Study of Plant Cell Wall Polymers Affected by Metal Accumulation Using Stimulated Raman Scattering Microscopy

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Project Goals:

This project employs newly-developed chemical imaging techniques to measure, in real-time, the concentration, dynamics and spatial distribution of plant cell wall polymers during biomass growth with inoculation of transgenic symbiotic fungi. The proposed new pathway of biomass production will: 1) benefit metal and radionuclide contaminant mobility in subsurface environments, and 2) potentially improve biomass production and process for bioenergy. The goal of this project will explore a new pathway of delivering detoxicified metal to plant apoplast using transgenic symbiotic fungi, which will enhance metal accumulation from soil, and potentially these metals may in turn be used as catalysts to improve the efficiency of biomass conversion to biofuels. We further develop chemical imaging tools to quantitatively analyze the key plant cell wall polymers such as cellulose and lignin.

In our previous project funded by BER lignocellulose imaging program, we have demonstrated that stimulated Raman scattering (SRS) microscopy, a new imaging method, which allows real time observation of biomass conversion processes (1-4). SRS microscopy offers chemical contrast based on the intrinsic Raman vibrational frequencies in a sample with much shorter imaging time and easier spectral identification. The SRS imaging technique for studying the conversion process *in situ* offers chemical specificity without exogenous labels, non-invasiveness, high spatial resolution, and real-time monitoring capability. We propose to further the recently emerging SRS techniques to imaging the distribution of plant cell wall polymers (e.g., cellulose and lignin) and cell wall architecture with different growth conditions including in heavy metal added soils and inoculated with symbiotic fungi. Specifically, we propose to:

- Establish a model system of plant-symbiotic fungi that produce metal-bound biomass by inoculating the poplar plants with transgenic fungus *Trichoderma harzianum* that carries secretory metal-binding protein.
- Evaluate fungal expression system growing in plant, and the efficiency of inoculation of fungi in intact plant cell walls. Specifically, we will examine if a closer contact between metals and cellulosic and lignin polymers caused by the entrapment of metals within plant cell, will alter plant cell wall structure and lead to a higher efficiency for cell wall degradation.

- Develop imaging tools to quantitatively analyze the distribution and deconstruction kinetics of plant cell wall polymers.

References

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This work is funded by the US Department of Energy, the Office of Biological and Environmental Research (BER).