Extraction of Lignin and Quantitative Sugar Release from Biomass using Efficient and Cost Effective Pyridinium Protic Ionic Liquids

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Supplementary Data

Compositional Analysis

Known weight (300 mg) of air-dried cellulose rich material (CRM) was put in a 100 mL pressure tube. After adding 3 mL of 72% sulfuric acid, the mixture was thoroughly stirred by a Teflon rod. After ensuring thorough mixing the pressure tube was transferred into a water bath (preheated at 30 °C). The sample was allowed to heat for 1 hour with a periodic stirring (every 15 minutes). The pressure tube was removed and 84 mL of deionized water was mixed to the contents of the tube and it was closed by a Teflon lid. The tube was autoclaved at 120 ^{o}C for 1 hour (Sanyo Labo Autoclave ML5 3020 U). It was then removed from the autoclave to be allowed to cool down to 25 ^{o}C. The solid portion of the tube was filtered off using a filter crucible of accurately measured mass. The filtrate obtained in the Buchner flask was taken into two different tubes for sugars and acid soluble lignin measurement. Sufficient amount of calcium carbonate was added to one tube to keep the pH in the range 6-6.5. This mixture was filtered using a syringe equipped with a 0.2 micrometer PTFE. The filtered sample was then taken into a 2mL HPLC vial for analysis of sugars. HPLC analysis of the sample was conducted using Shimadzu HPLC machine (Aminex HPX-97P Bio-Rad, 300 x 7.8 mm, purified water as mobile phase at 0.6 ml/min, column temperature 85°C, de-ashing columns were used as pre-filters). The standard solutions for sugars were prepared as 10 mL stock solutions of concentrations 0.1 mg, 1 mg, 2 mg, 4 mg and 8 mg per mL. The standard samples were also added along with the unknown samples for the HPLC analysis. Following formulas were used to measure the concentration of the sugar in the unknown samples (Eq.1 and Eq.2)

$$SRC = \frac{C_{HPLC} \cdot V}{initial\ weight} \quad \text{(Eq.1)}$$

$$\%sugar = \frac{c_{HPLC} \cdot V \cdot c \cdot corr_{anhydro}}{SRC \cdot ODW_{sample}} \cdot 100 \quad \text{(Eq.2)}$$

In the above formulas C_{HPLC} is the concentrations of different sugars measured using HPLC analysis, V denotes volume of the solution initially taken in the pressure tube (10.00 mL for the sugar recovery standards and 86.73 mL for the samples), initial weight designates the weight of the sugars in the original biomass, $corr_{anhydro}$ is used as the adjustment of any variation in mass of the sugars during hydrolysis of polysaccharides (0.90 for the C6 sugars glucose, galactose and mannose and 0.88 for the C5 sugars xylose and arabinose) and ODW designates oven dried mass of the untreated biomass or pulp in mg.

The contents of the second tube were used to calculate the amount of acid soluble lignin (ASL) in the sample. It was done by performing UV-Vis analysis at the 240 nm wavelength (Perkin Elmer Lambda 650 UV/Vis spectrophotometer). The amount of the acid soluble lignin was calculated using the formula given in Eq. 3.

$$\%ASL = \frac{A}{l \cdot \varepsilon \cdot c} \cdot 100 = \frac{A \cdot V_{filtrate}}{l \cdot \varepsilon \cdot ODW_{weight}} \cdot 100 \quad \text{(Eq. 3)}$$

Where %ASL is the percentage of acid soluble lignin in the original biomass, A denotes absorbance at the required wavelength (240 nm), l designates the UV cell path length (1 cm in this case), ε denotes coefficient of extinction (12 L/g cm), c is the concentration of the solution (mg/mL), ODW_{weight} represents the oven-dried mass of biomass sample in mg initially taken and $V_{filtrate}$ is the total filtrate volume (86.73 mL).

The solid residue in the crucible was washed well using purified water and it was placed in a convection oven (VWR Venti-Line 115) at 105 ^{o}C for 24 hours (drying off water). After removing the crucible from the oven it was instantly put into a desiccator for cooling to room temperature. When the crucible was cooled down it was weighed and the weight was recorded; finally, it was subjected to ashing in a muffle oven (Nabertherm controller P 330) at 575 ^{0}C. After ash formation the weight of the crucible was again measured. The percentage of ASL (acid insoluble lignin) was calculated using formula in Eq 4.

$$\%AIL = \frac{weight_{crucible\ plus\ air} - weight_{crucible\ plus\ ash}}{ODW_{ssample}} \quad \text{(Eq.4)}$$

