Chemical image of poplar stem using imaging mass spectrometry: ToF-SIMS and MALDI-MS

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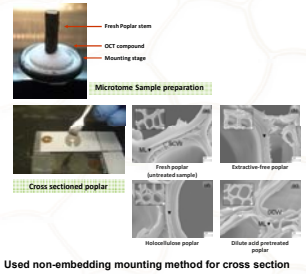
Abstract

Lignocellulosic materials have attracted extensive interest as a potential source of second-generation biofuel referred to as biomass-to-liquid technology (BLT). The structural heterogeneity and complexity of cell wall rely on the natural resistance of plant cell walls to microbial and enzymatic deconstruction referred to as biomass recalcitrance. Herein, we illustrated the chemical image using **imaging mass spectrometry (IMS)** in order to understand surface chemistry on the cell wall before and after treatment. **Time-of-flight secondary ion mass spectrometry (ToF-SIMS)** was applied to achieve the major component images and its quantitative variation after dilute acid pretreatment (DAP) on the surface of poplar stem. The ToF-SIMS images represented excellent spatial resolution for cell wall components under micron scale. In terms of quantification, the relative content of xylan after DAP increased by 30% on the surface of the poplar stem by ToF-SIMS, while bulk carbohydrate analysis showed that the relative concentration of xylose decreased ten fold in comparison with untreated poplar wood. In the high-mass range, **matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)** was applied to detect large size biopolymer, especially cellulose. Non-destructive cellulose poplar stem was obtained after acid hydrolysis and used for MALDI-MS. High intensity signals in the mass spectra of the cellulose poplar stem represented 162 Da mass differences between adjacent peaks, which reasonably must reflect losses of glucose units because of ~96% of glucose content by carbohydrate analysis. Finally, the MALDI-MS images of the cellulose poplar stem were obtained including spatial distribution of cellulose on the surface of poplar stem.

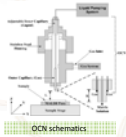
Sample preparation

a. ~2% H_2SO_4 160 ~ 175 °C 10 min in batch reactor
b. Holocellulose (cellulose + hemicelluloses) pulping: 4h with $NaClO_2$, CH_3COOH at 70 °C c. 2.5 N HCl at 100 °C 4h



- Used non-embedding mounting method for cross section
- Dried between glass slide to keep the flatness of sectioned sample
- Observed no damage of cell wall on the surface after dry process

Experimental method

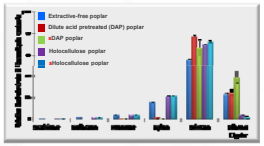


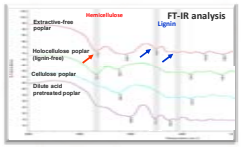
- Matrix were coated onto poplar stem surfaces using an oscillating capillary nebulizer (OCN) matrix applicaiton system
- MALDI-MS was performed using Voyager DE STR MALDI-TOF-MS (Applied Biosystems, USA) equipped with a 337 nm N2 laser
- Imaging MALDI-MS data were acquired using modified MMSIT without 32K data limitation.

- ToF-SIMS spectra and images were obtained by a PHI TRIFT III spectrometer (Physical Electronics Inc., USA) using a gallium liquid metal ion gun (LMIG, ^{69}Ga) as the primary ion source.
- The instrument was operated in positive mode (22kV) with 600pA of primary ion current with an ion microprobe mode.
- No matrix were coated onto poplar stem surfaces.

Results and Discussion

1. Bulk Composition analysis of poplar cellulose

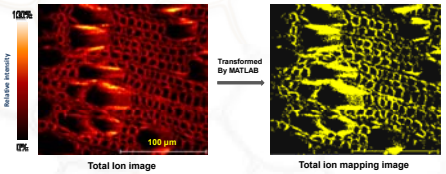


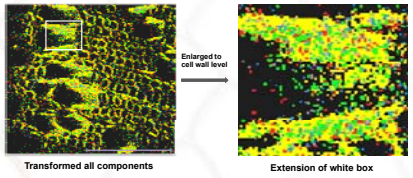


- Holocellulose poplar
  - -. Removed over 90% of Klason lignin
- Dilute acid pretreated & Cellulose poplar
  - -. Over 96% of glucose content in cellulose poplar
  - -. Over 96% of glucose content in DAP poplar
  - -. Removed most of hemicelluloses (~ 2%)
- Holocellulose & Cellulose poplar
  - -. Disappeared lignin signals (aromatic vibration)
  - -. Absorption bands at 1428 and 1510 cm^{-1}
- Dilute acid pretreated poplar
  - -. Disappeared hemicellulose signal
  - -. Absorption band at 1740 cm-1

