Advances in High Throughput Screening of Biomass Recalcitrance

Geoffrey B. Turner1,2, Stephen R. Decker1,2, Melvin P. Tucker1,2, Cody Law4, Crissa Doeppke3,2, Robert W. Sykes1,2, Mark F. Davis1,2, Angela Ziebell1,2 National Renewable Energy Laboratory; 2BioEnergy Science Center; (contact – angela.ziebell@nrel.gov)

Method Development

Advances in high throughput screening of biomass recalcitrance methods have resulted in improved conversion and replicate precision. Changes in plate reactor metallurgy, improved preparation of control biomass, species-specific pretreatment conditions, and enzymatic hydrolysis parameters have reduced overall coefficients of variation to an average of 6% for sample replicates. These method changes have improved plate-to-plate variation of controllable biomass-recalcitrance and improved confidence in sugar release differences between samples. With smaller errors plant researchers can have a higher degree of assurance more low recalcitrance candidates can be identified.

Method Development

Effects of plate metallurgy on sugar release: Sugar release is highly dependent on plate materials-of-construction. Initial plate reactors were made of aluminum with various corrosion-resistant coatings. The severe pretreatment conditions resulted in coating failure through cracks and peeling, resulting in aluminum leaching into the reaction mix during pretreatment.

The combined effects of inhibited sugar release, degradation of final products, and limitation of cellulase activity by metal ions resulted in ~3-fold lower sugar release for aluminum reactor plates versus Hastelloy, a high nickel alloy.

Biomass-specific pretreatment conditions:

- Target ~70% glucose release on poplar after 180°C pretreatment using low (x2), medium (x4) and high (x8) enzyme loading:
  - Digested using Spezyme and Novo 188 (70 and 3 mg enzyme / g biomass respectively)
  - Incubated at 40°C for 70 hours
  - Analyzed using GOPOD/XDH assays

- Enzyme age and composition was held constant as a template, pretreatment conditions were modified to achieve the desired level of sugar release for switchgrass.
  - Target 70% glucose release
  - Preferably keep either time or temperature the same as the poplar method

Different pretreatment time and temperatures were tested, resulting in a range of combined pretreatment/enzyme hydrolysis glucose conversion ranging from 13-83%. Pretreatment at 180°C for 17.5 min was chosen, as the glucose yield was well above `no pretreatment/no severity pretreatment` but was not so high as to limit our ability to detect increased conversion levels for improved variants. The first set large scale of switchgrass (~1450) is in progress.

Summary

Significant changes in plate reactor, control biomass preparation, pretreatment conditions and enzyme have shown significant reduced sample and control replicate variability.

- Reactor plate metallurgy significantly impacts sugar release
  - Metal leaching into reaction during pretreatment degrades sugars and inhibits enzyme activity
- Removal of starch and extractives significantly decreases control biomass variability
- New enzyme formulations give more consistent and higher levels
- Likely due to water activity impacts on enzyme structure and substrate interactions
- Not attempted here due to need to continually desalt and validate precise enzyme concentrations and activity

References:


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