Ultrastructure of Lignocellulose "Native-Pretreated-Deconstructed" by Advanced Solid-State NMR

Art J. Ragauskas[1,2], Marcus Foston[1,2], Yunqiao Pu[1,2], and Martin Keller [1,3]

*BESC[1]; School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA[2]; *Oak Ridge National Labs, Oak Ridge, TN[3]*

Introduction

Solid state nuclear magnetic resonance (NMR) methods can provide not only chemical information but atomistic structural details that are not easily accessible by other non-destructive high-resolution structural techniques. This makes solid state NMR methodology particular useful when studying structural problems in biological systems such as the changes occurring in the ultrastructure and supramolecular structure of biomass. The objective is to understand the mechanisms of recalcitrance and effect of dilute acid pretreatments on recalcitrance and the down steam processing of biomass by analysis of the changes in supramolecular structure upon pretreatment.

Pretreatment can govern:

- Accessibility of cellulose and hemicellulose
- Degree of crystallinity
- Relative % cellulose allomorphs
- Degree of acetylation
- Lignin and hemicellulose distributions
- Pore distributions
- Sugar yields

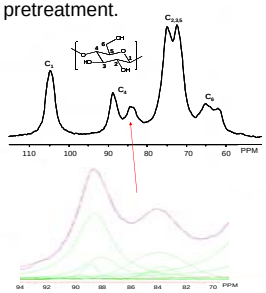

Figure 1. A representative ^{13}C CP/MAS spectrum of cellulose, which was isolated from unpretreated poplar and least-squared fitting of the C-4 region.

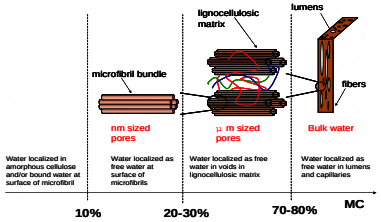

Figure 2. A schematic depicting the sequence of water removal from pores within biomass as a function of moisture content (MC).

Figure 3. 400 MHz NMR Spectrometer

Experimental

Pretreatment

Extractive-free poplar samples were prepared first by presoaking, at RT stirring in a ~1% dilute sulfuric acid solution at 5% solids for 4 hrs. The washed presoaked material was transferred to a 4560 mini-Parr 300ml reactor with ~1% dilute sulfuric acid solution at 5% dry solids The reactor was held at 160±2 ºC (6.4 atm) for the specified residence time ±30 sec. The reactor was quenched in an ice bath (~5 min).

NMR analysis

Holocellulose was isolated from pretreated samples by exposure to $NaClO_2$ (1.3g/1g lignocellulosic dry solids) under acidic condition in 375 ml of .14 M acetic acid at 70 ºC for 2 hrs. Cellulose was isolated from the holocellulose by hydrolysis for 4 hrs in 2.5 M HCl at 100 ºC.

The ^{13}C NMR samples were prepared by loading them into 4-mm cylindrical ceramic MAS rotors. Solid-state NMR measurements were carried out on a Bruker DSX-400 spectrometer operating at frequencies of 100.6 MHz for ^{13}C in a Bruker double-resonance MAS probehead at spinning speeds of 10 kHz. CP/MAS experiments utilized a 5 μs (90°) proton pulse, 1.5 ms contact pulse, 3 s recycle delay and 8K scans. All spectra were recorded on wet sample (60-80% water content). The line-fitting analysis of spectra was performed using NUTS NMR Data Processing software (Acorn NMR, Inc).

^{1}H T_2 NMR experiments on untreated and pretreated milled biomass pulp were performed on a magnetic resonance analyzer (Maran 23 ultra), operating at a ^{1}H frequency of 23 MHz using a permanent magnet. A minimum of 500 mg of milled pretreated or untreated biomass pulp was measured in 10-mm NMR tubes at a constant temperature of 25 ºC. The spin-spin relaxation times were determined using a standard Carr-Purcell-Meiboom-Gill (CPMG) sequence with 128 scans and τ=100 us. The relaxation delay was 2 sec, which was long enough based on the measurement of T_1. Diffusion coefficients were measured using a pulse field gradient (PFG) sequence with 128 scans, with a Δ= 0.1 s and taking 32 points varying δ between 0.0001 – 0.003 s.

^{2}H T1 NMR measurements were carried out on a Bruker DSX-300 spectrometer, operating at frequencies of 75.475 MHz for 2H in a Bruker double-resonance MAS probehead at non-spinning conditions. The inversion recovery experiments utilized a 5 μs (90°) ^{2}H pulse, 10 μs (180°) ^{2}H pulse, 1 s recycle delay and 128 scans. The NMR samples were prepared with milled pretreated or untreated biomass pulp into 7-mm cylindrical ceramic MAS rotors.

