Analytical BioEnergy Science Center Advances in Characterization of Biomass and Recalcitrance

Y. Pu, N. Jiang, H. Li, M. Foston, R. Samuel, J. Seokwons, A.J. Ragauskas, M. Keller

1School of Chemistry and Biochemistry, Institute of Paper Science and Technology, Georgia Institute of Technology, Atlanta, GA; 2Oak Ridge National Laboratory, Oak Ridge, TN; 3BioEnergy Science Center; 4Presenter (Yunqiao.Pu@ipst.gatech.edu)

Conclusions

- Sustainable supplies of renewable, carbon-neutral energy need viable, cost-saving biological energy production from plant biomass.
- Processing improvements are the best option to lower the high estimated cost of biofuels from lignocellulosics.
- 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

Background

- Perdeuterated ionic liquid for direct NMR
- 13C-1H HSQC 2D correlation NMR

Perdeuterated ionic liquid for direct NMR analysis of plant cell walls

- Plant cell wall samples were added into perdeuterated pyridinium ionic liquid-DMSO-d6 solution. The mixture was stirred vigorously at 70 °C for 1-4 h to form a homogeneous solution. The plant cell wall solution was then transferred to NMR tube for direct analysis.

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

- 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

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

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 (-20ºC)
- 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 to be not related to the crystallinity of cellulose
- Ionic liquids provided great potential for better NMR characterization of nonderivatized plant cell wall structures

Acknowledgements

This work was supported by the DOE Office of Biological and Environmental Research through the BioEnergy Science Center (BESC).

The BioEnergy Science Center (BESC) is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.