

Article

The Impact of Biomass and Acid Loading on Methanolysis during Two-Step Lignin-First Processing of Birchwood

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Abstract: We optimized the solvolysis step in methanol for two-step lignin-first upgrading of woody biomass. Birchwood was first converted via sulfuric acid methanolysis to cellulose pulp and a lignin oil intermediate, which comprises a mixture of lignin oligomers and C5 sugars in the methanol solvent. The impact of reaction temperature (140–200 °C), acid loading (0.24–0.81 wt%, dry biomass), methanol/biomass ratio (2.3/1–15.8/1 *w/w*) and reaction time (2 h and 0.5 h) was investigated. At high biomass loadings (ratio < 6.3/1 *w/w*), operation at elevated pressure facilitates delignification by keeping methanol in the liquid phase. A high degree of delignification goes together to a large extent with C5 sugar release, mostly in the form of methyl xylosides. Gel permeation chromatography and heteronuclear single quantum coherence NMR of lignin fractions obtained at high acid (0.81 wt%) and low biomass (15.8/1 *w/w*) loading revealed extensive cleavage of β -O-4' bonds during acidolysis at 180 °C for 2 h. At an optimized methanol/biomass ratio of 2.3/1 *w/w* and acid loading (0.24 wt%), more β -O-4' bonds could be preserved, i.e., about 33% after 2 h and 47% after 0.5 h. The high reactivity of the extracted lignin fragments was confirmed by a second hydrogenolysis step. Reductive treatment with Pd/C under mild conditions led to disappearance of ether linkages and molecular weight reduction in the hydrotreated lignin oil.



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1. Introduction

Concerns over anthropogenic climate change such as global warming are major drivers to accelerate the transition from fossil to renewable resources [1]. The development of alternative sustainable feedstocks for energy, chemicals and materials is regarded one of this century's most important societal challenges [2,3]. In this context, lignocellulosic biomass is a promising candidate because it is the most abundant renewable source of carbon on Earth with an estimated production rate of 180 billion tonnes per year [4]. Of these, about 33 billion tonnes correspond to wood and wood processing wastes (i.e., chopped trees or sawmill dust), while 14 billion tonnes are classified as agricultural crops and waste materials (such as straw, corn husks or bagasse) [5]. Appropriate processing of these lignocellulosic feedstocks into biofuels and biochemicals can lead to more sustainable energy and chemical industries. The economic feasibility of biomass conversion processes depends strongly on the effectiveness of exploiting the principal lignocellulosic components. More than 80% of the mass of lignocellulose comes from three of its constituent biopolymers: lignin (15–30 wt%, dry basis), cellulose (35–55 wt%, dry basis) and hemicellulose (10–35 wt%, dry basis) [6]. Lignin-free cellulose is currently utilized for paper manufacturing and increasingly for the production of second generation (2G) ethanol. In general, the

monomers of these biopolymers represent promising feedstocks for a sustainable chemical industry, specifically glucose from cellulose, predominantly xylose from hemicellulose and aromatics from lignin [7]. Although glucose and xylose can be fed into already developed biorefinery processes, the upgrading of lignin to useful products has not been achieved yet at the commercial scale despite the substantial demand for renewable aromatics. This is largely due to challenges in efficiently isolating lignin in a reactive form and its further depolymerization into its constituent monomers [8].

Current biomass fractionation technologies, which are mainly integrated into pulp and paper processes and emerging cellulosic ethanol biorefineries, aim at separating lignin from cellulose and hemicellulose fractions [9]. Kraft and sulfite pulping are the dominant technologies in pulp and paper processing. During the kraft process, the fibrous feedstock is digested with a mixture of sodium sulfide and sodium hydroxide at about 170 °C, while in the sulfite process this is achieved with an aqueous solution of a sulfite or bisulfite salt of sodium, ammonium, magnesium or calcium at a pH of either 1.5 or 4.0 at 140–170 °C [10]. Many new biorefinery processing steps aimed at obtaining lignin as a chemical intermediate involve treating the raw biomass in an alkaline environment [11] or with mineral acids at high temperature in water [12]. The organosolv process is another promising biomass pretreatment approach that utilizes organic solvents or mixtures of organic solvents with water (ethylene glycol, ethanol–water, acetone–water, butanol–water, etc.), sometimes catalyzed by acids such as hydrochloric, sulfuric, oxalic, salicylic and Lewis acids. Compared with conventional kraft and sulfite pulping, organosolv pulping can overcome several drawbacks such as severe water and air pollution [13] and result in a lower sulfur content of the isolated lignin [14,15]. Lignin from organosolv pulping is mainly recovered as a solid product by addition of acidified water. This type of lignin has a high purity, low ash content and has been the subject of catalytic conversion studies targeting biobased fuels and chemicals. However, typical monomer yields are low because undesired condensation reactions take place during lignin extraction and the downstream isolation steps. More specifically, the benzylic alcohols of lignin can be easily protonated and eliminated, producing reactive benzylic carbocations undergoing subsequent electrophilic aromatic substitution with nearby electron-rich quaiacyl and syringyl subunits, which leads to the formation of C-C linkages [9]. The high stability of these C-C bonds leads to low monomer yields after extraction and hydrogenolysis (generally < 5–10% (wt/wt) of the original Klason lignin content) [16,17]. The high condensation degree of such isolated lignins is comparable to others and evident from their high glass transition temperature ($T_g > 120$ °C), which prevents the valorization of lignin with a high added value [18].

Lignin-first processing is the broadly accepted term for solvent-based methods in which lignin preservation, together with that of the polysaccharides, is considered up-front, moving away from current practices of having to deal with an unattractive lignin material at the end of the biorefining process [19,20]. Lignin-first technology was developed as an active stabilization approach that liberates lignin from the plant cell wall and prevents condensation reactions through either catalysis or protection-group chemistry. To date, there have been several approaches reported for the lignin-first refinery. For instance, Xu et al. (2014) demonstrated that lignin in birchwood can be converted to phenolic monomers using a Ni-based catalyst in a combined fractionation–hydrogenation process [21]. Sels et al. (2015) reported the reductive conversion of birch sawdust into phenolic monomers over Ru/C catalyst in methanol through simultaneous solvolysis and hydrogenolysis [22]. Later, the influence of solvents, acid/base additives and different redox catalysts was investigated to improve the lignin monomer yield and carbohydrate retention during biomass fractionation [22–24]. Our group has recently demonstrated that metal triflates can effectively catalyze the release of lignin fractions from lignocellulose during reductive fractionation and that the dissolved lignin fragments are rapidly depolymerized to phenolic monomers by Pd/C-catalyzed hydrogenolysis [25,26].