Results and Discussion

Suggesting either hydrolysis localized in amorphous regions or crystallite growth occurs

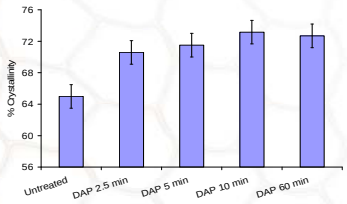

Figure 4. % Crystallinity of isolated cellulose as function pretreatment in ~1% dilute sulfuric acid solution at 160 ºC.

Ultrastructural changes occurring during pretreatment:

- Increase in % Crystallinity
- Increase in LFA and LFAD
- Increase in % *Para*-crystalline cellulose
- Increase in % I_β cellulose
- Decrease in % I_α cellulose

Suggesting crystallite growth occurs

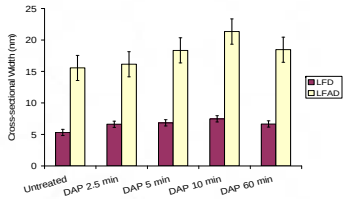

Figure 5. Lateral fibril and lateral fibril aggregate dimensions of isolated cellulose as function pretreatment in ~1% dilute sulfuric acid solution at 160 ºC.

Suggesting adsorbed water is more mobile or reduced water-biomass interaction occur due to pore expansion

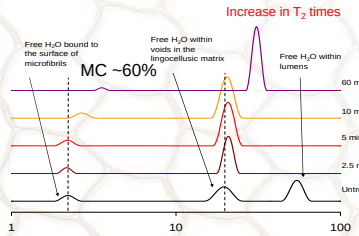

Figure 6. T_2 distribution water of adsorbed in poplar as a function of pretreatment in ~1% dilute sulfuric acid solution at 160 ºC.

Suggesting the pore systems become more tortuous with pretreatment

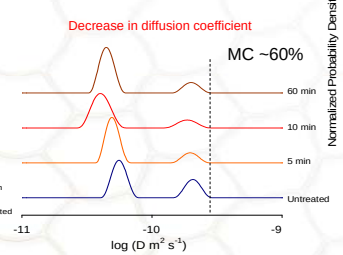

Figure 7. Diffusion coefficient distributions of water adsorbed in poplar as a function of pretreatment in ~1% dilute sulfuric acid solution at 160 ºC.

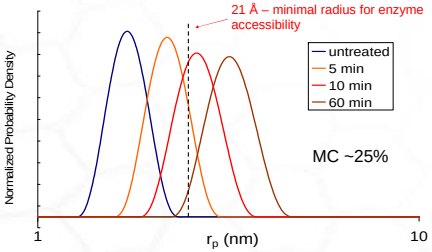

Figure 8. Pore size distribution of poplar as a function of pretreatment in ~1% dilute sulfuric acid solution at 160 ºC.

Suggesting pore expansion occurs upon pretreatment allowing for cellulase accessibility

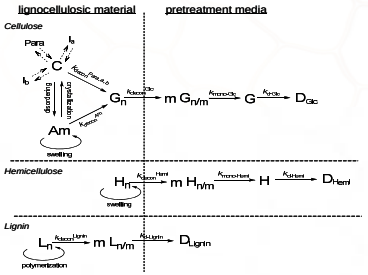

Figure 9. A schematic representing the complex ultrastructural and chemical processes occurring during pretreatment of biomass.

^{1}H T_2 Experiments

- Water can be found spatially localized:
  1. on and within cellulosic microfibrils
  2. exist as capillary water in the lumen or between fibers
  3. within the cellulose fibril matrix
- T_2 measurements show the nature of cellulose–water interactions change during dilute acid pretreatment.
- Displays a shift in T_2 times to longer relaxation or a more mobile state but also indicate the population of water with longer relaxation times increase.
- Suggest pore expansion occurs in the microfibril bundle at 10 min and in the lignocellulosic matrix at 60 min.

Conclusions

Carbohydrate and GPC

- Pretreatment removes the majority of the hemicellulose within 2.5 min.
- Pretreatment is fairly ineffective at lignin removal.
- GPC and sugar analysis indicate that pretreatment beyond 5 min leads to cellulose degradation.
- GPC suggest crystallinity increases is impart due to spatially localized hydrolysis in the amorphous regions.

^{13}C CP/MAS on isolated cellulose

- Complex interplay between several processes i.e. degradation, crystalline transformations, etc.
- Temperature is a key rate determining parameter
- Crystallinity index, LFD and LFAD increases with residence time.
- % I_α decreases, while the % para-crystalline and other crystalline allomorphs increase.

^{2}H T_1 Experiments

- By 2H T1 NMR, pore size distributions indicate that pore expansion in cellulose fibers occur during pretreatment.
- After 5 min the pore within the microfibril bundle expand such that cellulase has access to most of the cellulose fibrils.
- After 60 min pore surface area and volume increases by factors 3.5 of 6.5.

^{1}H Pulse Field Gradient Experiments

- Diffusion experiment suggest an increased tortuosity with pretreatment.

Acknowledgements

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