Exploring the Aging of Lignin Breakdown Products for Biological Conversion

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Project Goals: We aim to combine adaptive evolution and multi-omic approaches to identify aromatic tolerance mechanisms in *Rhodococcus opacus*. We aim to utilize identified genes and pathways to engineer an optimized *R. opacus* strain for the conversion of lignocellulose into valuable products.

Future commercial viability of second generation biofuel and biochemical production depends on lignin valorization. To this end, we have developed a hybrid (i.e., thermo-catalytic and biological) conversion platform to generate value-added products from lignin. This hybrid conversion platform consists of two sequential processes: a thermo-catalytic process that depolymerizes lignin into an aqueous-soluble lignin breakdown products (LBPs) and a biological process that converts and funnels compounds in LBPs into a single bio-product. To overcome well-known challenges to lignin depolymerization, LBPs are generated via a "lignin-first" depolymerization process using an activated carbon supported pallium catalyst and an alkaline solvent from milled poplar. In a practical hybrid conversion process, LBP may have to be stored prior to biological conversion. Moreover, complete microbial utilization of the LBP can take up to 72 hours. During our early efforts, we noted that over the course of several days, our LBP underwent a significant color change while a solid precipitant formed. As a result we studied, the stability of LBPs stored at different temperatures (room temperature and 4 °C) over the course of 32 days. We characterized the LBPs for compositional changes and changes in the growth and utilization of the LBP by the bacterium, *Rhodococcus opacus*, as a function of aging time.

This work is funded by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research, Biological System Sciences Division, award # DE-SC0018324.