Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Suppression of pseudo-lignin formation at severer dilute acid pretreatment conditions

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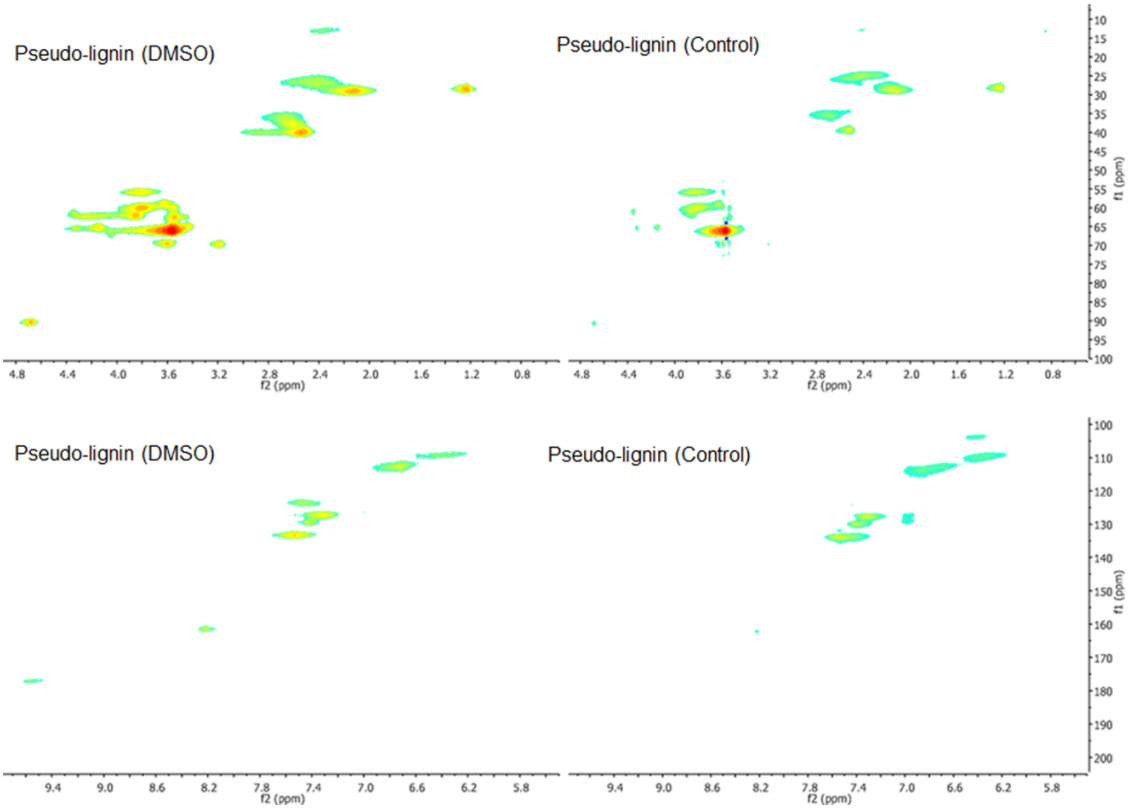
Fig. 1 2D HSQC NMR spectra of pseudo-lignin (DMSO) and pseudo-lignin (control).



Acid-insoluble lignin (K-lignin) and carbohydrate analysis

Samples for carbohydrate constituents and acid-insoluble lignin analysis were prepared using a two-stage acid hydrolysis protocol based on Tappi method T-222 om-88 with a slight modification. The first stage utilized a severe pH and a low reaction temperature (72 wt% H_2SO_4 at 30 °C for 1 h). The second stage was performed at lower acid concentration and higher temperature (3 wt% H_2SO_4 at 121 °C for 1 h) in an autoclave. The resulting solution was cooled to room temperature and filtered through a G8 glass fiber filter (Fisher Scientific, USA). The remaining residue that is considered as acid-insoluble lignin was oven-dried and weighed to obtain the acid-insoluble lignin content. The filtered solution was analyzed for carbohydrate constituents determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using Dionex ICS-3000 (Dionex Corp., USA).

Molecular weight analysis of isolated pseudo-lignin

Dry pseudo-lignin (20 mg) was acetylated by stirring with 1:1 (v/v) acetic anhydride/pyridine mixture (2.00 ml) at room temperature for 72 h. The solvent mixture was removed under reduced pressure at 50 ºC. The acetylated lignin was dissolved in chloroform (50 ml) and washed with water (20 ml). The chloroform phase was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. Prior to gel permeation chromatography (GPC) analysis, the dried acetylated pseudo-lignin samples were dissolved in tetrahydrofuran (1.00 mg/ml), filtered through a 0.45 µm filter and placed in a 2 ml auto-sampler vial. The molecular weight distributions of the acetylated pseudo-lignin samples were analyzed by Agilent GPC SECurity 1200 system equipped with four Waters Styragel columns (HR0.5, HR2, HR4, HR6), Agilent refractive index (RI) detector and Agilent UV detector (270 nm). Tetrahydrofuran was used as the mobile phase (1.0 ml/min) and the injection volume was 30.0 µl. A calibration curve was constructed based on 10 narrow polystyrene standards ranging in molecular weight from 1.2×10^3 to 5.5×10^4 g/mol. Data collection and processing were performed by Polymer Standards Service WinGPC Unity software (Build 6807). The number-average and weight-average molecular weights (M_n and M_w) were calculated by the software relative to the universal polystyrene calibration curve.

FT-IR ATR spectroscopic analysis

Spectrum One FT-IR system (Perkin Elmer, Wellesley, MA) with a universal attenuated total reflection (ATR) accessory was used to characterize the isolated pseudo-lignin samples. Each sample was pressed uniformly and tightly against the diamond surface using a spring-loaded anvil. FT-IR spectra were obtained by averaging 64 scans from 4,000 to 650 cm^{-1} at 4 cm^{-1} resolution. Baseline and ATR corrections for penetration depth and frequency variations were carried out using the Spectrum One software supplied with the equipment.

NMR spectroscopic analysis

NMR experiments were performed using a Bruker AMX-400 spectrometer operating at a frequency of 100.61 MHz for ^{13}C NMR analysis. Quantitative ^{13}C NMR spectrum was acquired using dimethylsulfoxide (DMSO)-d_6 (450 μL) as the solvent for the samples (120 mg) at 298 K with an inverse-gated decoupling sequence, 90° pulse angle, 12-s pulse delay and 8000 scans. Distortionless enhancement by polarization transfer (DEPT) NMR spectra were recorded using a 135º pulse angle, 3-s pulse delay, and 12000 scans for the same samples from quantitative ^{13}C NMR analysis. 2D heteronuclear single quantum coherence (HSQC) correlation NMR analysis was performed using a standard Bruker pulse sequence with a 90° pulse, 0.11 s acquisition time, a 1.5-s pulse delay, a $^{1}J_{C\text{-}H}$ of 145 Hz and acquisition of 256 data points.