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Introduction to Session 2: Enzyme Catalysis and Engineering

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The role of enzymes in biomass-to-fuels is rapidly expanding from a narrow focus on the hydrolysis of cellulose remaining after pretreatment to a more comprehensive approach, where increasing attention is focused on noncellulase activities, including hemicellulases, lignin-modifying, and accessory enzymes. This transition is being driven from several directions. Foremost is the realization that pretreatment may be best viewed as an activation or preconditioning step in the hydrolysis of biomass. Pretreatment conditions severe enough to produce highly accessible biomass for subsequent enzymatic conversion may be saddled with the significant problems of high cost, yield losses, and inhibitor formation. Mild severities may significantly reduce these problems; however, lower severity pretreatment results in higher recalcitrance, and often higher chemical complexity compared to more severely pretreated biomass.

The broadening diversity of biofuel feedstocks, the varied thermochemistry of the dozen or so pretreatment technologies, and a wide range of severities for each pretreatment all contribute to a complex picture when assessing the nature of the substrate to be converted by biomass-to-fuels enzymes. The likelihood that a single enzyme system will be able to efficiently deconstruct a wide variety of highly complex pretreated substrates is considered to be low. This complexity, however, can be addressed through several variations in the enzyme system employed, including more aggressive enzyme loadings, increased specific activities, altered substrate specificities, and tailoring enzyme cocktails to specific causes of recalcitrance. Although each approach has its limitations and benefits, it is readily apparent that matching the enzyme activities to a given substrate may be a route for cost-effective minimization of the drawbacks of more severe pretreatments, while maintaining the yields and efficiencies required for an economical biomass conversion process.

Session 2, Enzyme Catalysis and Engineering, addressed many of the issues detailed above. The main session focused on the functionality of natural enzyme systems in the

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hydrolysis of a diverse range of feedstocks under different process conditions. Hydrolysis of insoluble chitin and soluble chitosan by a three-enzyme system was shown to be directly affected by the binding residues near the catalytic site. More insights were gained into the mechanism of multi-enzyme cellulose hydrolysis by *Trichoderma reesei*. In more process-related research, the effect of ethanol on enzymatic hydrolysis of biomass was investigated, as was the conversion of two niche-feedstocks, barley straw and canary grass, to ethanol. Session 2A examined the role of hemicellulase activities such as ferulic acid esterase, β-xylosidase, β-xylanase, and α-L-arabinofuranosidase in the hydrolysis of biomass. Session 2B focused on the noncatalytic parameters involved in enzyme hydrolysis of biomass. The effect of water was a dominant theme in these talks and a new mechanism of binding by the Family 1 carbohydrate binding module based on molecular modeling was presented.

Overall, Session 2 provided a very good panoramic snapshot of the current research focusing on enzymes for biomass conversion. The diversity and quality of the work indicate that this area of biomass conversion research is very vigorous and is continuing to expand in scope. As feedstock diversity and pretreatment protocols continue to expand, the role of enzymes will become increasingly critical to maintaining high yields, low losses, and efficient conversion rates. The synergy between the biomass-to-fuel trinity of feedstock diversity, pretreatment thermochemistry, and enzyme activity will be the cornerstone of a successful biomass conversion industry.