2. Surface analysis by Imaging Mass spectrometry

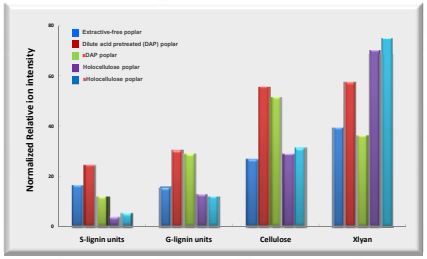
2.1.1 ToF-SIMS Imaging Mass spectrometry





- Transformed images expressed detailed positions of each species (e.g. cellulose, xylan, and lignins).
- **Abundance of xylan appeared on the surface while bulk carbohydrate analysis indicated ~2% xylan remained.**

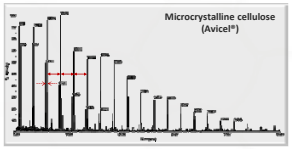
2.1.2 Semi-quantitative ToF-SIMS information

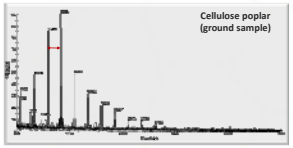


- **After dilute acid pretreatment, relative intensity of xylan increased two fold, while bulk carbohydrate analysis indicated ~ 2% xylan remained.**
- After holocelluose pulling, relative intensity of G-lignin units slightly decreased, while S-lignin units decreased by 80%.

2.2 MALDI-MS Imaging Mass spectrometry

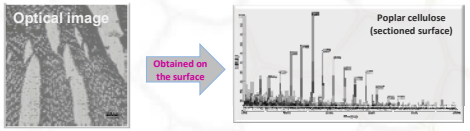
- Microcrystalline cellulose as a model compound
- Observed series of poly-glucose fragment ions with sodium adduct in microcrystalline cellulose and poplar cellulose
- Observed 162Da of mass differences ($C_6H_{10}O_5$) with adjacent peaks (red arrows)
- Observed the most intense peak at *m/z* 1338 (DP 8)



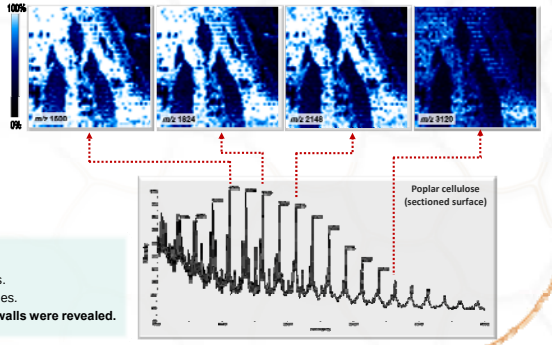


- Applied linear positive ionization mode for IMS
- Generated molecular images at selected mass ions.
- Optical image is matching to the molecular ion images.
- **The spatial distribution of cellulose across cell walls were revealed.**

2.2.3. Direct analysis of cross section of poplar stem



- Observed series of positive poly-glucose ions in reflection mode
- The most intense peak of stem sample shifted (*m/z* 1824) to high mass range comparing to ground samples



Conclusions

- ToF-SIMS has been successfully applied to analyze surface of native plant cell.
- Surface analysis by ToF-SIMS showed different chemistry compared to bulk analysis after various treatments.
- ToF-SIMS images showed spatial distribution of major cell wall components with high resolution.

- MALDI-MS/ MALDI-MSI protocols for insoluble cellulose were optimized
- Series of ions represented oligo- or poly-glucose units with an interval of 162 amu (anhydroglucose units) were obtained.
- The molecular distribution of celluloses in poplar stem samples were highly correlated with optical images.
- The molecular images of celluloses demonstrated their distributions across the cell walls

Acknowledgements

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