



Lignin residue from production of bio-ethanol using spruce as raw material: Fractionation and characterization by 2D NMR and GPC

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ABSTRACT

For gaining more structural information about the lignin residue (hydrolysate lignin, HL) from acid hydrolysis of wood *e.g.* lignocellulose bio-ethanol production a fractionation study was made and the soluble fractions was analyzed with GPC and 2D NMR. Structural knowledge of the different technical lignins are important for identifying possibly new routes for further depolymerization or other applications of HL in the future biorefinery processes.

Keywords: *lignocellulose ethanol production; hydrolysate lignin; structure characterization; quantitative HSQC; 2D NMR.*

INTRODUCTION

Today sugar cane and corn are the major raw materials for production of bio-ethanol. In the future wood residues is a potential important raw material for this production and large research efforts has been done in order to produce ethanol from wood. Reasonable good carbohydrate conversion degree has been obtained, but wood is a rather complex material and the process needed is much more complicated compared to when the process is based on sugar cane or corn. Consequently, the total production cost will be much higher if wood residue is used as raw material and cannot, at this point, compete on the world market. For making the approach using wood residue economical feasible other high value products have to be gained from the side streams *e.g.* lignin residue or hydrolysate lignin (HL). The aim of this study was therefore, as a first step, to characterize the HL but also to make it more soluble by *i.e.* alkaline treatment or by fractionation with organic solvents. For gaining more insight of the HL chemical structure the soluble fractions was analyzed by gel permeation chromatography (GPC) and quantitative and qualitative two-dimensional nuclear magnetic resonance [2D NMR, ¹H-¹³C Heteronuclear Single Quantum Coherence (HSQC) and Q-CCAHSQC].



EXPERIMENTAL

The HL used in this study was from spruce wood chips pretreated with SO₂, steam exploded (210 °C, 5 min), depolymerized by enzymatic hydrolysis and the solid fraction filtered off to retain HL (Stenberg *et al.* 1998). Spruce HL was further fractionated with organic solvents [methanol and THF (60 °C, 16 w/v%) and dioxane (95 °C, 14 w/v%)] and 1M aq. NaOH [rt. and 95 °C (16 w/v%) and 170 °C (5 w/v%)] for 3h, filtered and dried at 40 °C (3 days) before further analysis. The lignin fractions was analyzed with GPC (DMSO/10mM LiBr, Polar-Gel columns, Pullulan standards) for determination of Mw distribution and polydispersity (PD). Structure determination of lignin fractions (100 mg/0.75 mL DMSO-*d*₆) was done with solution phase NMR qualitative phase sensitive ¹³C-¹H HSQC and quantitative Q-OCCHSQC was run at high field NMR 800 MHz equipped with 5 mm TXO cryoprobe (Koskela *et al.* 2010, Zhang and Gellerstedt 2007).

RESULTS AND DISCUSSION

HL was first subjected for a solubility study, yielding partly solvation in DMSO, pyridine and aq. NaOH. The solubility was rather poor in all these cases which indicates a highly condensed structure with low phenolic content of HL. Therefore, a fractionation approach was applied for retain structural information about HL. HL was fractionated with different organic solvents (60-95 °C, MeOH, dioxane and THF) yielding a minor part as soluble HL fraction (9-18%), a sugar fraction (approx. 10%) and an insoluble HL residual fraction (approx. 80%). By instead using alkaline conditions (1M NaOH) yielded slightly higher yield of HL soluble fraction (12-24%, rt., 95 °C and 170 °C). However, lower yields of residual lignin (46-65%) indicating that alkaline environment induce reactions of HL. For a better understanding of the chemical properties that influence the solubility the soluble HL fractions was further analyzed by GPC and qualitative and quantitative 2D NMR.

HL received from bio-ethanol production plant was analyzed with GPC for determination of Mw, DMSO soluble HL lignin yielded a biphasic peak at 5.4 kDa and PD of 5.8. It has to be noted that the heavier Mw fragments in HL was not dissolved. Thereto, the fractionated organic solvent and alkaline soluble HL fraction was found to differ significantly in Mw distribution. The organic solvent extracted soluble HL fractions (MeOH, THF and dioxane) was found to have lower Mw (3.3-4.4 kDa, PD 3.9-5.0) vs. the alkaline extracted (10-17 kDa, PD 4.6-5.0). This difference in Mw between organic solvent vs. alkaline fractionation indicates that different HL lignin fractions have been extracted out or that alkaline conditions induce reactions/polymerization of HL.

Investigation of structure of the soluble part of the HL by 2D NMR indicate major changes in the lignin structure. During the harsh acidic conditions have the known oxygen-aliphatic network (inter-unit linkages) in HL been degraded/fragmented (Table 1). The most in wood abundant aryl ether β-O-4' structure could not be detected at all. Faint signals of β-β' could be found but the chemical shifts of γ-CH₂ indicate some modifications in this inter-unit linkage as well. The only unchanged lignin oxygen aliphatic structural motif was β-5' phenylcoumaran structure. Which seems to be more resistant to the acidic conditions and thus are accumulated. However, the γ-CH₂ structures next to a hydroxyl and ether linkage could still be found, indicating that a part of the oxygen network are left or included as end groups in HL.