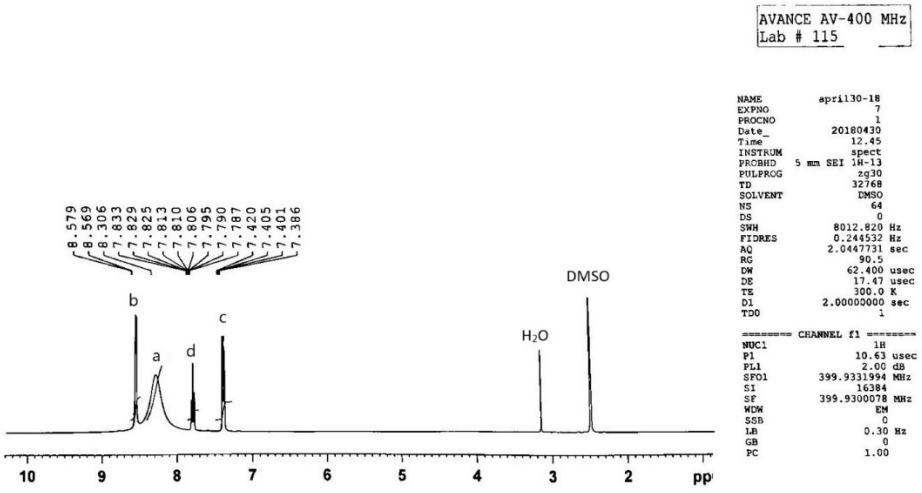
Where $Weight_{crucible\ plus\ air}$ denotes the weight of oven dried crucible plus the acid insoluble lignin and ash and $W_{crucible\ plus\ ash}$ designates the weight of the crucible after ash formation.

Saccharification

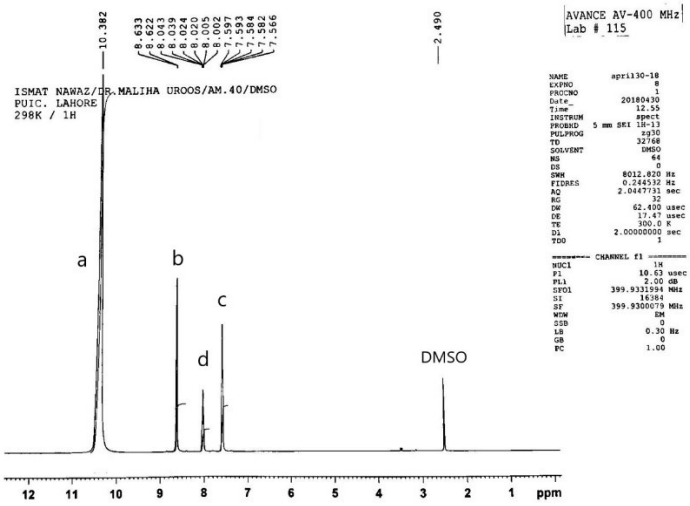
100 mg oven dried weight (ODW) of the untreated biomass and delignified pulp samples were taken in Sterilin tube and the measured weight was recorded. 9.9 mL of the enzyme solution was added to the above tube. This mixture was prepared by mixing 5 mL buffer solution (100 mM sodium citrate and citric acid, pH 4.8), 40 mL of solution of tetracycline in ethanol (10 mg / mL of 70 % ethanol), 30 µL cycloheximide solution in deionized water (10 mg / mL), 50 µL enzyme mixture ((Novozymes NS-22201) and 50 mL purified water (deionized).

The sterilin tubes were then sealed and put into an incubator (Stuart Orbital Incubator S150) at 50 ^{o}C and speed of 250 rpm. Three blank referral samples (having only 100 µL of water were) were also subjected to incubation to correct any discrepancy occurring due to residue sugars in the enzyme mixture. The sample tubes were kept for 7 days for incubation under the above mentioned conditions and removed after 7 days. These were then allowed to cool down.