Even though these lignin-first processes are promising, there are still a lot of obstacles that need to be overcome before they reach the commercialization stage. Some of them

have been already addressed in previous research, as summarized hereafter. The recovery of heterogeneous catalysts remains a challenge because of the presence of cellulose pulp residue and other residues. Magnetic separation has been explored to remove Raney Ni catalysts [21–27]. An alternative approach is to avoid direct contact between the heterogeneous catalyst and the solid feedstock. Reductive biomass fractionation and lignin hydrogenolysis can be separated in two beds in a continuous flow process. Lignin can be extracted in the first bed and converted to monomers in a reductive environment in the second catalyst bed [28,29]. The delignification and lignin depolymerization processes can also in principle be carried out in two consecutive batch processes. Luterbacher et al. (2016) demonstrated a two-step method involving a HCl-catalyzed extraction of lignin in the presence of formaldehyde in the first step, thereby suppressing condensation reactions. High monomer yields close to the theoretical values were achieved under Ru/C catalysis [30]. We also investigated a two-step process system in the absence of a stabilization agent as a feasible route for commercializing this technology. We carried out a preliminary optimization study by investigating different types of acids (H_2SO_4 , H_3PO_4 , HCl, $\text{Al}(\text{OTf})_3$, *p*-TSOH) during the fractionation step, aimed at minimizing lignin repolymerization and investigating the influence of the quality of the lignin oil intermediate on the outcome of the second hydrogenolysis step using Pd/C as a heterogeneous catalyst. Despite the overall progress, two important process parameters were not investigated, namely the solvent/biomass ratio (w/w) and the acid loading per dry weight of biomass. Their influence on the process economics and the quality of the extracted lignin fragments can be significant. The solvent/biomass ratios (w/w) employed previously in lignin-first processing were between 15.8/1 and 10/1 w/w , while the acid loading varied from 10 to 60 wt% (based on dry biomass) [1,6,9,19,22–26,30,31]. The solvent/biomass ratio is an important parameter for operational costs (i.e., reactor sizing, total throughput, solvent regeneration) and for solubilization of lignin components [32]. For the organosolv process, it has already been stressed that high solvent loadings lead to intensive solvent recycling and storage capacity needs. This will increase the overall fixed capital and operational costs [33]. Demonstration-scale organosolv processes (i.e., Organocell, Alcell) have been optimized to operate in a liquor/solids ratio below 10/1 (w/w) [34]. Additionally, the acid loading during biomass fractionation is of great importance because it can influence the delignification degree and rate of polymerization reactions of the extractives (i.e., lignin and hemicellulose).

The objective of this work was twofold. First, we aimed to optimize the first delignification step in terms of solvent/biomass ratio and acid loading by targeting the maximum delignification and fractionation efficiency. Methanol was selected as solvent because it is cheaper and a better solvent for lignin than other alcohols (i.e., ethanol, 1-propanol, 1-butanol) [35–39]. This optimization builds on an earlier work where we investigated a two-step catalytic process for oak sawdust fractionation and evaluated the delignification efficiency using different homogeneous acids [40]. We found that for a methanol/biomass ratio of 1/20 w/v , a temperature of 160 °C and a reaction time of 2 h, delignification and sugar cellulose retention strongly depend on the type of acid. Among the investigated homogeneous acids, sulfuric acid showed the best performance. Here we focused on exploring the limitations of our fractionation process by applying higher biomass loadings than reported before, which is important with respect to the development of an economically viable process. The intermediate crude lignin oils (CLOs) were evaluated based on the fractionation results and analyzed for their quality with gas chromatography/gas chromatography-mass spectrometry (GC/GC-MS), gel permeation chromatography (GPC) and heteronuclear single quantum coherence NMR (HSQC NMR). Second, we gauged important structural changes of the extracted lignin fractions (i.e., β -O-4 content) and evaluated their reactivity by subjecting the resulting lignin oils to a second reductive hydrogenolysis step for obtaining phenolic monomers.

2. Results and Discussion

2.1. Acid Methanolysis of Birchwood

Solvolytic fractionation of birchwood was evaluated as a method to extract oligomeric lignin from biomass. We used methanol as a solvent and sulfuric acid as the acid catalyst for the direct conversion of biomass to a lignin oil composition. We optimized the acid solvolysis of birchwood towards the highest delignification degree by varying the methanol/biomass ratio, temperature and sulfuric acid concentration with retention of cellulose as another important aim. Table 1 summarizes the fractionation results under different pretreatment conditions.

Acid solvolysis of birchwood was first conducted at a methanol/biomass ratio of 15.8/1 *w/w*, an acid concentration of 4 mM H₂SO₄ and different reaction temperatures. The amount of acid catalyst has been optimized for the indicated ratio based on a one-step catalytic reductive depolymerization approach [26]. The reaction time was kept constant at 2 h during the whole optimization study. At 140 °C, a lignin oil yield of 40.4 wt% was achieved with a delignification degree of 64.8 wt%. At 160 °C the oil yield increases to 60.5 wt%, while 93.5 wt% of lignin can be extracted from birchwood. Generally, a higher acid concentration and temperature can improve the lignin extraction efficiency [34]. We observed that a further increase of the temperature to 180 °C resulted in a higher lignin oil yield (67.4 wt%) and delignification degree of 119 wt%. The fact that the delignification degree exceeds 100% can be attributed to the involvement and presence of sugar-derived products in the condensation reactions that occur during the pretreatment process. This has been explained by Huijgen et al. (2011) who studied the catalytic effect of sulfuric acid on organosolv (ethanol/water) pretreatment of wheat straw at temperatures between 160–180 °C, an acid loading of 1.1 wt% and a reaction time of 120 min [35,36]. The conversion of xylan derivatives to products like humins or lignin-furfural condensation products was observed, while some glucose present due to partial cellulose hydrolysis was also involved in humin formation. Cellulose degradation was also observed in our experiments, as the retention of the wood residue (i.e., the cellulose pulp) decreased with a yield of 34.7 wt% (entry 3), compared to the actual glucan content of birchwood (39.6 wt%). The use of higher biomass loading at 160 °C (entry 4) resulted in a lignin oil yield of 46.8 wt% and a delignification degree of 71.7 wt%, while at 180 °C 103 wt% of the available lignin was extracted. Reactions at a methanol/biomass ratio of 6.3/1 *w/w* at 160 °C at the same acid concentration yielded a low delignification degree of 37.8 wt%. We also increased the pretreatment severity of the process by increasing the temperature to 180 °C (entry 7). It has been reported that delignification during organosolv pretreatment improves with pretreatment severity, which is usually represented by the combined severity (CS) factor [34–37]. This factor depends on acidity, reaction time and temperature. Despite this, the delignification degree was lower (31.5 wt%), and the achieved lignin oil yield was only 12.8 wt%. To resolve this, we introduced 3 MPa of nitrogen gas to keep methanol in the liquid phase. Ragauskas et al. (2013) emphasized the importance and participation of organic solvents (like methanol or ethanol) in the main mechanism of acid-catalyzed organosolv pretreatment [34]. The hydrolysis of lignin hemicellulose linkages and internal lignin bonds results in hemicellulose and lignin solvolysis by cleavage of 4-*O*-methylglucuronic acid ester bonds to the α -carbons of lignin and cleavage of α - and β -*O*-aryl ether linkages, respectively. We have previously demonstrated that methanol exhibits the highest solvolytic efficacy of lignin among a set of linear alcohols, including heavier lignin fractions originating from condensation reactions [38]. Thus, it is crucial to keep the organic solvent in the liquid phase to maximize lignin extraction and dissolution. In conventional organosolv pretreatment processes, this is achieved using aqueous organic solutions. The concentration of solvent in water ranges from 40 to 80%, which reduces the vapor pressure during operation while maximizing lignin dissolution at the optimum liquor/solids ratio (4/1 *w/w*) [34]. The effect of the additional cold pressure at a methanol/biomass ratio of 6.3/1 *w/w* is evident in our process (entry 8). The delignification degree increased drastically to 98.8 wt% at a yield