Table 1. Lignin regions and structural motif detected with quantitative 2D NMR of the soluble HL fractions compared with a Kraft lignin.^a

Structure	δ_C/δ_H (ppm)	MeOH 60 °C	NaOH rt.	NaOH 95 °C	NaOH 170 °C	Kraft lignin ^b
β -O-4' (CH _{α})	71.7/4.8-4.7	nd	nd	nd	nd	2
β -5' (CH _{α})	87.5/5.4-5.5	3	2	nd	nd	3
β - β' (CH _{α}) ^c	85.8/4.6-4.7	<1	nd	nd	nd	1
Carbohydrates (CH ₁)	90-104/4.0-5.5	<1	<1	nd	nd	1
Guaiacyl G ₂	112-110/7.2-6.9	10	6	7	1	12
Guaiacyl G ₅	115.4- 116.6/6.6-6.9	29	25	26	17	31
Guaiacyl G ₆	118.6- 120.7/7.0-6.7	4	4	4	1	8
Condensed G ₂	113-114/6.6-6.7	4	4	4	2	3
Condensed G _{5/6}	121-122/6.6-6.5	8	8	7	5	10
Guaiacyl ox. G ₂ '	114-109/7.6-7.2	6	4	6	5	4
G' (-COOH, -COR)	123-127/6.8-7.8	20	8	9	9	4
Cond. CH _{β} -alkyl	44-52/1.7-2.2	18	11	9	4	3
Cond. CH _{α} α -5/6	49-53/3.9-4.6	2	3	2	nd	nd
β -O-4' "like" γ -CH ₂ ^c	60.2-61.3/3.2- 3.5	10	8	7	6	22 ^d

^aIntegrated and reported relative to methoxyl signal (δ_C/δ_H 55.76-56.47/63.92-3.60 ppm). ^bLignoBoost. ^csymmetrical/2. ^dCH₂/2. ^e δ_H 3.17-3.63 ppm used: nd, not detected.

Analysis of the aliphatic region shows a crowd of different CH₂-groups connected α and β to an aromatic ring together with γ -CH₃ end groups. Of course the extractives contribute to these signals (*i.e.* fatty acids and resin acids) but indications of dihydroconiferyl alcohol like structures (Ar-C-C-CH₂-OH/R) and carboxylic end groups (Ar-CH₂-COOH) could be detected. In addition, the methine carbons (tertiary CH, Table 1) formed by condensation reactions could be detected in HL in fairly high amount compared with Kraft lignin (which of course also are condensed from the Kraft process). This leads to the conclusion that HL are more condensed than Kraft lignin. The insoluble part of HL is most likely even more condensed, but this remains to be investigated.

The known guaiacyl lignin structure, contains G₂, G₅ and G₆ aromatic CH. The signal strength in G₂ and G₆ regions was lower and shifted to G₅ region. This together with signals in the condensed region (Figure 1 and Table 1) indicates that new carbon-carbon bonds have been formed leading to a more substituted aromatic structure (higher degree of condensation). In addition, other new structures formed are oxidation of lignin side chains to α -carbonyl end groups at G₂ and G₆ position (G₂' and G', Table 1). This could be a position for further chemical depolymerization of the soluble HL fraction.

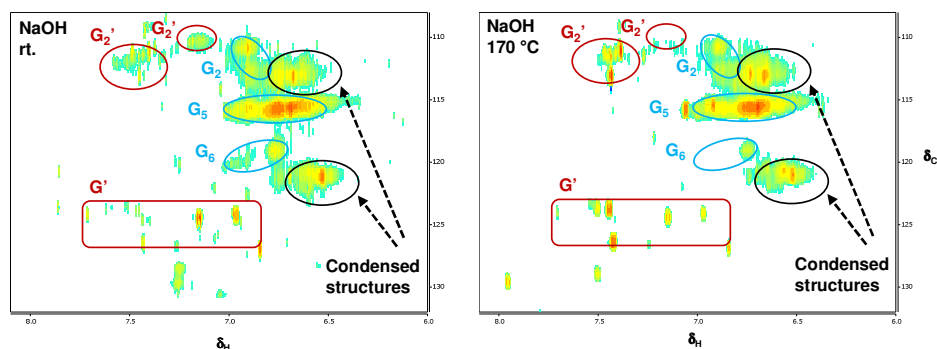


Fig. 1. 2D NMR of aromatic region for aq NaOH soluble HL fractions.

CONCLUSIONS

1. The soluble fraction (9-20%) of lignin obtained from steam exploded/enzymatic hydrolysis (HL) have been analyzed with 2D NMR. It was found that the lignin aliphatic-oxygen network have largely been modified/cleaved and no sign of the major aryl ether β -O-4' structures was found. The only non-modified structure left was phenylcoumaran β -5' structures.
2. HL is condensed both in aromatic region (C-C) and in aliphatic region (tertiary CH).
3. Oxidation of the lignin side chain α - and β -position to carbonyl functional groups (-COOH/R, -COR and -CHO) have been taking place in spruce HL.
4. Fractionation with methanol vs. aq. NaOH yields different structures and Mw of spruce HL lignin. Alkaline extraction could possibly contribute to base catalyzed polymerization to larger HL structures. Indicating that other reactive positions (not β -O-4') are left in HL for future modifications.

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REFERENCES

- Koskela H, Heikkilä O, Kilpeläinen I, Heikkinen S. (2010) Quantitative two-dimensional HSQC experiment for high magnetic field NMR spectrometers. *Journal of Magnetic Resonance*, 202(1),24-33.
- Stenberg K, Tengborg C, Galbe M, Zacchi G. (1998). Optimisation of steam pretreatment of SO₂- impregnated mixed softwoods for ethanol production. *Journal of Chemical Technology & Biotechnology*, 71(4), 299-308
- Zhang L, Gellerstedt G. (2007). Quantitative 2D HSQC NMR determination of polymer structures by selecting suitable internal standard references. *Magnetic Resonance in Chemistry*, 45(1), 37-45