to the substrate surface (see sidebar, The Cellulosome, p. 102).

Fermentation and Recovery

Use of microbes for fermentation is the most common method for converting sugars produced from biomass into liquid fuels. To develop commercially viable processes for cellulose bioconversion to ethanol, an organism is needed that uses all sugars (cofermentation of C-5 and C-6 sugars) produced from biomass saccharification at rates and in high-alcohol concentrations that match or surpass current yeast-based glucose fermentations. These capabilities, along with process-tolerant traits, involve multiple genes and pathways that are not readily resolved. Today, the capability to introduce and control multiple gene changes simultaneously in an organism is limited.

New classes of fermentation organisms and enzymes capable of metabolizing both C-5 and C-6 sugars resulting from biomass deconstruction are required to advance bioprocessing. Vast and largely untapped biochemical potential in the microbial world may be accessible through the sequencing of new microbial genomes and community "metagenomes." As first-generation organisms are being tested and improved, the focus will be on advances that allow elimination of whole steps in the conversion process. For example, thermophilic microorganisms will be examined for their ability to ferment biomass sugars at elevated temperatures, allowing development of optimal and simultaneous saccharification and cofermentation (SSCF) and thus increasing overall process efficiency. Metabolic engineering with advanced biological diagnostics will be used to develop strains