of extracted lignin oil of 42.7 wt%. We further increased the liquid/solids ratio to 3.9:1 *w/w* under the same conditions and 3 MPa nitrogen (entry 9). This led to a drop of the delignification degree to 50.2 wt%. After increasing the acid concentration to 8 mM, a lignin oil yield of 52.8 wt% was achieved, while 94.2 wt% of lignin was extracted from birchwood (entry 10). We then explored the limits of the acid methanolysis process by applying a methanol/biomass feeding ratio of 2.3/1 *w/w* at 180 and 200 °C. The delignification degrees were 66.3 and 68.8 wt%. After fixing the process severity by increasing the acid concentration to 10 mM H₂SO₄, a lignin oil yield of 38.4 wt% and delignification of 86.4 wt% was achieved after acid solvolysis at 180 °C.

Table 1. Fractionation by acid methanolysis of birchwood as a function of the methanol/biomass ratio, temperature, pressure and acid loading.

Entry	Pretreatment Conditions ^b				Fractionation Results ^d					
	Ratio ^a	Acid Loading (wt%)	Acidity (mM H ₂ SO ₄)	Temperature (°C)	Wood Residue ^c (wt%)	Liquid Fraction			DL ^e (wt%)	Total (wt%)
						Lignin Oil (wt%)	Lignin (wt%)	Methyl Glycosides (wt%)		
1	15.8:1	0.81	4	140	53.3	40.4	15.7	6.3	64.8	94
2	15.8:1	0.81	4	160	41.7	60.5	22.6	17.8	93.5	102
3	15.8:1	0.81	4	180	34.7	67.4	28.9	15.5	119	102
4	9.5:1	0.49	4	160	52.8	46.8	17.3	7.2	71.7	99
5	9.5:1	0.49	4	180	41.8	40.8	25.0	20.5	103	94
6	6.3:1	0.32	4	160	80.5	18.8	9.1	1.5	37.8	99
7	6.3:1	0.32	4	180	75.6	12.8	7.6	6.7	31.5	88
8	6.3:1	0.32	4	180	53.8	42.7	24.0	10.0	98.8	97
9	3.9:1	0.20	4	180	75.2	15.3	13.3	0.0	50.2	92
10	3.9:1	0.40	8	180	42.9	52.8	22.7	11.8	94.2	95
11	2.3:1	0.24	8	180	66.2	26.6	16.2	1.7	66.3	93
12	2.3:1	0.24	8	200	61.8	27.6	16.6	5.2	68.8	91
13	2.3:1	0.30	10	180	58.7	38.4	20.6	12.0	86.4	97

^a Methanol/biomass ratio (*w/w*); ^b In entries 1–7, no additional nitrogen pressure was applied, and in entries 8–13, 3 MPa of nitrogen pressure was applied; ^c glucan content of birchwood feedstock is 39.6 wt% as determined by the NREL method [37]; ^d The two main fractionation products of the pretreatment process are the wood residue and the lignin oil. The lignin oil comprises the lignin fraction and methylated glycosides, together with other carbohydrates that could not be identified and thus are not presented in this table. ^e The degree of delignification (DL) was determined using Equation (2) in Section 3.3 and is based on the Klason lignin content of birchwood (i.e., 24.2 wt%).

The use of an acid hydrolysis step also releases some of the polysaccharides. In general, the retention of C₆ sugars is expected to be much higher than that of C₅ sugars during acid delignification because of the high crystallinity of cellulose [40–42]. The C₅ and C₆ sugars extracted during reaction in methanol are soluble in the lignin oil phase and mainly identified as their methyl analogues (methyl glycosides stream, Table 1). Figure 1 presents the type and yields of the methyl glycosides in the lignin oil fractions derived after acid methanolysis of birchwood at 180 °C at various biomass loadings. Methyl β-D-xylopyranoside is the main C₅ sugar-derived product in all cases. Comparison of the delignification degree (Table 1) and the methyl xyloside yield (Figure 1) shows that a high degree of delignification is always accompanied by a high degree of C₅ sugar release, which is in agreement with previous findings [38]. Further, a small amount of methyl-glucopyranoside was observed when high biomass loadings were utilized. It is likely that the combination of high acid loading and high temperature promotes the conversion of cellulose. Previous studies have also reported the presence of methyl-glucopyranosides during acid-catalyzed and methanol-mediated reductive fractionation of birchwood [24,25,43]. The released carbohydrates can also be dehydrated to furfural-type products, such as furfuraldehyde, 5-hydroxyethylfurfural and 5-(methoxymethyl)furfural in an acidic solution [35,36,44]. This can explain the higher lignin oil yields compared to the yield of methylated glycosides. These additional products were not observed, presumably due to the occurrence of lignin-furfural condensation reactions [45].

