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Dynamic Modeling of Synthetic Microbial Consortia to Optimize the Co-fermentation of Glucose and Xylose

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Abstract
Second-generation biofuels have the potential to replace fossil fuels in the energy economy without negatively impacting the food supply. An effective biocatalyst must be able to convert all sugars found in lignocellulosic hydrolysates to biofuels. Few microbes exist in that have both a wide substrate range and high ethanol yields necessary for this process. Mixed culture biotechnology is a promising alternative to the use of single organisms in the production of fuels from lignocellulosic biomass. These systems mimic natural processes for the degradation of lignocellulose and exploit the native capabilities of each microbe. The segregation of metabolic pathways allows for the individual optimization of each step in the process. Preliminary work with a consortium capable of saccharification and fermentation showed promise, but the dynamics were poorly understood. Metabolic modeling is a powerful tool for understanding the interactions between microbes in mixed cultures. The development of accurate models of mixed culture metabolism will help

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drive the engineering of these systems for industrial applications.

In this dissertation, dynamic flux balance analysis is applied to mixed culture systems by combining mathematical reconstructions of pure culture metabolism. By tuning the inoculum to sugar concentration, simulations of *Saccharomyces cerevisiae* and *Escherichia coli* mutants engineered to ferment a specific substrate display the potential for improved ethanol production over pure cultures. A framework for translating model predictions to experimental systems was developed for a co-culture of *S. cerevisiae* and xylose-specific *E. coli*. The consumption of sugar mixtures was optimized through this method, but the inability of the predicted gains in ethanol production to be replicated in experimental systems reveals the importance of selecting microbes with similar optimal growth conditions. The more compatible microbes *S. cerevisiae* and *Scheffersomyces stipitis* were modeled under microaerobic conditions to optimize ethanol production from a mixture of glucose and xylose. To further demonstrate the ability of these systems to ferment lignocellulosic hydrolysates, the effect of furan inhibitors on pure and co-cultures was assessed through modeling and experiment. The work presented here represents the first steps towards engineering and optimizing a microbial consortium for the production of ethanol from lignocellulosic biomass.

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