**Effects of lignin on cellulase during lignocellulosic biomass saccharification**

Non-productive binding of enzymes to lignin is thought to impede the saccharification efficiency of pretreated lignocellulosic biomass to fermentable sugars. Due to a lack of suitable analytical techniques that track binding of individual enzymes within complex protein mixtures and the difficulty in distinguishing the contribution of productive (binding to specific glycans) versus non-productive (binding to lignin) binding of cellulases to lignocellulose, there is currently a poor understanding of individual enzyme adsorption to lignin during the time course of pretreated biomass saccharification. Researchers in the DOE’s Great Lakes Bioenergy Research Center utilized an FPLC (fast protein liquid chromatography)-based methodology to quantify free Trichoderma reesei cellulases (namely CBH I, CBH II, and EG I) concentration within a complex hydrolysate mixture during the varying time course of biomass saccharification. Three pretreated corn stover samples were included in this study: Ammonia Fiber Expansion, dilute acid, and ionic liquid pretreatments, which are the pretreatments being researched by the 3 DOE-funded BRCs. The relative fraction of bound individual cellulases varied depending not only on the pretreated biomass type (and lignin abundance) but also on the type of cellulase. Acid pretreated biomass had the highest levels of non-recoverable cellulases, while ionic liquid pretreated biomass had the highest overall cellulase recovery. CBH II has the lowest thermal stability among the three T. reesei cellulases tested. By preparing recombinant family 1 carbohydrate binding module (CBM) fusion proteins, they showed that family 1 CBMs are highly implicated in the non-productive binding of full-length T. reesei cellulases to lignin. Their findings aid in further understanding the complex mechanisms of non-productive binding of cellulases to pretreated lignocellulosic biomass. Developing optimized pretreatment processes with reduced or modified lignin content to minimize non-productive enzyme binding or engineering pretreatment-specific, low-lignin binding cellulases will improve enzyme specific activity, facilitate enzyme recycling, and thereby permit production of cheaper biofuels.

**References:** Gao, D., Haarmeyer, C., Balan, V., Whitehead T.A., Dale, B.E., Chundawat, S.P.S. “Lignin triggers irreversible cellulase loss during pretreated lignocellulosic biomass saccharification”. Biotechnology for Biofuels(2014). 7:175

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