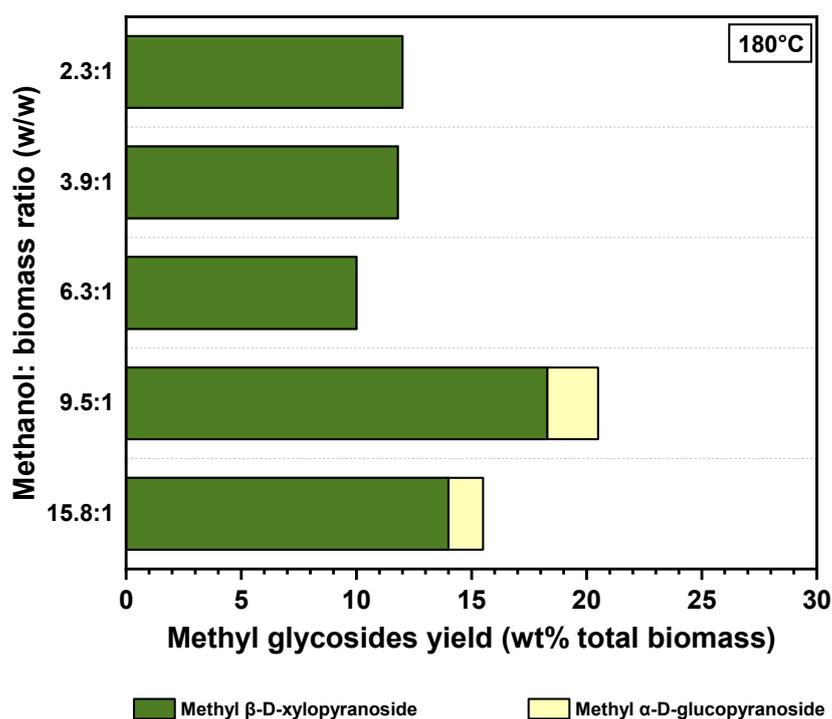


Figure 1. Yield of methylated glycosides in the lignin oil fraction derived from acid methanolysis of birchwood under different methanol/biomass ratios, at 180 °C and a reaction time of 2 h.

The formation of methyl-D-glycosides under these conditions is not well understood. Hemicelluloses are plant cell-wall heteroglycans built up by a β -linked glycan backbone of, e.g., xylose, mannose, galactose and/or glucose [42]. D-xylose is the primary monosaccharide in hardwoods and grasses [34]. It is likely that the pyranosides are formed via the Fisher glycosylation mechanism (Figure 2). Fisher glycosylation is the reaction of an unprotected carbohydrate refluxed in an alcohol (e.g., methanol) in the presence of an acid catalyst to give the corresponding glycosides [43]. From a mechanistic point of view, the reaction occurs in four steps: (i) formation of furanosides as intermediate; (ii) anomerization of furanosides; (iii) ring expansion of furanosides to pyranosides; and (iv) anomerization of pyranosides [45–49]. Methyl xylosides have also been synthesized in the lab from D-xylose and methanol in the presence of sulfuric acid [46]. When ethanol was used as solvent ethyl xylofuranosides and ethyl xylopyranosides were the intermediates and final products. The latter has also been reported in a similar study after reaction of birchwood in ethylene glycol (EG) and sulfuric acid. Similar sugar compounds were detected containing a xylopyranose entity coupled with EG via an ether bond at the C1 position [23]. The methyl glycosides can be isolated from the lignin fragments either by liquid–liquid extraction using ethyl acetate/water or by water precipitation. They can be later valorized as surfactants [44], or even upgraded to valuable platform chemicals such methyl levulinate [47].

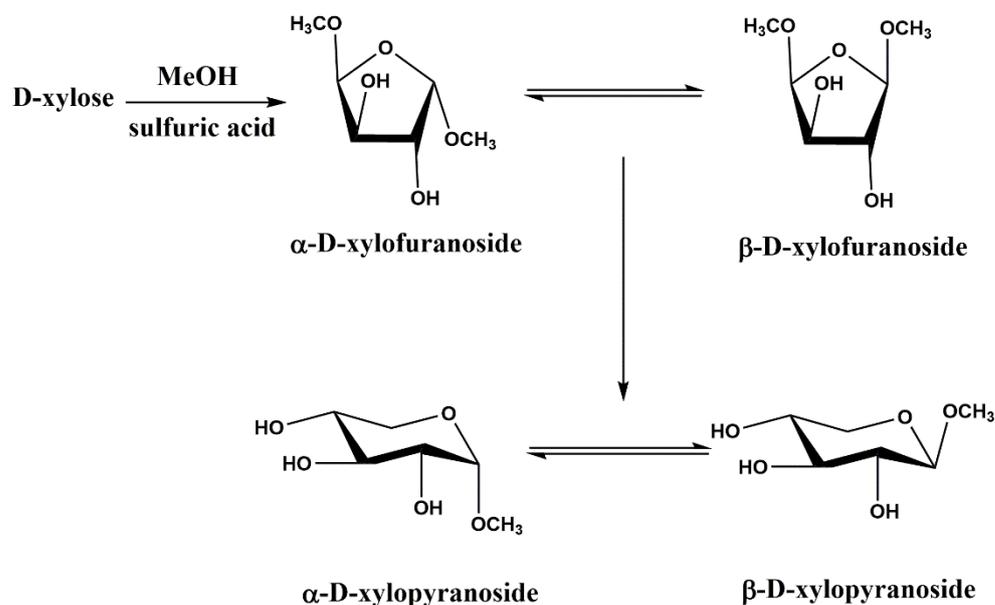


Figure 2. Methanolysis of D-xylose to pyranosides via the Fischer reaction [44].

2.2. Effect of Acid Loading

Acid pretreatment of woody biomass involves the use of concentrated or diluted acids to break down the rigid structure of the lignocellulosic material [48]. Sulfuric acid is the most frequently used catalyst for different types of organosolv pretreatments. Treatment can occur either at low acid concentration and high temperature (dilute acid pretreatment) or high acid concentration and low temperature (concentrated acid pretreatment). The choice mainly depends on the reactor used for the organosolv extraction. Batch organosolv extractions require moderate sulfuric acid loading. The acid loading is based on the percentage dry weight of the biomass, and typical values are in the range between 0.5 and 1.75 wt% [34]. Higher acid loadings might lead to more delignification, but also to more substantial degradation of the extractives. The use of a higher acid loading is possible in continuous flow-through organosolv extraction at low temperatures because fresh solvent and acid are continuously added to the system. Zijlstra et al. (2019) investigated the mild organosolv (ethanol) lignin extraction from cedar and beechwood in a flow reactor using 180 mM H_2SO_4 at 120 °C, yielding a lignin with high β -O-4 content [49]. Here, we used acid loading of at least an order of magnitude lower (4–10 mM H_2SO_4) during the optimization of batch acid methanolysis extraction at 180 °C. Figure 3a shows how the lignin oil yield depends on the sulfuric acid and biomass loading. At a low biomass loading (15.8/1 *w/w*) and the highest acid loading of 0.81 wt%, a lignin oil yield of 67.4 wt% was obtained, which comprises not only solubilized lignin oligomers but also products derived from solubilized hemicellulose including degradation products. We further note that lower acid loadings result in a lower delignification degree but also a lower oil yield. Compared to the lowest biomass loading (15.8/1 *w/w*), the lignin oil yield at a ratio of 3.9/1 *w/w* decreased to 52.8 wt%, while for the lowest ratio of 2.3/1 *w/w* the lignin oil yield dropped to 38.4 wt%.