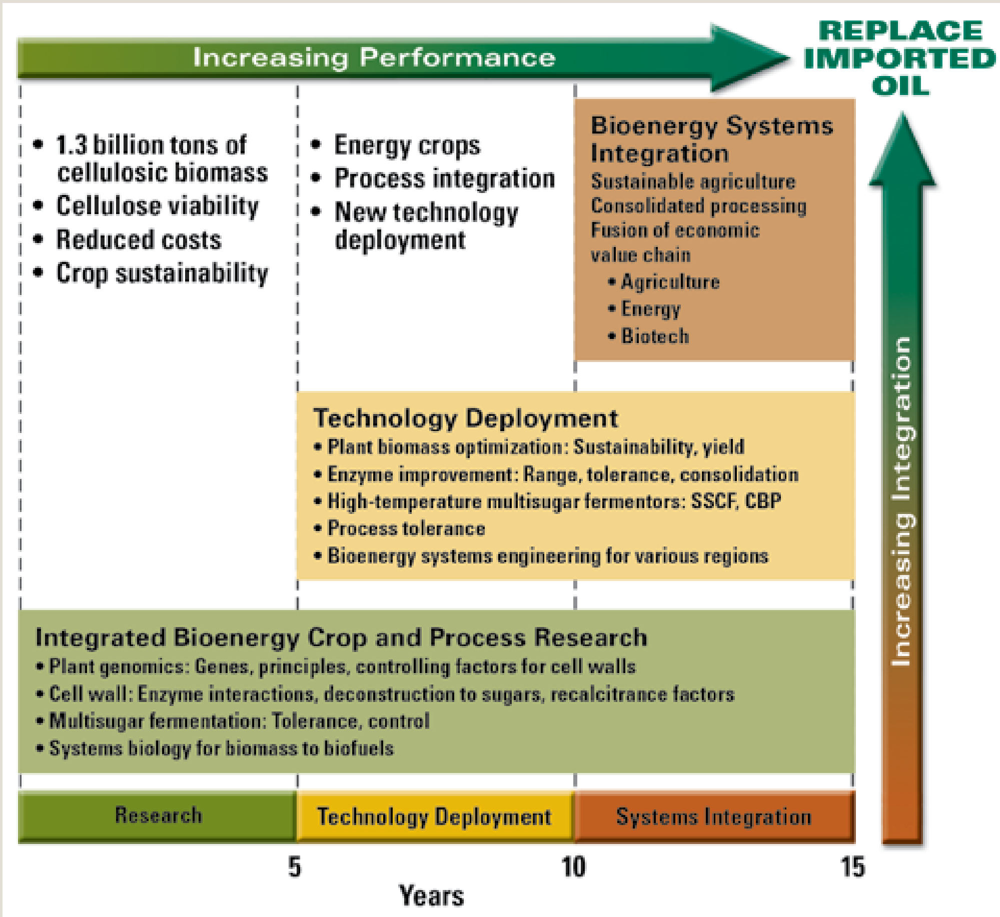

Fig. 1. Phased Development of Bioenergy Systems. Over the next 5 to 15 years, research and technology advancement will occur in three general phases. In the Research Phase, application of genome-based systems biology will provide the basis of knowledge, concepts, and tools for implementation in the Technology Deployment Phase. In the Systems Integration Phase, both fundamental and applied research and technology will support multiple bioenergy systems through the concurrrent development of crops and biorefinery processes for the various U.S. agroecosystems. Details are outlined in Table 1, p. 33.

with high tolerance to process stresses, inhibitors created in pretreatment, and high-alcohol concentrations. Genomic, proteomic, metabolomic, and imaging technologies, coupled with modeling and simulation, will elucidate the regulation and control of microbial metabolism and provide a predictive understanding of cell-design principles to support system engineering of integrated bioprocessing (see Fig. 1. Phased Development of Bioenergy Systems, this page, and Table 1. Technical Strategy Timeline, p. 33).

Technology Deployment Phase (within 10 years)

A new generation of dedicated energy crops with composition and structure better suited for breakdown to sugars for fermentation, high yield, and robustness will be essential in achieving energy security. Newly engineered deconstruction enzymes will enhance or supplant thermochemical processing to deconstruct more efficiently a broad range of biomass feedstocks. Ultimately, this will lead to enhanced energy-efficient and environmentally attractive processes for carrying out traditional pulping processes and other wood-processing techniques.

Table 1. Technical Strategy Timeline

<table>
<tr><th>Research Phase (0 to 5 years)</th><th>Technology Deployment Phase (5 to 10 years)</th><th>Systems Integration Phase (10 to 15 years)</th></tr>
<tr>
<td>Legacy Resources and Early Energy Crops (E-Crops)
Drivers
Expand biomass resource base and increase utilization
• Early E-crops
• Cellulose-processing viability
• Cost reduction</td>
<td>Transition: Modular Technology Deployment
Drivers
• Need for new energy crops to reach or exceed 1 billion tons of biomass
• Process simplification and improvement in modules
• Use of systems biology, chemistry
• Bioprocess engineering
• Rational systems design</td>
<td>Integration and Consolidation
Fusion of economic value chains
Drivers
• Consolidated bioenergy systems
• Technologies tailored for various regions
• Buildout</td>
</tr>
<tr>
<td>Feedstocks
E-crop sustainability
• Impacts on soil ecosystems, nutrients
• E-crop model development
• Genes, principles, E-crop subsystem controls
– Cell-wall makeup and structure
– Ties to deconstruction and fermentation</td>
<td>Feedstocks
• Understand plant as system
• Domesticate E-crops
• Enhance sugars, minimize lignin and toxic inhibitors
• Increase yield and soil sustainability</td>
<td rowspan="3">End-to-End Concurrently Engineered Biofuel Systems (Biome-E Crop Processing)
• Systems tailored to regions and fully consolidated processing
• Includes E-crops with enhanced composition
• Tool kits for plant engineering
• Consolidated processing tied to biofuel systems
– Tailored deconstruction enzyme mix
– Engineered microbial metabolic systems
– Stress and process tolerance
– Full system control
• Tool kits for rapid manipulation and diagnosis</td>
</tr>
<tr>
<td>Feedstock Deconstruction
• Reduce enzyme costs
• Understand enzyme-lignocellulose interactions
– Cell-wall recalcitrance
• Survey natural enzyme diversity
• Establish fundamental enzyme limits
• Develop ligninases and hemicellulases
• Develop gene-transfer systems for cellulolytic machines and entire pathways</td>
<td>Deconstruction
Deploy
• Improved enzymes (rate, specificity)
– Broadened substrate range
– Reduced inhibition
– E-crop concept
• Tools to diagnose and manipulate enzyme substrate interactions
• Tools to design and improve enzymes</td>
</tr>
<tr>
<td>Fermentation to Ethanol and Recovery
• Study use of all sugars, including direct cellulose utilization
• Study stress response and inhibitors
– High-alcohol and -sugar concentrations
• Understand regulation and control
• Survey natural diversity</td>
<td>Fermentation and Recovery
Deploy
• Cofermentation of C-5 and C-6 sugars
• New strains (multiple)
– Stress tolerance
– High temperature
• Tools for full regulatory control
• Tools for rapid analysis and manipulation
• Testing of consolidated organisms possessing celluloytic and ethanologenic properties</td>
</tr>
</table>