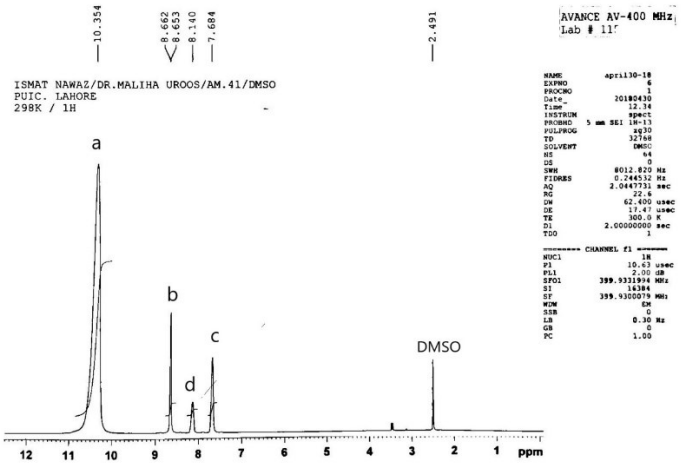
The samples were then subjected to HPLC analysis to measure the amount of the released glucose. To do this, 1 mL of the solution was taken in the HPLC vial after filtration using a syringe having 0.2 micrometer syringe filter (Shimadzu HPLC system equipped with RI detector and aminex HPX-87P (Biorad 300 x 7.8 mm) column). Deionized water was run as the mobile phase (column temperature 85 ^{0}C, acquisition time 40 min). The standard sugar solution mixtures (0.1, 1, 2, 4 mg/mL of glucose, xylose, mannose, arabinose and galactose) and 8 mg/mL of only glucose were run for calibration of the instrument.



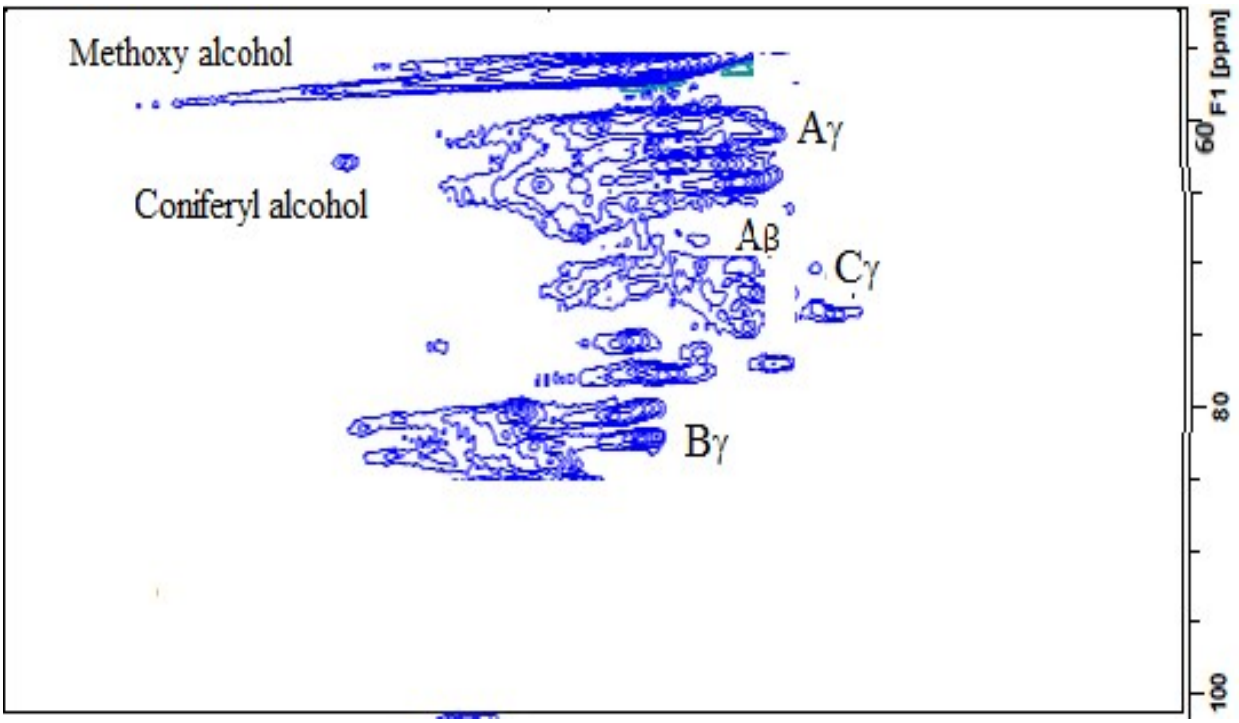
S1: 1H NMR spetrum of Pyridinium dihydrogen sulfate (P1)