Figure 3b shows how the acid concentration impacts the molecular weight distributions of the lignin product extracted at different methanol/biomass ratios at 180 °C. At the highest acid loading (0.81%), several shoulders at both low- and high- M_w (molecular weight) ends develop in the gel permeation chromatogram. The increased signal in the lower M_w ranges (200–220 and 400–500 g/mol) points to the formation of depolymerized lignin fragments. The acid catalyst is not only active in cleaving α - and β -aryl ether bonds in the lignin-hemicellulose network but can also attack similar linkages in the lignin network itself, which constitute up to 70% of the total linkages in lignin. Efficient cleavage of these type of linkages will depolymerize lignin into its monomers/dimers and oligomers [50]. The presence of methanol-soluble oligomers is reflected by the shoulder of the M_w range of

700 to 1000 g/mol. We also observe the appearance of high M_w fragments in the tail of the chromatogram, which indicates that the rate of repolymerization of intermediate fractions is high, leading to the formation of heavier compounds than the starting lignin. In the absence of a capping agent or hydrogenation of reactive double bonds, repolymerization cannot be prevented. For instance, Luterbacher et al. (2019) reported a two-step method involving the HCl-catalyzed extraction of lignin in the presence of formaldehyde, thereby suppressing repolymerization [9]. When lower acid loadings (0.40% and 0.24%) were utilized, the shoulders at the low- M_w end decreased, indicating lower rates of ether-bond cleavage and lower quantities of monomers and oligomers. The development of a shoulder in the M_w area of 1800 g/mol is indicative of repolymerization reactions. The high M_w tail (>1800 g/mol) in the gel permeation chromatogram for the low acid loadings cases was shifted to the left, resulting in a smaller M_w distribution.

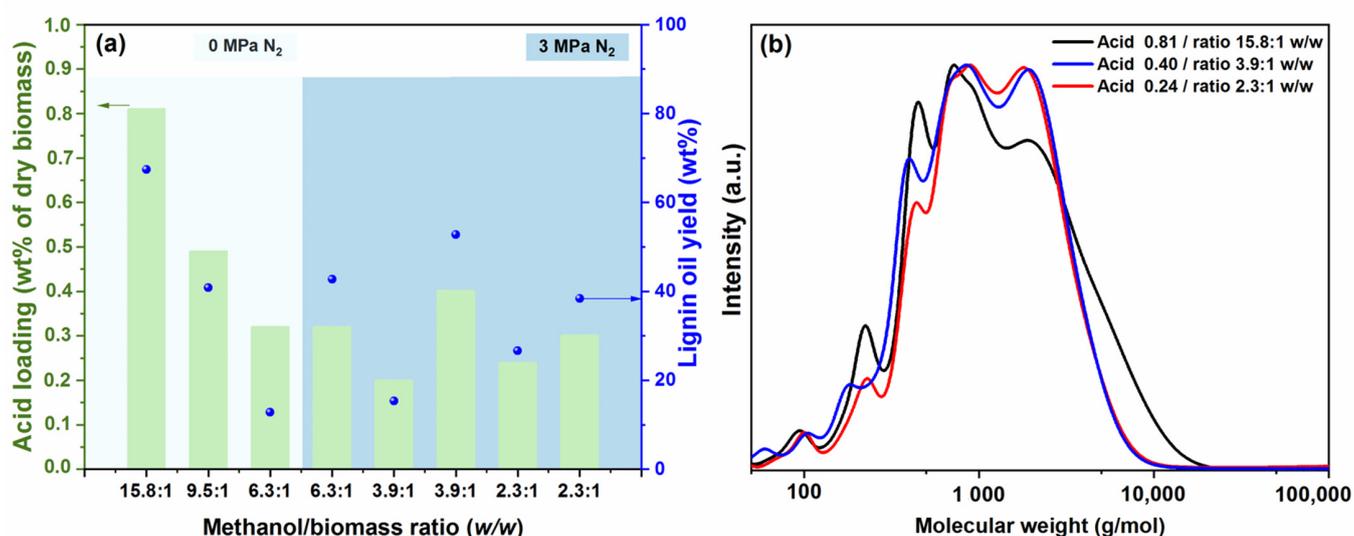


Figure 3. Acid solvolysis of birchwood at 180 °C for 2 h: (a) influence of acid loading and methanol/biomass ratio on the lignin oil yield; (b) molecular weight distribution of lignin fractions from entries 3, 10, 11 (Table 1).

Two-dimensional HSQC NMR is a useful technique to follow the structural changes of lignin during acid-catalyzed delignification. Relevant to the current work, Ouyang et al. (2018) showed using this technique that treatment of lignin oils derived from the same birchwood by sulfuric acid methanolysis at a solvent/biomass ratio of 15.8/1 *w/w* consumed all β -O-4 bonds [40]. We characterized the lignin fractions of the parent lignin by 2D HSQC NMR (Figure 4) and employed a semi-quantification method to understand the effect of acid loading on the retention of the native aryl-ether structure in the extracted lignin (Table 2). In the aromatic region of all 2D HSQC NMR spectra (δ_C/δ_H 100–146/5.0–7.5 ppm), the cross-signals show that the lignin oil from birchwood contains syringol (S) and guaiacyl (G) units, which is a typical feature of hardwood. In the side-chain region (δ_C/δ_H 50–95/2.5–6.0 ppm), signatures of the main lignin–lignin intra-linkages (β -O-4', β - β' , β -5) can be observed for the lignin obtained by sulfuric acid methanolysis of birchwood under different acid and biomass loadings (Figure 4a–c). At the highest acid loading and methanol/biomass ratio (Figure 4a), the cross signals of C_α - H_α (A_α) (δ_C/δ_H 71.8/4.86 ppm) and C_α - H_α (A'_α) (δ_C/δ_H 81.5–86/4.44–4.84 ppm) of β -O-4' linkages were not observed. This is an indication of acid solvolysis of ether linkages and is an agreement with the gel permeation chromatogram (Figure 3b), where low M_w fragments were identified. As can be seen from the semi-quantification analysis (Table 2), no ether bonds could be found, while a small quantity (6.6%) of condensed linkages (β -5) were present. The C–H correlation signals of the β -O-4 structure were clearly visible when the reaction was carried out at lower sulfuric acid loadings. At a loading of 0.40 wt% acid, the number of linkages per 100 aromatic units amounted to 11.2, while the β -5 linkages were largely