Mid-term technology will begin the consolidation of process steps. For example, improved organisms will be engineered with the ability to ferment mixed sugars, demonstrate resistance to toxic substances, and produce effective deconstruction enzymes. Systems biology and a new generation of synthetic and analytical organic chemistry will be critical for understanding these bioenergy systems and for predicting and modifying their function.

Feedstocks

Plants intended for biomass production and downstream processes involving conversion to sugars and, ultimately, ethanol will be understood and designed as a system. New breeds of energy crops will be introduced with enhanced sugar content and optimized cell-wall structures for processing, including minimization of lignin and inhibitor precursors. Plant domestication and sustainable agroecosystems based on perennials engineered to increase yield, productivity, and tolerance to such stressors as drought and salinity will reach a mature state. Multiple crops will be developed for various regional and global agroecosystems.

Deconstruction

This phase will result in deployment of improved pretreatment procedures and saccharifying enzymes with enhanced catalytic rate and substrate specificity, a broader range of applications, and reduced inhibitor production. Improved understanding of cell-wall recalcitrance and enzyme action will provide design specifications for new energy crops. Advanced high-throughput biological and chemical tools will be available to diagnose and manipulate enzyme-substrate interactions. Improved biocatalysts with desirable traits can be rationally designed for specific feedstocks and incorporated into molecular machines such as cellulosomes.

Fermentation and Recovery

New strains of industrial-processing organisms with such novel capabilities as cofermentation of C-5 and C-6 sugars and high tolerance to inhibitors, alcohol end product, and temperature will contribute to a more energy and product efficient bioprocess. Systems biology investigations will have produced a predictive understanding of cellular metabolism and regulatory controls in key fermentation microbes. This knowledge will serve as a foundation for rational development of new strains with consolidated subsets of pretreatment, hydrolysis, and fermentation capabilities. High-throughput biological and chemical tools, including computational modeling for rapid analysis and manipulation in the laboratory and in production environments, will be available.

Systems Integration Phase (within 15 years)

Bioenergy systems will include a concurrently engineered set of designer energy crops for specific agroecosystems, deconstruction and saccharification enzymes, and robust fermentation. Incorporated as multiple processes in plants or microbes, these methods will accelerate and simplify the end-to-end production of fuel ethanol, enabling flexible biorefineries that can operate on a regional scale.

Integration and Consolidation

Creation of fully consolidated bioenergy systems tailored for specific regional climate and soil characteristics will allow buildout of these sustainable bioenergy economies. These systems will meld feedstocks, biomass deconstruction, bioprocess engineering, and fermentation research and development, yielding optimal two-step processes. The first step is based on consolidated feedstock traits, and the second is based on consolidated microbial traits. A concurrently engineered end-to-end biofuel system using advanced systems biology and chemical-analysis capabilities will be practicable. Toolkits for plant and agroecosystem engineering will support systems tailored to regions and consolidated processing. Companion consolidated bioprocess engineering will be tied to agroecosystems; with tailored enzyme mixes, engineered microbial metabolic systems will incorporate stress and process tolerance and permit full system control. Instrumentation available both in facilities and in the field will enable rapid diagnosis and manipulation of all critical aspects of the integrated biorefinery.