Enhanced Conversion of Lignocellulose to Biofuels: Bioprocess Optimization from Cellulose Hydrolysis to Product Fermentation

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Our program will address several of the major bottlenecks impeding the practical production of biofuels, such as ethanol and butanol, from cellulosic feedstocks. The program has several inter-related components, which will interface closely with complementary research performed throughout the EBI. The scope of the program spans the discovery and application of new thermophilic organisms as enzyme sources and/or for biofuel production, protein engineering and kinetic modeling of improved cellulases, cellular engineering for improved solvent tolerance, and bioprocess engineering to optimize fermentation. The specific components of the program are summarized below.

Bioprospecting for High-Temperature Conversion of Lignocellulose to Ethanol

Lignocellulose degradation systems from extremely thermophilic microorganisms are ideal candidates for the development of more active, cost-effective enzymes for cellulose processing. Elevated operating temperatures would also be beneficial in fermentations to produce biofuels. In addition to lower risk of microbial contamination, a higher temperature would reduce cooling costs and facilitate ethanol (or, for example, butanol) removal and recovery. To enable translation of these advantages to practice, we propose to isolate and characterize multisubunit extracellular and periplasmic glycolytic enzymes in several extremely thermophilic bacterial strains specifically adapted for cellulose and hemicellulose degradation. We will also isolate,novel extreme thermophiles that produce ethanol and/or butanol from enrichments of hot spring samples previously collected in Eastern Russia and the continental US. Prospecting for cellulose/hemicellulose degradation systems will be assisted by whole genome sequencing of novel isolates. Another component of the proposed effort is the development of a simultaneous saccharification and fermentation process for operation near the boiling point of ethanol. Ethanol production during saccharification of cellulose/hemicellulose at 75ºC will increase process efficiency, minimize contamination, and facilitate evaporative removal of the fuel product. Individual thermophilic bacterial strains with high rates of specific lignocellulose digestion will be sequenced and the cellulose and xylanase genes will be expressed in productive recombinant host strains.

High-Throughput Solid-Substrate Cellulolytic Screens and Directed Evolution of Improved Cellulases

Protein engineering has proven to be a powerful tool in creating enzymes with new and improved properties; however, designing and employing methods to screen or select cellulase mutants using *solid* cellulosic substrates remains a largely unmet challenge. This proposal seeks to overcome this challenge, as well as that of developing more cost-effective cellulases, by developing high-throughput solid substrate assays and applying them in the directed evolution of thermophilic cellulases (e.g., T_{opt} = 80ºC). The methodology developed will be applicable to

the generation and study of improved cellulases that can be used in various process configurations for the production of biofuels from cellulosic biomass.

Mechanistic Kinetic Modeling for Optimal Cellulase Design and Cellulose Hydrolysis

Accurate kinetic models of cellulose hydrolysis by cellulases (Figure 1) are of critical importance for evaluating cellulase-component compositions and for designing and optimizing processes for cellulose conversion to biofuels. Such models will also aid in the development and characterization of improved cellulolytic systems generated by protein engineering and synthetic biology. We propose to develop a comprehensive model of cellulose hydrolysis that can be used to predict cellulase performance, guide cellulase design, and optimize the hydrolysis of various cellulosic substrates, including those obtained from EBI investigators. Reaction rate data will be collected in batch reactors for mesophilic and thermophilic cellulases and analyzed using the model to determine the kinetics of hydrolysis and the corresponding rate law parameters.

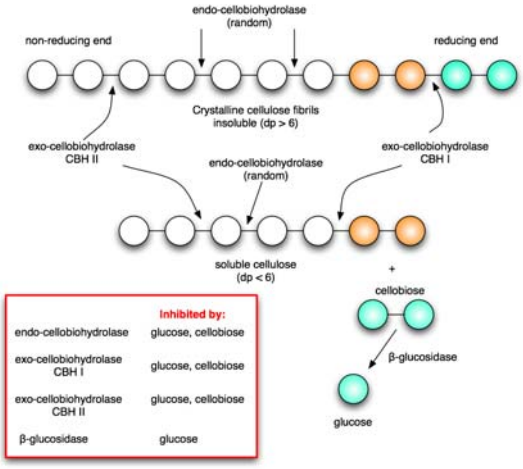

Figure 1. The celluose chain is randomly cleaved by endo-cellulase to provide both reducing and non-reducing free ends. Exocellulases bind to these and release cellobiose which is subsequently hydrolyzed to glucose monomers.

Alleviating Product Toxicity in Biofuel Production

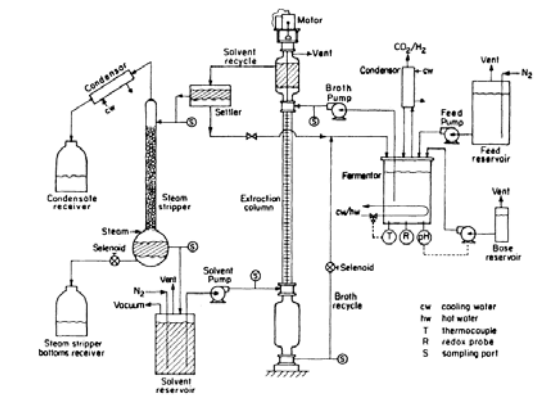

Figure 2. Schematic design of experimental apparatus for continuous *in situ* extraction of biofuels during fed-batch fermentation.

The development of new microbes with greater tolerance toward the final fuel product, e.g., butanol, could lead to substantial improvements in the cost effectiveness of producing biofuels from cellulosic biomass. We propose to engineer enhanced tolerance toward butanol into *E. coli* and solventogenic organisms, including yeast and *Clostridia* sp. By building upon previous studies in our laboratory showing that the effects of product inhibition during acetone-butanol fermentations can be reduced by extractive fermentation (Figure 2), an extractive fermentation system will be set up and used to optimize *in situ* product removal in fed-batch fermentations by the high solvent-producing strains. Higher intrinsic butanol tolerance combined with extractive fermentation is expected to result in extremely high production rates and volumetric productivities of biobutanol.