Investigation of fate of poplar lignin during autohydrolysis pretreatment to understand the biomass recalcitrance

Reichel Samuel[a], Shilin Cao[a], Birinchi K Das[a], Fan Hu[a], Yunqiao Pu[b], and Arthur J.Ragauskas[a b]

[a]*BioEnergy Science Center, School of Chemistry and Biochemistry* [b] *Institute of Paper Science and Technology, Georgia Institute of Technology, 500 10th St., Atlanta, GA 30332, USA*

Supplementary information

Experimental Section

Materials

Populus trichocarpa used in this study was harvested at Oak Ridge National Laboratory, TN. The biomass size was reduced in a Wiley mill to pass a 2 mm screen based on Tappi method T 257 cm-02 and soxhlet extracted with 1:2 ethanol-toluene mixture for 24 h. The *p*-dioxane used in this study was distilled over sodium borohydride prior to use and all other chemicals were used as received.

Enzyme lignin isolation

Wiley milled, extractive free poplar was ball-milled for one week in a porcelain jar using ceramic balls under nitrogen. Cellulase treatment was carried out following literature methodology.[17-18] In brief, poplar (12.00 g) was suspended in acetic acid-ammonium acetate buffer (200.00 mL, pH: 4.8), cellulase and β-D glucosidase (1:2) were added, (cellulase 40 FPU/g cellulose) and the resultant mixture was continuously stirred for 48 h at 50 ^{o}C, upon completion of the reaction the solution was centrifuged and the process was repeated once more and the residue obtained was washed with deionized water, centrifuged and freeze dried. The enzyme treated residue was extracted with *p*-dioxane-water (96%, 10.00 mL/g biomass) for 48 h × 2, centrifuged and the clear solution was freeze dried. The crude lignin was further purified via dissolution in 90% acetic acid, followed by precipitation in water, and centrifugation washing with deionized water until neutral pH. The solid residue was freeze dried and finally dried under vacuum at 40 ^{o}C overnight. Gravimetric yield: ~10% based on starting biomass.

Lignin Characterization

All NMR experiments were carried out on a Bruker Avance 400 MHz NMR spectrometer. HSQC NMR analysis was carried out by dissolving 30 mg lignin in 0.5 mL DMSO-d_6, the DMSO peak (δ_C/δ_H 39.5/2.5 ppm) was used for chemical shift calibration. The ^{31}P NMR spectra were acquired after *in-situ* derivatization with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane(TMDP) and N-hydroxyl-norbornene-2,3-dicarboximide as an internal standard. The acquisition parameters were 90 ^{o} pulse angle, 0.98 s acquisition time, 25 s pulse delay and 128 transients at room temperature.[19] The molecular weights of lignin samples were determined by GPC, prior to this analysis lignin was derivatized using acetic anhydride and pyridine according to a literature procedure.[20] The lignin acetate was then dissolved in tetrahydrofuran (THF, 1 mg/mL) and filtered through a 0.45-μm membrane filter. GPC analysis was carried out on a PSS-Polymer Standards Service (Warwick, RI, USA) GPC SECurity 1200 system featuring Agilent HPLC 1200 components equipped with four Waters Styragel columns (HR1, HR2, HR4, HR6) with an ultraviolet detector, THF was the mobile phase (1.0 mL/min) with injection volumes of 30 μL. Data collection and processing were performed using PSS WinGPC software (Build 6807). The number average molecular weights (Mn), weight average molecular weights (Mw) and polydispersity are calculated against a calibration curve. The calibration curve was created by fitting a second order polynomial equation to the retention volumes obtained from a series of narrow molecular weight distribution polystyrene standards, dioctyl phthalate (M_w=390 g/mol), 2,2'-dihydroxy-4,4'-dimethoxyl-benzophenone (M_w=274 g/mol), 2-phenylhydroquinone (M_w=186 g/mol) and phenol (M_w=94 g/mol).

