Dynamic Visualization of Cell Wall Deconstruction during Pretreatment and Enzymatic Hydrolysis Using MRI

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Introduction

Studies continue on the fundamental understanding of the micro-architecture of the cell walls during the pretreatment and hydrolysis using MRI in order to resolve the critical issues in bioconversion, e.g. high recalcitrance and low enzyme efficiency of the cell walls. Based on the previous studies using other imaging modalities (Chundawat et al., 2011; Ding et al., 2012), this project aims to use non-invasive and high resolution MRI to track dynamic water transportation inside sugarcane bagasse cell wall during phosphoric acid pretreatment and enzymatic hydrolysis in order to: 1) reveal effects of dilute phosphoric acid pretreatment on deconstruction of sugarcane bagasse cell wall; and 2) elucidate the relationship between the cell wall deconstruction and enzymatic hydrolysis efficiency.

Experimental

MR microscopy (MRM), a subset of MRI method, is used to reveal the micro-anatomical structures of lignocellulosic cell wall. Given the cylindrical structures of the plant cells and thereby anisotropic water diffusion in them, the microstructural changes of the cell walls can be monitored non-invasively by 3D visualization or by employing T1, T2, and diffusion weighted imaginging (DWI). The MR microimages will be used to dynamically monitor cell wall deconstruction and digestion preference and direction during acid pretreatment and enzyme hydrolysis process. The sugarcane bagasse will be treated with different phosphoric acid concentrations (0.5%-1.5%). The attempted MRI sequences will include conventional T1, T2, and DWI.

Results and Discussion

The preliminary results show that the DWI can be applied to delineate the internal structure of sugarcane bagasse. The vascular bundle is clearly observed consisting of xylem, phloem and sclerenchyma fibers cells (Fig. 1A). The signal from parenchyma cells surrounding the vascular is relatively stronger than other cell types. However, sclerenchyma fibers showed hypointense signal presumably because of their higher cell density in cell walls. Similarly, the relatively higher signal intensity of sclerenchyma fibers in treated sugarcane bagasse, is probably due to the relocation or reduction of lignin content (Fig. 1D). Additionally, the boundary of xylem and phloem became fuzzy because of the deconstruction by the pretreatment, which would increase the accessibility of cellulase to the cell wall. To address the micron-scale changes in the samples, the coils with higher sensitivity will be used to observe the dynamic changes of the cell walls.

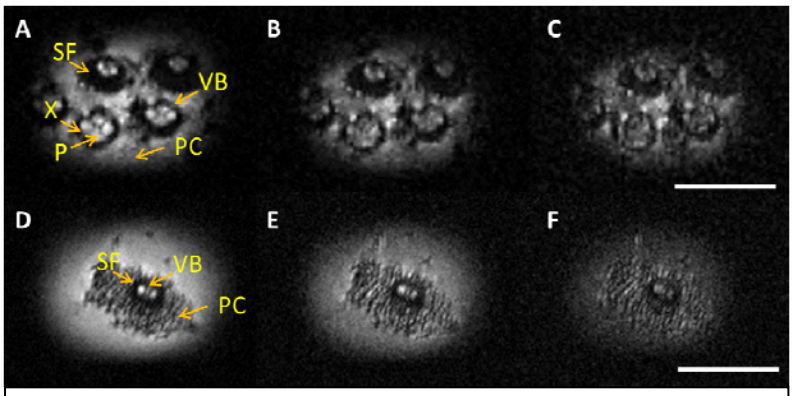

Figure 1. Diffusion-weighted MR images of raw sugar cane (A-C) and pretreated biomass (D-F) at the b-values of 0 (A & D), 600 (B & E), 1,000 (C & F) [s/mm^2]. Scale bar [500 µm]. Sclerenchyma Fibers(SF), Parenenchyma Cells (PC), Vascular Bundles (VB), Xylem (X), Phloem (P).

Conclusions

DWI technique can be used as a tool to visualize structure of lignocellulosic cell wall. The dynamic changes of cell wall structures during pretreatment (delignification) and enzymatic digestion will be investigated in the future.

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References

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(2) Ding, S.-Y. et. al., *Science*, **338**(6110), 1055-1060 (2012).