Cellulose DP and accessibility contribute to cellulase enzyme action on biomass substrate

Background

• The enzymatic hydrolysis of lignocellulosic biomass proceeds initially at a fast rate followed by a rapid decrease in the conversion rates. The exact mechanisms leading to this rate decrease are not fully understood.

Approach

- *Populus* and switchgrass biomass samples were treated with dilute acid and alkaline pretreatments and subjected to enzymatic hydrolysis for 72 h.
- Cellulose accessibility and degree of polymerization (DP) of pretreated and incomplete hydrolyzed biomass were characterized to understand the fundamentals of biomass recalcitrance and limitations occurring during enzymatic hydrolysis.
- "Restart" hydrolysis, which involves disruption of batch hydrolysis and addition of fresh buffer/enzyme, was used to assess if substrates lose their reactivity with conversion.

Outcomes

- DP analysis during hydrolysis suggested a synergistic action of endo- and exo-glucanases that contribute to the occurrence of a "peeling off" mechanism.
- Cellulose accessibility analysis showed that limited accessible surface area of cellulose is probably not a major limiting factor that causes the decline of hydrolysis rate in its late stage.
- Restart hydrolysis experiment suggested that enzyme related factors such as enzyme inactivation or steric hindrance of enzymes should be responsible to the reduction in hydrolysis rate given the large size of cellulase enzymes.

Significance

• The results obtained here should provide insight into the mechanism of enzymatic hydrolysis of heterogeneous lignocellulosic biomass, and along with the method by which pretreatment aids enzymatic hydrolysis, they should be extremely helpful for the selection or development of biomass pretreatment for different biomass substrates.





Changes of cellulose accessibility measured by Simons' stain of untreated and pretreated poplar and switchgrass during 72 h enzymatic hydrolysis.



One-hour hydrolysis rate versus cumulative cellulose conversion for uninterrupted and restarted hydrolysis of DAP poplar (a), DAP switchgrass (b), Alkaline poplar (c), and Alkaline switchgrass (d).



3