Table 1 Assignment of ^{13}C-^{1}H correlation signals in the HSQC spectrum of poplar lignin [21-23]

δ_c/δ_H (ppm)	Assignment
20.0/1.9	Acetyl CH_3
53.6/3.1	C_β/H_β in resinol (β-β) substructure (C)
55.7/3.8	C/H in methoxyl group
60.2/3.6	C_γ/H_γ in β-O-4 substructure (A)
62.8/3.8	Cγ/Hγ in phenylcoumaran substructure (B)
71.8/4.8	C_α/H_α in β-O-4 linkage (A)
85.8/4.2	C_β/H_β in β-O-4 linkage (A_s)
84.7/4.7	C_α/H_α in resinol substructure(C)
87.1/5.5	C_α/H_α in phenylcoumaran substructure **(**B**)**
104.3/6.7	$C_{2,6}/H_{2,6}$ in etherified syringyl units (S)
105.5/7.2	$C_{2,6}/H_{2,6}$ in oxidized C_α=O (S´)
111.4/7.0	C_2/H_2 in guaiacyl units (G)
115.4/6.8	C_5/H_5 in guaiacyl units (G)
119.3/6.8	C_6/H_6 in guaiacyl units (G)
130.0/7.7	$C_{2,6}/H_{2,6}$ in *p*-hydroxybenzoyl units (PB)

A: β-O-4 ether linkage; B: β-5/α-O-4 phenylcoumaran; C: resinol, G: guaiacyl unit; S: syringyl unit; S´: oxidized syringyl with C_α=O; PB: *p*-hydroxybenzoyl