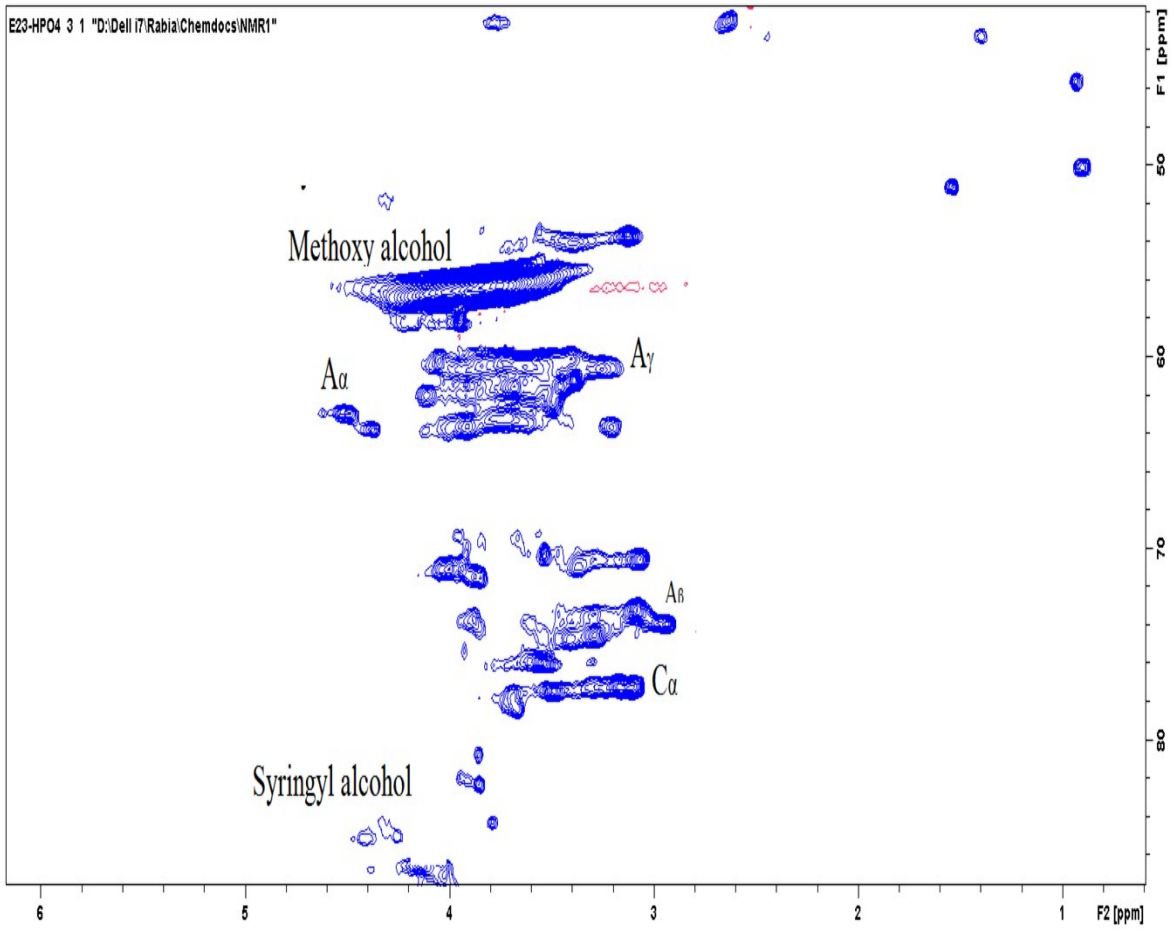
S2: 1H NMR spectrum of Pyridinium dihydrogen phosphate monophosphoric acid (P2)



S3: 1H NMR spectrum of Pyridinium dihydrogen phosphate diphosphoric acid (P3)

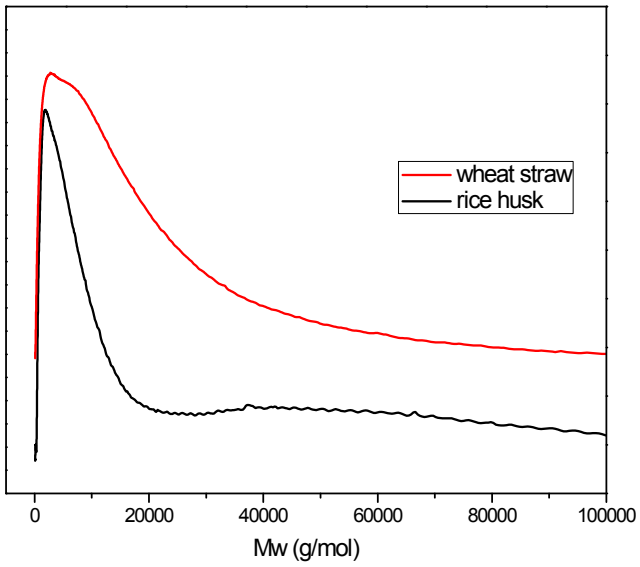


(a)



(b)

S4: Aliphatic portion of HSQC NMR spectrum a) wheat straw; b) rice husk



S5: GPC profile of the lignin extracted from wheat straw and rice husk at 100 ^{o}C, after 2 hour pretreatment with 5% loading.