preserved. At higher methanol/biomass ratio (2.3/1 *w/w*), the effect of lower acid loading (0.24 wt%) was more profound. More β -O-4' bonds were preserved, i.e., about a third of the total lignin linkages. Further, the formation of more β - β bonds can be evidence of repolymerization in line with the GPC data in Figure 3b. According to the literature, the formation of new C–C linkages in acidic conditions can occur via two mechanisms. First, β -O-4 linked dimers without methoxy groups (1-phenyl-2-phenoxy-1,3-propanediol and 1-(4-hydroxyphenyl)-2-phenoxy-1,3-propanediol) are prone to self-condensation through intramolecular condensation. The presence of methoxy groups in G/S units can effectively limit this type of repolymerization [51]. Second, protonation of hydroxyl groups located at the α position (A_α) is followed by dehydration to form the benzylic cation intermediates, which can react with aromatic monomers to form new C–C bonds. To investigate whether the number of aryl-ether bonds can be increased, thereby limiting condensation and C–C bond formation, an additional experiment was performed in the same conditions as entry 13 but with shorter reaction time of 30 min. The pretreatment time contributes to the process severity as part of the CS factor [34]. As expected for lower process severity, the delignification degree was 69.6 wt%, but we observed an increase in the total number of β -O-4 linkages (47.4%). The contribution of β -5 linkages decreased to 1.8%, while the β - β linkages amounted to 8.4%. Overall, the higher number of native aryl-ether bonds at higher biomass loadings can be attributed to the lower rate of nucleophilic attack required to break ether bonds in the lignin fractions. We have shown that methanol promotes cleavage of β -O-4 bonds during lignin solvolysis in similar operating conditions [36].

Table 2. The number of linkages per 100 aromatic units present in lignin extracted after acid methanolysis at 180 °C, at different acid loadings, methanol/biomass ratios (*w/w*) and reaction times.

Linkage	Number of Linkages Per 100 Aromatic Units			
	Ratio 15.8/1 <i>w/w</i> H ₂ SO ₄ 0.81 wt% 2 h	Ratio 3.9/1 <i>w/w</i> H ₂ SO ₄ 0.40 wt% 2 h	Ratio 2.3/1 <i>w/w</i> H ₂ SO ₄ 0.24 wt% 2 h	Ratio 2.3/1 <i>w/w</i> H ₂ SO ₄ 0.24 wt% 30 min
β -O-4' (A_α)	0.0	3.0	7.6	15.4
β -O-4' ($A'\alpha$)	0.0	8.2	27.1	32.0
β - β' (B_α)	0.0	0.0	7.1	8.4
β -5' (C_α)	6.6	5.9	4.0	1.8

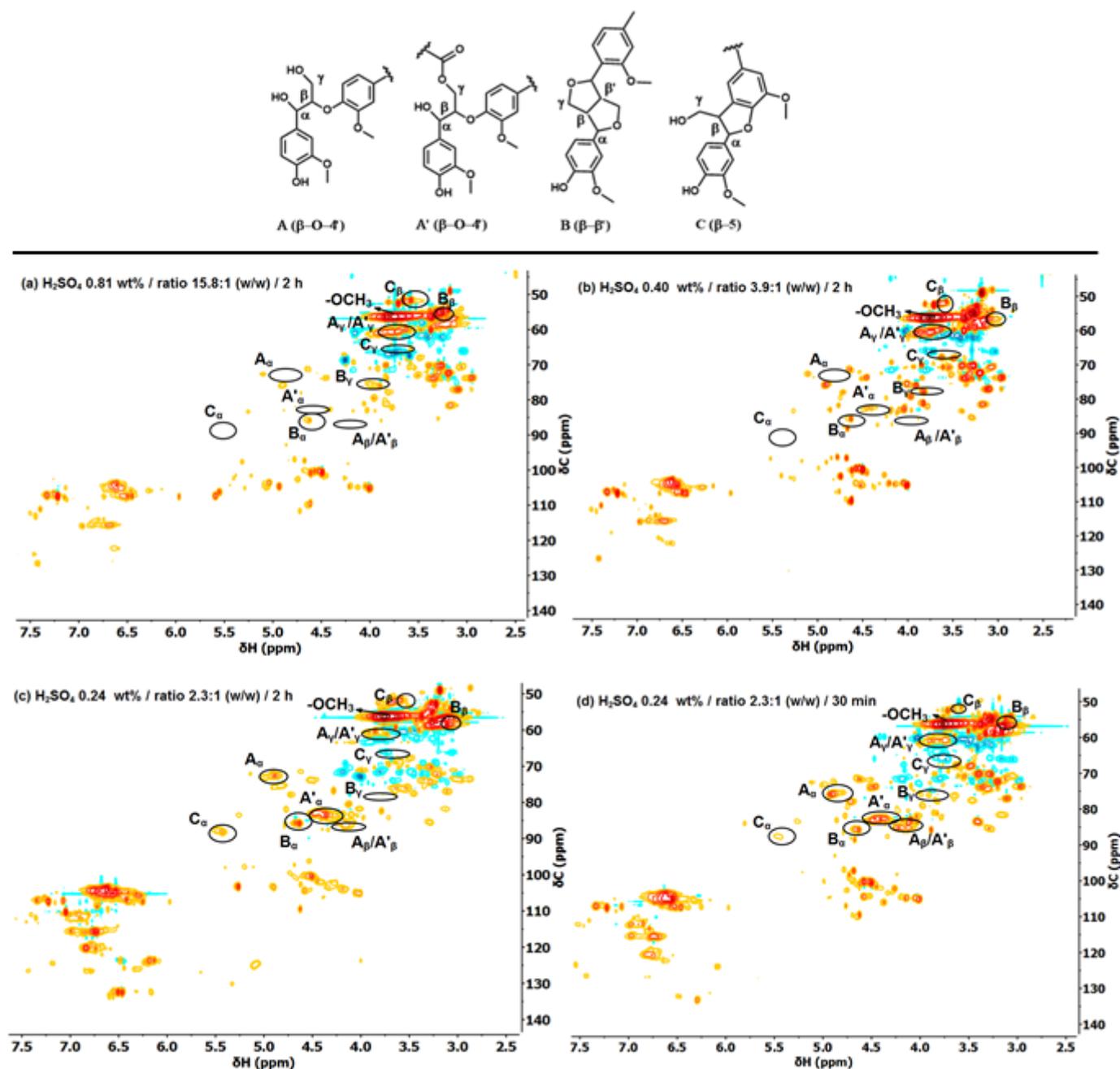


Figure 4. HSQC NMR analysis of lignin oil obtained after acid methanolysis of birchwood at 180 °C (a) for 2 h, 0.81 wt% sulfuric acid and ratio of 15.8:1 *w/w*; (b) for 2 h, 0.40 wt% sulfuric acid and ratio of 3.9:1 *w/w*; (c) for 2 h, 0.24 wt% sulfuric acid and ratio of 2.3:1 *w/w*; (d) for 30 min, 0.24 wt% sulfuric acid and ratio of 2.3:1 *w/w*.

