Analytical BESC Advances in Characterization of Biomass and Recalcitrance

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Background

- Sustainable supply of renewable, carbon-neutral energy needs viable, cost-savings biological energy production from plant biomasses.
- Processing is considered to be responsible for the high estimated cost of biofuels from lignocellulosics.
- BESC is aimed to develop improved plant materials with low energy needs viable.
- Analytical characterization can provide detailed knowledge of:
  - The physical and chemical properties of biomass contributing to biomass recalcitrance.
  - Fundamental understanding of the relationship between plant polysaccharides, lignin and how these biopolymers are integrated in the plant cell wall.
  - How biomass properties change during pretreatment and how such changes affect biomass deconstruction by enzyme/microorganisms.

Lignin characterization through NMR spectroscopy

- Baseline and transgenic alfalfa: C3H and HCT gene down-regulation
- Ball-mill lignin isolation
- One-dimensional 1H and 13C NMR
- One-dimensional 31P NMR
- Two-dimensional (13C-1H) heteronuclear correlation spectra

One-dimensional NMR analysis

Solid-state CPMAS 13C NMR analysis

- The alfalfa samples were first holopulped to removed lignin.
- Holopulp samples were then treated with 2.5 M HCl to remove hemicelluloses.
- Samples were packed and spun at 8 kHz for CPMAS NMR.

Perdeuterated ionic liquid for direct NMR analysis of plant cell walls

- Plant cell wall samples were added into perdeuterated pyridinium ionic liquid-dx/DMSO-d6 solution. The mixture was stirred vigorously at 70°C for 1.5 h to form homogeneous system.
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Microtome sample: MALDI-mass image analysis

- Spatial analysis of biomass chemical constituents across the plant cell wall for native and deconstructed biomass.
- The fresh samples are attached on the mounting head and sectioned into 20~80 um slices in a cryostat (-20°C).
- Extractive free lignin free and hemicellulose free samples are prepared.
- Microtome samples are analyzed using MALDI-mass image analysis.

Conclusions

- Transgenic alfalfa showed significant changes of lignin structure as revealed by NMR.
- Reduced recalcitrance appeared not related to crystallinity of cellulose.
- Ionic liquids provided great potential for better NMR characterization of nonderivatized plant cell walls.

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