Advances in Lignin Valorization via Biological and Catalytic Transformations

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

In most biorefinery designs, lignin is slated to be burned for heat and power because its inherent heterogeneity and recalcitrance make it difficult to valorize. Despite nearly a century of lignin depolymerization research, most catalytic strategies to break down lignin yield a heterogeneous slate of aromatic compounds, which makes purification of single, high-yielding co-products from lignin quite daunting.

Reductive catalytic fractionation (RCF) has emerged as a leading biomass fractionation and lignin valorization strategy. We will highlight recent work using flow-through reactors to investigate RCF of poplar. A new flow-based RCF process enables the acquisition of intrinsic kinetic and mechanistic data essential to accelerate the design, optimization, and scale-up of RCF processes. We examined time-resolved product distributions and yields obtained from experiments with different catalyst loadings to identify and deconvolute events during solvolysis and hydrogenolysis. Multi-bed RCF experiments provided unique insights into catalyst deactivation, showing that leaching, sintering, and surface poisoning are causes for decreased catalyst performance. The onset of catalyst deactivation resulted in higher concentrations of unsaturated lignin intermediates and increased occurrence of repolymerization reactions, producing high molecular weight species. This initial study demonstrates the concept of flow-through RCF, which will be vital for scale-up of this promising approach as well as enabling the survey of many biomass samples to understand how lignin chemistry affects RCF yields, such as those from the Center for Bioenergy Innovation's Genome-Wide Association Study (GWAS) libraries, which will be pursued in future work.

Additionally, some microbes have evolved catabolic pathways that enable the utilization of lignin-derived aromatic molecules as carbon and energy sources. Aromatic catabolism most commonly occurs via Upper Pathways that act as a “biological funnel” to convert heterogeneous lignin-derived substrates to Central Intermediates, such as syringate, protocatechuate, or catechol. These compounds subsequently undergo oxidative ring cleavage and are further converted to central carbon metabolism. In the Center for Bioenergy Innovation, we employ the robust aromatic-catabolic microbe, *Pseudomonas putida* KT2440, to understand, harness, and expand these powerful metabolic pathways to convert both aromatic model compounds and heterogeneous, lignin-enriched streams to value-added compounds such as ß-ketoadipate. Here, we will present several recent insights into lignin depolymerization and aromatic catabolism by *P. putida* KT2440.

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