Enzymatic Synthesis of Xylan Microstructures

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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 addresses 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, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Xylans are a family of hemicellulosic polysaccharides found within the cell walls of many plants and algal species and consist of a linear backbone of β 1,4 linked xylosyl residues that are often substituted with other glycosyl or acetyl substituents. Xylans are known to interact with cellulose and lignins in the plant cell wall and are a known contributor to biomass recalcitrance to enzymatic conversion for fuels and products.

Here we demonstrate the ability of a recombinantly expressed xylan synthase to produce xylan microcrystalline aggregates with a well-defined morphology *in vitro,* using UDP-xylose as a glycosyl donor and short xylan oligosaccharides as acceptors. We have investigated the conditions under which these aggregates form and the kinetics of these aggregation events with varied acceptor concentrations. Microcrystals produced from the enzymatic polymerization and aggregation of xylan chains appear to form hexagonal structures with an average diameter of 6-8 μM; these microcrystals exhibit birefringent properties when subjected to cross-polarized light. Furthermore, xylan microcrystal crystallinity, morphology, and properties are influenced by fine structural elements, such as the structure of sidechain branching on the xylan acceptor. Together, these results suggest a route to new biobased materials and help to inform our understanding of polysaccharide dynamics and interactions.

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