

# 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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# Bioprocess Systems Engineering and Economic Analysis

Complete process modeling should be initiated to guide the scientific work described in this Roadmap, including systems engineering and economic analyses, to evaluate the most-probable scenarios; to coordinate advances and needed research across the feedstock, deconstruction, and fermentation domains; and to reduce risk as the development cycle proceeds. Results and methodologies should be made available to the community through the web and should be subject to continuous improvements based on community feedback.

Biomass-conversion literature has many examples of pretreatment, hydrolysis, and fermentation systems that are technically effective but have no real chance to be competitive economically. A common tendency among biotechnologists is to attempt processes that will achieve, for example, the highest yields, rates, and titers. Process modeling, however, very often reveals that different combinations of these parameters are needed to produce a commercially viable process. Disciplined systems engineering and economic analysis using mass balances and standard analytical methods can eliminate ineffective approaches rather quickly, narrowing the focus to the most-promising options. Current advanced saccharification enzyme systems have demonstrated what a concerted, focused program of enzyme development can achieve. These systems, however, were designed for a specific acid-based pretreatment and the biomass raw material of corn stover. Other combinations of pretreatments and biomass materials will need to be analyzed and subjected to process engineering and economic analyses as they mature.

## Research Goals

This work seeks to integrate improvements in the molecular biology of plant materials and those in pretreatment, enzyme hydrolysis, and fermentation while conducting rigorous bioprocess systems engineering (incorporating new processes into a model refinery) and economic analysis. The approach integrates all elements of enhanced and cost-effective means to convert biomass into ethanol and other bioproducts.

Practical options for lignocellulose conversion to liquid fuels are sharply constrained by the need to produce fermentable sugar costing a few cents per pound, preferably with minimal sugar loss and degradation. Furthermore, in a biorefinery, all downstream processes will be affected by pretreatment choice. Process integration, therefore, is crucial. Finally, no evidence suggests that biological methods alone (i.e., without a thermochemical pretreatment

step) will be able to obtain the high-yield, low-cost conversions of cellulose and hemicellulose required for economic viability. Research elements would include (1) correlation of cell-wall chemistry and structure with the efficiency of cellulase polysaccharide depolymerization, with and without pretreatment; (2) multidisciplinary studies of specific targeted lignocellulosic materials with promising pretreatments and advanced enzymes, using standard analytical methods and detailed mass balances; (3) examination of the effects of individual and combined enzymatic activities on pretreated substrates, with the primary technical-evaluation criterion of maximum sugar yield at minimum enzyme loadings; and (4) process engineering and economic modeling of these integrated pretreatment and hydrolysis studies to identify promising combinations and eliminate less-attractive options. This work must be coordinated with all domains of the research portfolio to have the desired effect—optimal process development.

Considering the goal to develop one or more commercial processes for ethanol production from biomass, a proper evaluation of most-probable future scenarios is needed. For example, several groups envision simultaneous saccharification and fermentation (SSF) for a more viable process. Since saccharification enzyme mixes work better at 50°C and a pH between 4 and 6, then as indicated by other study groups, “*none of the current ethanol producers’ microorganisms is suitable.*” Under this scenario the parameters of saccharification must be included in designing new production microorganisms. Process modeling is needed to evaluate the importance and sensitivity of SSF vs a two-step process. Similarly, ethanol fermentations will be evaluated by three main parameters: Yield from carbohydrates, rate of production, and final titer.

Modeling will be very useful in evaluating the feasibility of consolidated bioprocessing. As an example, current specific-activity values of the three enzymes used today to degrade cellulose (i.e., exoglucanases, endoglucanases, and beta-glucosidases) may possibly allow prediction of the protein amount that a “consolidated strain” will have to produce per unit of time to degrade cellulose and liberate sugars quickly enough to sustain fast rates of ethanol production.

## Milestones

### Within 5 years or less

Initial research should address correlation of changes in plant cell-wall structure with ease of hydrolysis. This could be done through chemical composition or nanoscale and molecular-scale imaging (such as would be available from National Renewable Energy Laboratory). The relationship between changes in plant cell-wall structure (e.g., lignin composition) would enable better formulation of more-effective and more-productive advanced enzymes in their interactions with a solid substrate. Emphasis would be on coupling changes in plant cell-wall structure, changes in pretreatment conditions, and decreases in enzyme usage to achieve conversion extent and rate currently attained with much-higher enzyme loadings.

### **Within 10 years**

Once scientists determine the impact of changes in plant cell-wall structure on hydrolysis extent and the ease with which a plant cell wall can be deconstructed, further mechanistic studies for improving the efficiency of enzyme hydrolysis will be carried out. Certain types of plant cell walls may have lignin content higher than or different from current biomass materials; utilization of enzymes including lignases, hemicellulases, and cellulases could be considered. A model would be helpful, for example, in which a lignin-rich material is converted efficiently into its fractions of lignin, cellobiosaccharides, and xylans. Lignin could be used as a source of energy for a cellulose-to-ethanol processing plant. The proper composition balance in lignin to polysaccharides could make such a facility self-sufficient with respect to energy usage. Systems integration of these various steps will be carried out in a longer-term project. Scientists expect initial improvements in the efficiency of cellulose-to-ethanol conversion in an existing plant (such as a dry mill) to be possible within 10 years.

### **Within 15 years**

Engineering, molecular evolution of new enzymes, and genetic modification of agronomic traits should be combined to yield a vertically integrated technology for converting biomass materials to ethanol and other bioproducts. Combining molecular biology with process engineering may yield plants that are robust in the field but easily accessible for conversion using specialized enzymes added after harvest. Transportation of biomass materials is known to be a key cost factor, so enzymes integrated into plant material could make it more compressible after harvest. Lignin-degrading enzymes may make possible high-lignin plants (including certain types of grasses) that are easily deconstructed once exposed to proper conditions in a processing plant, yielding an adequate amount for powering the facility.

### **The Role of GTL and OBP Facilities and Capabilities**

The challenge in using advanced analytical and modeling capabilities is to integrate research vertically across several domains into a viable, technically and economically sound biorefinery concept. Research results must be rapidly assimilated into an overall systems model that will provide insight into the impact of new phenomena or processes; concomitant technologies must be incorporated into the system to optimize these effects. Such analysis will guide a vertically integrated research portfolio that supports all aspects of bioprocess engineering. A summary follows.

### **Protein Production**

Protein production capabilities will be critical in generating and characterizing new proteins and biomarkers for imaging and for identifying key protein-protein interactions, including enzymes for lignin, polysaccharide, and hemicellulose ester degradation.

## **Proteomics**

Proteomic measurements can provide valuable information about proteins and mechanisms by which monosaccharide conversion to ethanol and other products is achieved. Combinations of native and genetically modified organisms can achieve better conversion in existing ethanol facilities.

## **Cellular Systems**

Cellular systems analysis and modeling may be employed to generate and monitor microbial cells and communities that would enable expression of new types of enzymes or proteins so sufficient quantities are available for research.

## **DOE Joint Genome Institute**

DOE JGI will be important in discovering new microorganisms and enzymes that may be useful in converting more-recalcitrant forms of cellulosic materials to monosaccharides.