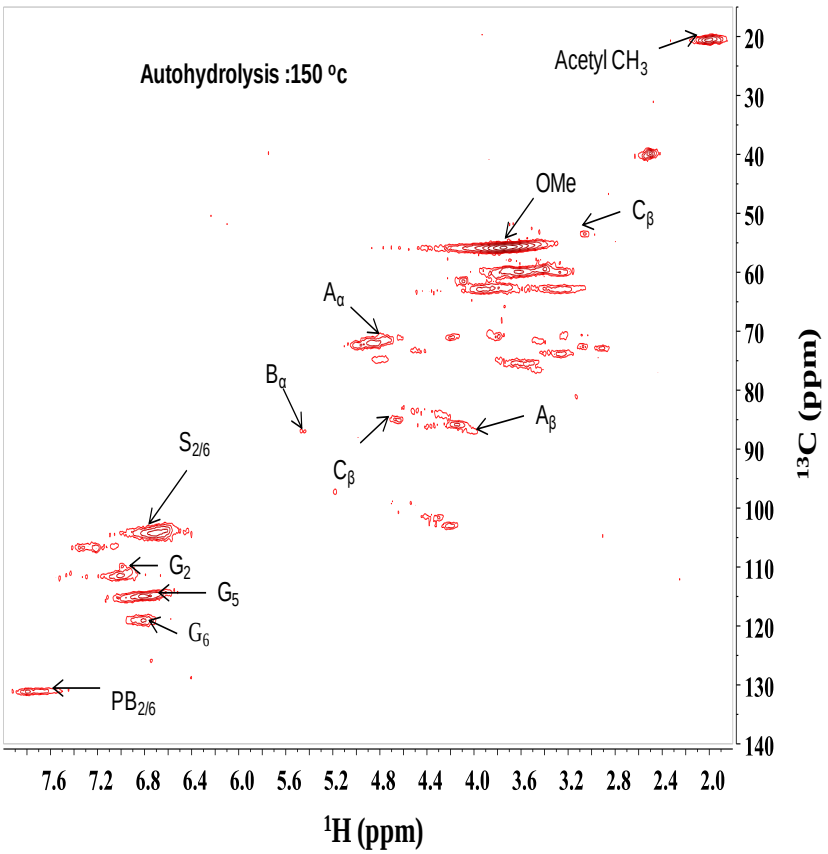

Fig. 1 HSQC spectrum of autohydrolysed poplar enzymatic lignin in DMSO -d_6

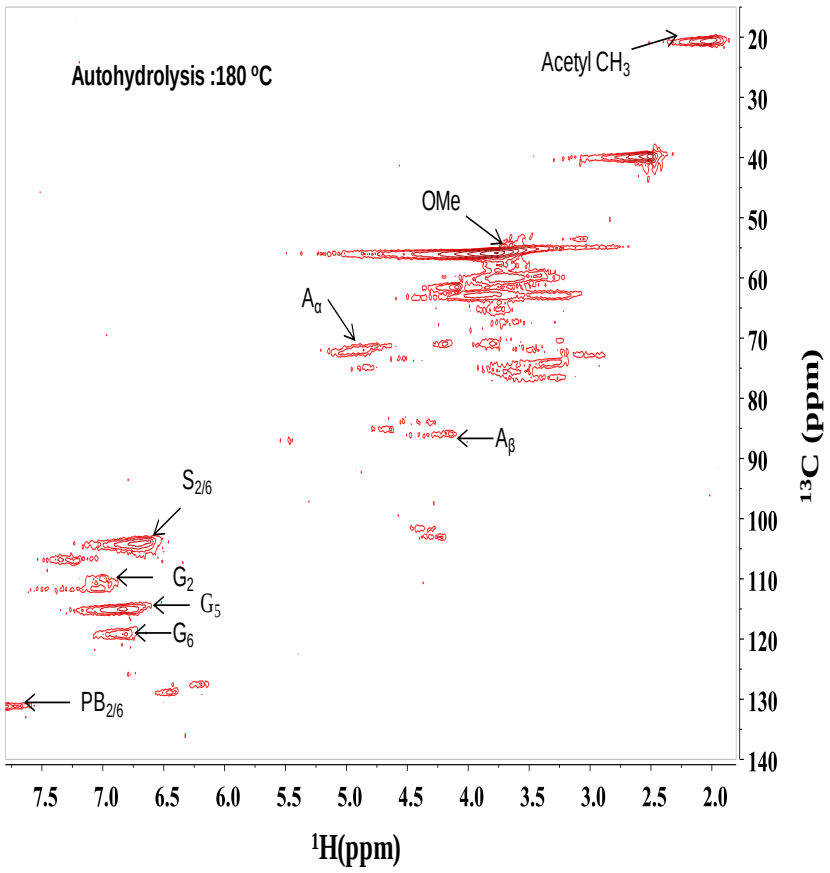

Fig. 2 HSQC spectrum of autohydrolysed poplar enzymatic lignin in DMSO -d_6

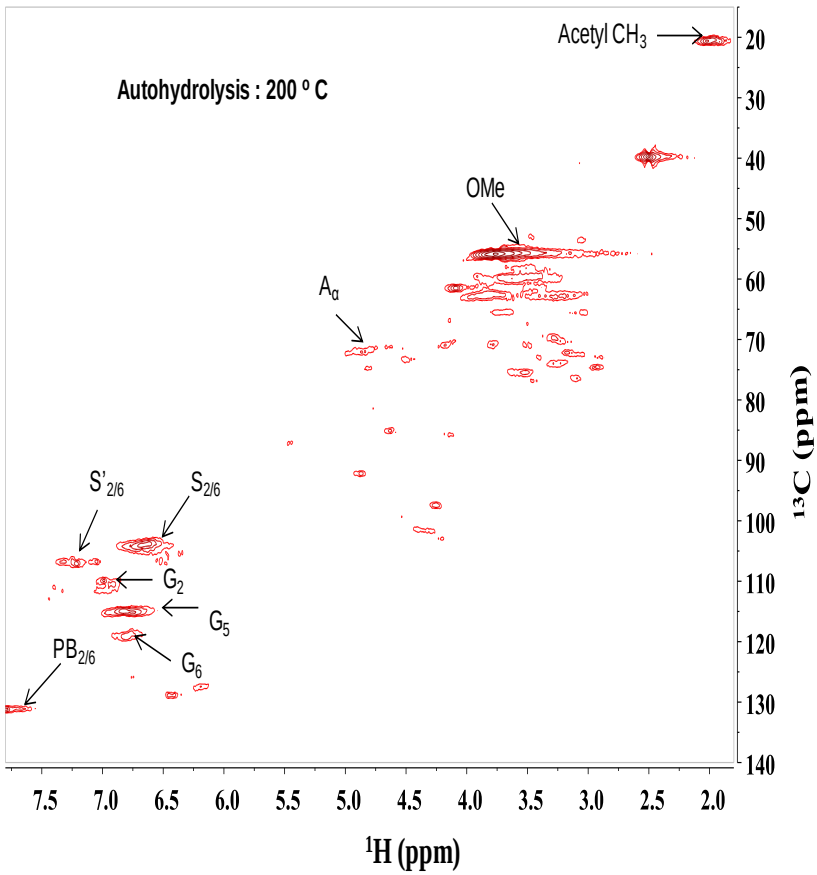

Fig. 3 HSQC spectrum of autohydrolysed poplar enzymatic lignin in DMSO -d_6

Experimental procedure

All pretreatments were conducted in a 300 mL glass lined Parr reactor equipped with a 4842 temperature controller. Enzymatic lignin (100.00 mg) was charged in the reactor with 100.00 mL preheated DI water (60 ^{o}C) and sealed. The impeller speed was adjusted to 100 rpm and heated to 150 ^{o}C (~4 ^{o}C/min). The reactor was quenched in an ice bath and the cooled pretreated slurry was freeze dried and finally vacuum dried prior to analysis. The above pretreatment was repeated at 180, 200 ^{o}C with zero residence time at the maximum temperature and 200 ^{o}C for 30 min residence times. The pretreatment yield ranged between 90-75%.