

# Characterization Of Biomass By Imaging Mass Spectrometry

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Bioethanol has been highlighted as an alternative renewable energy source, which plays significant roles in reducing greenhouse gas emission and ensuring fuel security. Recently lignocellulosic biomass (e.g., non food-based agricultural residues and forestry wastes) has been promoted to use a source of bioethanol instead of food-based materials (e.g., corn and sugar cane), however to fully realize the benefits an improved understanding of lignocellulosic recalcitrance must be developed. Therefore, enhanced characterization methodology is required to measure the chemistry, structure, and interactions of the biomass components and it can contribute to understand biomass recalcitrance.

In this dissertation, imaging mass spectrometry (IMS) is applied for biomass to characterize the surface upon pretreatment processes. Time of flight secondary mass spectrometry (TOF-SIMS) and matrix-assisted laser desorption/ionization (MALDI) are employed and analytical methodologies specially for biomass are developed. Juvenile hybrid poplar stem grown in a greenhouse is used for a model substrate.

The first study focuses on the development of sample preparation for surface analysis by IMS. A cross sectioned sample is then treated under various methods such as dilute sulfuric acid pretreatment, flowthrough pretreatment, and cellulose isolation. To determine the different chemistry between surface and bulk upon dilute acid pretreatment in batch reactor, TOF-SIMS is firstly applied to characterize the surface of the pretreated poplar stem in Chapter 5. TOF-SIMS probes the surface of cross sectioned poplar samples, providing two-dimensional (2D) molecular images of major components (i.e., cellulose, hemicelluloses, and lignin) and their relative ion counts. 2D molecular images present the lateral distribution of major components before and after dilute acid pretreatment while the comparison of the relative ions provides semi-quantitative information on the surface. Thereafter, these surface data are compared to bulk chemical composition by sugar analysis in order to prove different chemistry between surface and bulk.

The second study is focused on tracking the lateral lignin distribution on the surface of poplar stem using TOF-SIMS technique developed earlier. Cross sectioned poplar stems are flowthrough pretreated under different conditions, providing different bulk lignin contents. Flowthrough pretreatment provides a number of advantages such as the removal of pretreatment products prior to quenching process thus avoiding precipitation of products. To understand lignin changes at cell wall layers, high resolution TOF-SIMS imaging process is optimized under burst mode. As a result, 2D lignin ion images present the lateral distribution under submicron scale, providing lignin localized area in cell corner after flowthrough pretreatment. Semi-quantitative information also presents an evidence of different chemistry between surface and bulk upon flowthrough pretreatment process.

The third study is focused on the development of three-dimensional (3D) molecular imaging method using TOF-SIMS. Extending the usefulness of TOF-SIMS for biomass recalcitrance, a 3D molecular imaging is firstly introduced to biomass by acquiring multiple 2D images in a stack. This is accomplished by reconstruction, stacking the 2D molecular images layer by layer. Consequentially 3D molecular image provides both lateral and vertical distribution of characteristic species from surface to sub-surface. This

spatial molecular information can be used to directly determine the chemical change between surface and bulk without relative comparison of other data such as bulk sugar profile. Stress-induced tension wood in poplar stem is used as a model substrate. Tension wood is not only defined by the presence of this gelatinous (G-) layer but also is ideal for demonstrations of chemical imaging because this cellulose rich area can be readily distinguished from the more chemically complex surroundings.

The last study is focused on the development and optimization of MALDI-MS/IMS method for insoluble cellulose. MALDI has a capability to detect large molecules (theoretically unlimited) while SIMS can only detect relatively lower mass species. This feature allows detecting different molecular size of cellulose oligomers. Microcrystalline cellulose is used to optimize a cellulose detection protocol such as matrix application. Thereafter, cellulose poplar isolated from holocellulose poplar is introduced to MALDI-MS. To visualize lateral distribution of different DP of cellulose on the surface of poplar stem, a cross-section of cellulose poplar is used. Series of cellulose oligomers in poplar stem are firstly generated using MALDI-IMS