Final Scientific/Technical Report for DE-FG02-07ER64500
"Study of Lignocellulosic Material Degradation with CARS Microscopy"

Executive Summary

The program of research undertaken by our Harvard group, in collaboration with Dr. Ding at the National Renewable Energy Laboratory (NREL) in Golden, CO, seeks to introduce, validate and apply a new analytical technique to study the conversion of lignocellulosic biomass into ethanol. This conversion process has been the subject of intense interest over the past few years because of its potential to provide a clean, renewable source of energy to meet increasing global demand.

During the funding period, we have clearly demonstrated visualization of lignin and cellulose using intrinsic vibrational contrast with simulated Raman scattering (SRS) microscopy, developed at Harvard (Figure 1). Our approach offers high spatial resolution and time resolution that is sufficient to capture the kinetics of a pre-treatment process. This is reflected by the publications listed below, as well as the use of SRS microscopy at NREL as a routine analysis tool for research on lignocellulosic biomass.

In our original proposal, we envisioned moving to near-field CARS imaging in order to perform chemical mapping at the nanoscale. However, given the dramatic progress made by our group in SRS imaging, we concentrated our efforts on using multi-component SRS (lignin, cellulose, lipid, water, protein, deuterated metabolites, etc.) to quantitatively understand the spatially dispersed kinetics in a variety of plant samples under a variety of conditions.

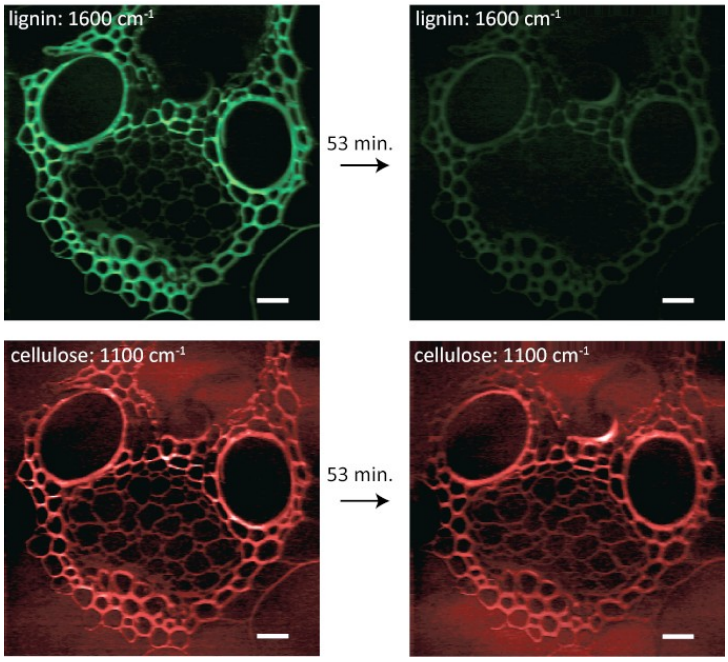

Figure 1. Before and after images of wild-type corn stover after dilute acid pre-treatment. Top images (green) show the lignin channel before and after 53 minutes of dilute acid pre-treatment. The signal drops by about an order of magnitude. Bottom images (red) show simultaneous acquisition of the cellulose signal. In this channel, no substantial change in the signal level is seen. These results indicate that this pre-treatment process selectively degrades lignin, as desired. Scale bar: 20 microns.

In addition, we built a next generation laser system based on fiber laser technology that allowed rugged and portable instrumentation for SRS microscopy. We also pursued new imaging approaches to improve the acquisition speed of SRS imaging of lignocellulose without sacrificing signal-to-noise ratio. This allowed us to image larger volumes of tissue with higher time resolution to get a more comprehensive picture of the heterogeneity of this chemical process from the submicron up to the centimeter scale.

Publications supported by this Grant

Min, Wei; Lu, Sijia; Holtom, Gary R.; Xie, X. Sunney "Triple-Resonance Coherent Anti-Stokes Raman Scattering Microspectroscopy" *ChemPhysChem*, **10**, 344-347 (2009).

Kieu, Khanh; Saar, Brian G.; Holtom, Gary R.; Xie, X. Sunney; Wise, Frank W. "High-power picosecond fiber source for coherent Raman microscopy" *Optics Letters*, **34**, 2051-2053 (2009).

Saar, Brian G.; Holtom, Gary R.; Freudiger, Christian W.; Ackermann, Chrisita; Hill, Winfield; Xie, X. Sunney "Intracavity wavelength modulation of an optical parametric oscillator for coherent Raman microscopy" *Optics Express*, **17**, 12532-12539 (2009).

Zeng, Yining; Saar, Brian G.; Friedrich, Marcel G.; Chen, Fang; Liu, Yu-San; Dixon, Richard A.; Himmel, Michael E.; Xie, X. Sunney; Ding, Shi-You "Imaging Lignin-Downregulated Alfalfa Using Coherent Anti-Stokes Raman Scattering Microscopy" *Bioenerg. Res.* (2010)

Saar, Brian G.; Zeng, Yining; Freudiger, Christian W.; Liu, Yu-San; Himmel, Michael E.; Xie, X. Sunney; Ding, Shi-You "Label-Free, Real-Time Monitoring of Biomass Processing with Stimulated Raman Scattering Microscopy" *Angew. Chem. Int. Ed.* **49**, 5476-5479 (2010).

Inventions/Patents:

“Frequency Modulation for Ultrafast Laser Sources”; Inventor: Xie, Xiaoliang Sunney; Co-Inventors: Holtom, Gary L.; Saar, Brian ; Evans, Conor L. (reported to DOE 5/13/2008).