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**Saccharification of thermochemically pretreated cellulosic biomass using native and engineered cellulosomal enzymes**

Using microbes like *Clostridium thermocellum* for consolidated bioprocessing of biomass enables one-pot production of fuels

**The Science**

Pretreating lignocellulosic biomass using microbes such as *C. thermocellum* enables a one-pot process for breaking down sugars and fermenting those sugars for fuel and chemicals. In this study, we examined the bacterium’s efficiency in breaking down cellulose in industrially relevant pretreated biomass, finding that pretreatments that remove both lignin and hemicellulose can help improve the specific activity of the bacterium’s cellulosomal enzymes.

**The Impact**

Our research on *C. thermocellum*, a bacterium that deconstructs biomass using large, multi-enzyme complexes called cellulosomes, provides insight into how to overcome the challenge of deconstructing lignocellulosic-derived sugar polymers, and may lead to the more efficient and cost-effective use of cellulosic biomass in making fuels and chemicals.

**Summary**

Researchers in the Great Lakes Bioenergy Research Center compared the hydrolytic activity of the most abundant cellulosomal enzymes from *C. thermocellum* and investigated the importance of enzyme complexation using a model engineered protein scaffold called a “rosettasome.” We tested the hydrolytic performance of non-complexed enzymes, enzyme-rosettasome/rosettazyme complexes, and cellulosomes on distinct cellulose allomorphs formed during pretreatment of biomass. The scaffold-immobilized enzymes always gave higher activity than the free enzymes; however, cellulosomes exhibited higher activity than rosettazyme complexes. This was likely due to the greater flexibility of the native versus engineered scaffold, as deciphered using small angle X-ray scattering. Scaffold-tethered enzymes gave lower saccharification yields on industrially relevant lignin-rich switchgrass than cellulose alone, as well as comparable activity on all the cellulose allomorphs tested. These results indicate that the type of pretreatment can significantly impact the saccharification efficiency of cellulosomal enzymes for various consolidated bioprocessing scenarios, and pretreatments that remove both lignin and hemicellulose can help improve the specific activity of cellulosomal enzymes.

**Contacts (BER PM)**

N. Kent Peters  
Program Manager, Office of Biological and Environmental Research  
[kent.peters@science.doe.gov](mailto:kent.peters@science.doe.gov), 301-903-5549

**(PI Contact)**

Bruce E. Dale  
Michigan State University  
bdale@msu.edu

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**Publications**

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