2.3. Reductive Catalytic Upgrading of Lignin Oil

In order to check the reactivity of the extracted lignin after acid methanolysis of birchwood in the lowest methanol/biomass ratio of 2.3/1 *w/w* and lowest acid loading (0.24 wt%) (entry 13), we subjected the derived lignin oil to a second depolymerization step over the Pd/C catalyst at 180 °C under 3 MPa H₂. We did not neutralize the acid after the first step because the presence of acid also promotes the depolymerization of the lignin oil [26]. Table 3 lists the main results in comparison to our earlier work [42]. Figure 5 shows the structural changes of depolymerized lignin as probed by 2D HSQC NMR, the M_w distribution of the lignin oils before and after hydrogenolysis and the lignin monomers formed during hydrogenolysis.

Table 3. Results of reductive depolymerization of birchwood-derived lignin oils obtained under different fractionation conditions.

Entry	Feedstock	Fractionation Conditions	Yield Monomer Products (wt%) *	Mass Monomer Products (mg) *
1	Birchwood-derived lignin oil (entry 13, Table 1)	Methanol/biomass ratio 2.3/1 <i>w/w</i> , Sulfuric acid loading 0.24 wt%	12.0	345.4
2	Birchwood-derived lignin oil (Ouyang et al.) [40]	Methanol/biomass ratio 15.8/1 <i>w/w</i> , Sulfuric acid loading 3 wt%	17.8	54.7

* Calculated based on the Klason lignin weight of the starting biomass.

Although the phenolic monomer yield after the second hydrogenolysis step for the higher biomass loading was slightly lower (12 wt%) than the reference case (17.8 wt%), the quantity of lignin monomers was much higher (345.4 vs. 54.7 mg). The use of the optimized two-step approach not only provided higher biomass loading during the first fractionation step but also preserved more reactive lignin (more β -O-4 linkages), explaining the increased number of phenolic monomers released during the second hydrogenolysis step. In this experiment, four types of lignin monomers were obtained, namely 2-methoxy-4-propyl phenol, 2,6-methoxy-4-propyl phenol, 2-methoxy-4-hydroxyphenyl acetate and 2,6-methoxy-4-hydroxyphenylacetate (Figure 5c). Comparing the gel permeation chromatograms (Figure 5b) for the lignin oil before and after hydrogenolysis, a clear decrease of the molecular weight can be observed, and a shoulder appears in the M_w area of 250 g/mol. The average molecular weight (M_n), weight average molecular weight (M_w) and dispersity (\mathfrak{D}) for both fractions are given in Table 4. The M_w of the isolated lignin after acid methanolysis of birchwood under 0.24 wt% sulfuric acid loading was 1546 g/mol. This value was reduced to 927 g/mol after the lignin oil intermediate was subjected to reductive depolymerization with 3 MPa H_2 at 180 °C for 2 h over the Pd/C catalyst. The \mathfrak{D} of the depolymerized lignin fraction decreased from 2.51 to 1.92, compared to the parent lignin, also suggesting the formation of lower M_w fragments.

Table 4. GPC analysis of the lignin fraction after delignification (entry 13) and after reductive depolymerization at 180 °C, 2 h, 3 MPa H_2 over Pd/C catalyst.

Entry	M_n (g/mol)	M_w (g/mol)	\mathfrak{D} (-)
Lignin fraction (entry 13, Table 1)	614	1546	2.51
Lignin fraction (entry 13, Table 1) (after catalytic depolymerization over Pd/C)	483	927	1.92

We further characterized the lignin oil with 2D HSQC NMR to better understand whether structural changes occurred during the second hydrogenolysis step (Figure 5a). Examination of the HSQC-NMR spectra, specifically the cross signals of C_{α} - H_{α} (A_{α}) (δ_C/δ_H 71.8/4.86 ppm) and C_{α} - H_{α} (A'_{α}) (δ_C/δ_H 81.5–86/4.44–4.84 ppm) in β -O-4' linkage structures, revealed complete disappearance of the β -O-4 signals, indicating that most ether bonds were cleaved during reductive hydrogenolysis. Combined with the GPC results (Figure 5b and Table 4), it is evident that depolymerization occurred.

