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### Title

Understanding Ionic Liquid Pretreatment of Lignocellulosic Biomass by Hyperspectral Raman Imaging

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<https://escholarship.org/uc/item/9gs4p17m>

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### Publication Date

2009-12-16

## **Understanding Ionic Liquid Pretreatment of Lignocellulosic Biomass by Hyperspectral Raman Imaging**

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Pretreatment of lignocellulosic biomass is essential for breaking down the highly interwoven matrix of cellulose, lignin and hemicellulose, which facilitates enzyme accessibility and adsorption to cellulose for efficient saccharification. Ionic liquids are a new class of non-volatile solvents exhibiting excellent solvating properties and have shown great promise for lignocellulosic biomass pretreatment with easy recovery of cellulose by rapid precipitation with anti-solvents. Ionic liquids have been demonstrated to be very effective in cellulose solubilization in bulk, and have shown to swell cell walls perhaps by breaking inter and intra chain hydrogen bonding. However, to date, molecular level understanding of ionic liquid pretreatment on lignin and its impact on different tissue and cell types of biomasses is lacking. The aim of this research is to develop a fundamental understanding of ionic liquid pretreatment by monitoring the compositional changes during the pretreatment process. Raman microscopy based on molecular vibrational spectroscopy is a label-free imaging technique capable of real-time and noninvasive examination of plant cell walls with chemical selectivity. In this research, we employed hyperspectral Raman imaging to study tissue and cell type specific distribution of cell wall components and the impact of ionic liquid pretreatment on various cell types of corn stover to identify signatures for predicting deconstructionability and understand pretreatment dynamics. The Raman mapping results have shown that the distribution of lignin and cellulose varies significantly across different tissue and cell types in the following order: sclerenchyma cells and tracheids > epidermal cells > bundle sheath cells > parenchyma cells. Lignin content decreases rapidly in tracheids and sclerenchyma cells and slowly in parenchyma cells during ionic liquid pre treatment. Significant cell wall swelling of various cell types during ionic liquid pretreatment was revealed by confocal fluorescence microscopy.

This work was part of the DOE Joint BioEnergy Institute (<http://www.jbei.org>) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.