Analytical BESC Advances in Characterization of Biomass and Recalcitrance

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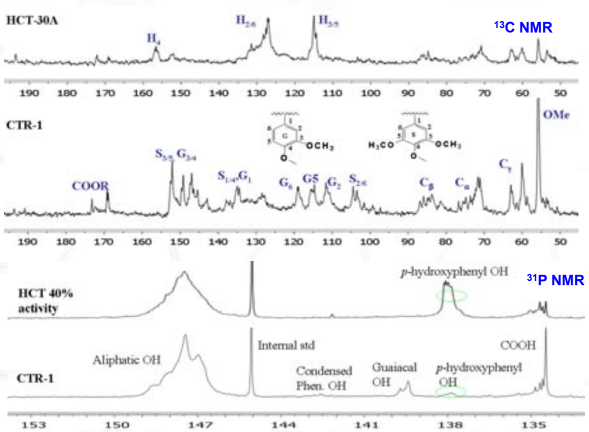
Background

- Sustainable supply of renewable, carbon-neutral energy needs viable, cost-savings biological energy production from plant biomass.
- 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 recalcitrance and consolidated bioprocessing methods that facilitate cost-effective conversion of biomass to fermentable sugars.
- 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

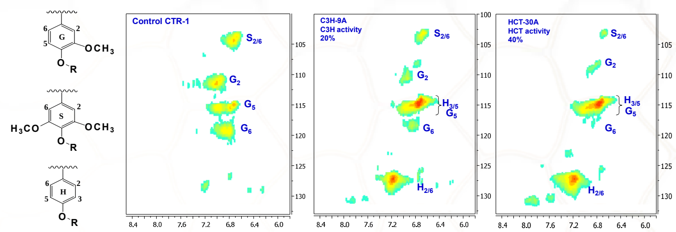
- Baseline and transgenic alfalfa: C3H and HCT gene down-regulation
- Ball-mill lignin isolation
- One-dimensional ^1H and ^{13}C NMR
- One-dimensional ^{31}P NMR
- Two-dimensional (^{13}C-^1H) heteronuclear correlation spectra

One-dimensional NMR analysis

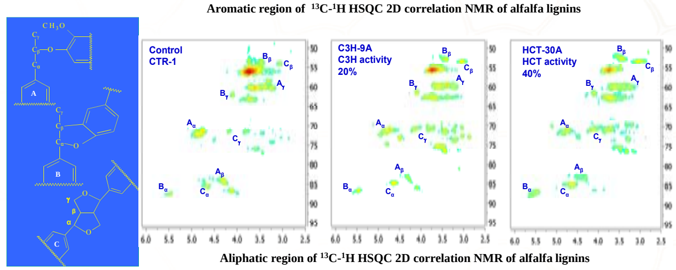


One-dimensional ^{13}C and ^{31}P NMR spectra of isolated alfalfa lignins

^{13}C-^1H HSQC 2D correlation NMR



Aromatic region of ^{13}C-^1H HSQC 2D correlation NMR of alfalfa lignins

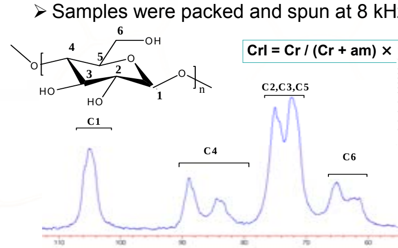


Aliphatic region of ^{13}C-^1H HSQC 2D correlation NMR of alfalfa lignins

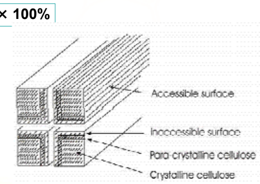
Solid- state CPMAS ^{13}C NMR analysis

- 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

$CrI = Cr / (Cr + am) \times 100\%$

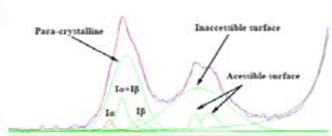


CPMAS ^{13}C NMR spectrum of alfalfa



aggregated cellulose fibrils model*

*T. Liitia. Doctoral thesis, University of Helsinki, Finland, 2002

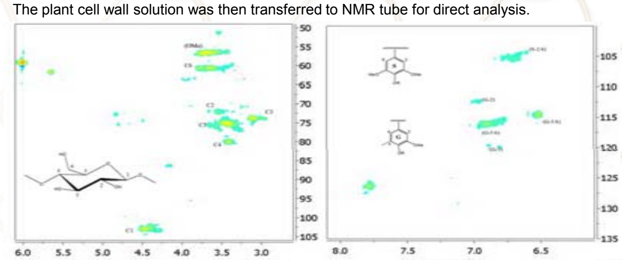


Line-fitting of CP/MAS ^{13}C-NMR spectrum

Assignment	Chemical shift (ppm)	FWHH (Hz)	Intensity (%)	Line type
Cellulose Iα	89.75	55	2.08	Lorentz
Cellulose Iα + β	88.88	73	8.31	Lorentz
Para-crystalline Cellulose I	88.74	215	36.59	Gauss
Cellulose Iβ	87.87	109	4.17	Lorentz
Accessible fibril surface	84.52	68	2.66	Gauss
Inaccessible fibril surface	84.26	426	41.27	Gauss
Accessible fibril surface	83.60	120	4.92	Gauss

Perdeuterated ionic liquid for direct NMR analysis of plant cell walls

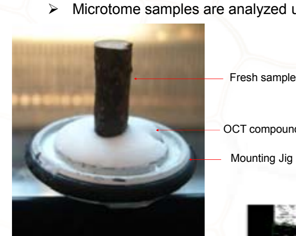
- Plant cell wall samples were added into perdeuterated pyridinium ionic liqiud-dx /DMSO-d6 solution. The mixture was stirred vigorously at 70 °C for 1-4 h to form homogeneous system. The plant cell wall solution was then transferred to NMR tube for direct analysis.



2D HSQC of poplar in 1:2 [Cmpry]Cl-d7/DMSO-d6

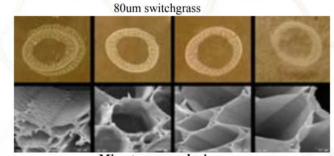
Microtome sample: MALDI-mass image analysis

- 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.

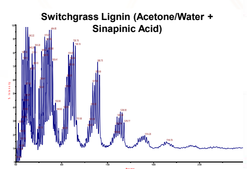


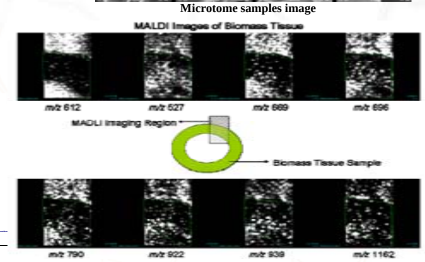
Sample mounting on microtome jig

80um switchgrass



Microtome samples image





MALDI-mass imaging using 20um microtome fresh switchgrass

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 wall structures

Acknowledgements

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