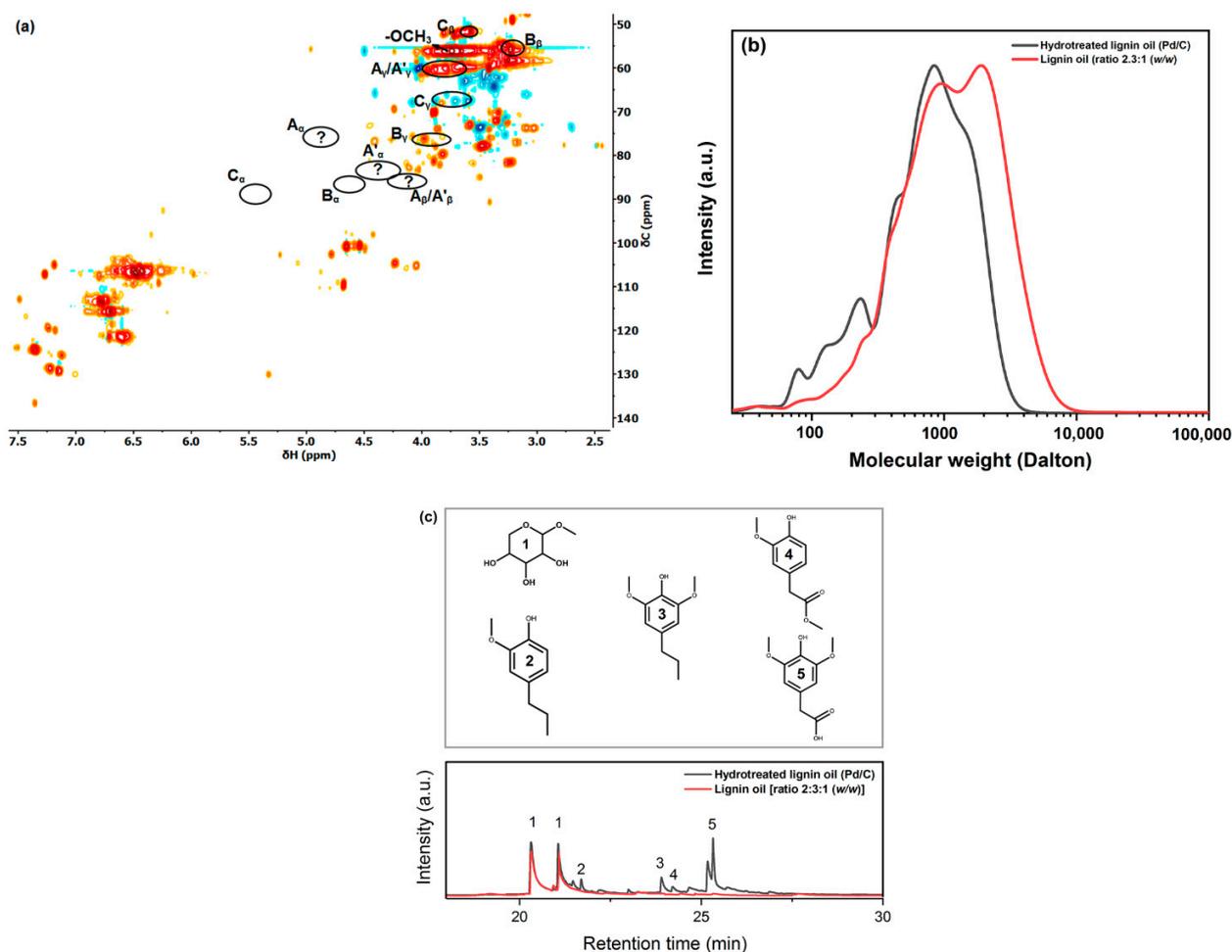


Figure 5. (a) HSQC NMR analysis of lignin oil obtained from catalytic hydrogenolysis of lignin oil from entry 13; (b) GPC analysis of lignin oils from entry 13 obtained from the first fractionation step (red curve) and the second hydrogenolysis step (black curve); (c) gas chromatograms and peak identification of the lignin oil fractions obtained after the first fractionation step (red line) and the second catalytic hydrogenolysis step (black line).

3. Materials and Methods

3.1. Materials

Birchwood chips (native hardwood species from the genus *Betula*) were purchased from Sappi Benelux (Lanaken, Belgium). Anhydrous methanol, ethyl acetate, THF, d_6 -DMSO and H_2SO_4 (95–97%) were purchased from Merck Group, Amsterdam, The Netherlands. The 5 wt% Pd/C was purchased from Sigma-Aldrich, Amsterdam, The Netherlands. N-dodecane was purchased from Alfa Aesar GmbH, Karlsruhe, Germany.

3.2. Feedstock Pretreatment

Birchwood chips were first ball milled to small particles using a Retsch Planetary 4-Station ball mill (conditions: 500 rpm, 1 h) and then sieved manually to obtain particles with a size between 125–300 μm . The birchwood sample was pretreated to remove the extractives with water (24 h) and ethanol (24 h) in a Soxhlet extractor. After extraction, the wood sample was dried at 105 $^\circ\text{C}$ overnight to remove residual solvent.

3.3. Experimental Procedures

In a typical experiment and depending on the solvent/biomass ratio, 3–12 g of extracted birch sawdust, 15–60 mL methanol and a certain amount of sulfuric acid were introduced into a 100 mL Parr autoclave. For example, for a methanol/biomass ratio of

15.8/1 *w/w*, 3 g of dried and grinded biomass was added to 60 mL of methanol (47.5 g on mass basis), together with 0.81 wt% sulfuric acid (based on weight of dry biomass). The autoclave was sealed and flushed three times with nitrogen to remove any oxygen. The mixture was heated to 160–200 °C (ca. 10 °C min⁻¹), and the stirring speed was 500 rpm. After 2 h acid delignification, the autoclave was cooled down to room temperature in an ice bath. The extracted lignin oil (comprising lignin and sugar fractions) and cellulose pulp were separated by vacuum filtration through a glass PORE4 filter crucible (VitraPOR®). The reactor walls and the stirrer were cleaned carefully with a spatula to recover all the wood residue fractions. The cellulose pulp was washed with approximately 60 mL of fresh solvent, removing all residual extractives. The cellulose pulp was dried for 24 h at 60 °C until no further weight changes were observed. Aliquots of the filtrate were analyzed by GC and GC-MS. For the determination of the yield of the extractives, the filtrate was evaporated using a rotary evaporator (45–70 °C, 500 Pa) for 1 h. The intermediate product fraction represents the solubilized lignin oligomers and carbohydrates of the parent birch sawdust. The product fraction was dried at 80 °C until stable weight. To remove the carbohydrate fractions, liquid–liquid extraction with ethyl acetate–water was applied. The lignin oligomers were extracted in the organic ethyl acetate layer, while the sugars remained in the aqueous phase. Therefore, a mixture of 100 mL ethyl acetate and 60 mL H₂O was added into the dried product fraction (oligomers and carbohydrates). After rigorous mixing until complete dissolution of the lignin and carbohydrate fractions in the extraction solvents, the total mixture was left for 24 h in order to achieve phase separation. The resulting two phases were separated from each other using a separatory funnel. The ethyl acetate phase comprising the lignin fraction was subjected to rotary evaporation (45 °C, 500 Pa) and yielded the final lignin oligomeric fraction.

For the hydrogenolysis step, the filtrate (extracted lignin oil after fractionation), 500 mg Pd/C and 30 µL *n*-dodecane were mixed in the autoclave. After flushing the autoclave with nitrogen, it was pressurized with H₂ to 3 MPa. After a leak check, the reaction mixture was heated to 180 °C (ca. 10 °C min⁻¹). The stirring speed was 500 rpm. After reaction, the autoclave was cooled in an ice bath and depressurized. Aliquots of the liquid phase were analyzed with GC and GC-MS. We determined the following main parameters.

$$\text{Yield of lignin oil (wt\%)} = \left(\frac{\text{lignin oil fraction after methanol removal}}{\text{weight of birch wood}} \right) \times 100\% \quad (1)$$

$$\text{Degree of delignification (wt\%)} = \left(\frac{\text{final lignin oligomeric fraction}}{\text{weight of birch wood} \times \text{Klason lignin content in birch wood}} \right) \times 100\% \quad (2)$$

$$\text{Yield of monomers (wt\%)} = \left(\frac{\text{weight of monomers (calculated from GC – FID)}}{\text{weight of starting birch wood} \times \text{Klason lignin content in birch wood}} \right) \times 100\% \quad (3)$$

3.4. Analytical Procedures

3.4.1. GC-MS Analysis

The liquid phase product mixture was analyzed using a Shimadzu 2010 GC-MS system equipped with an RTX-1701 column (60 m × 0.25 mm × 0.25 µm) and a flame ionization detector (FID), together with a mass spectrometer detector. Identification of products was achieved based on a search of the MS spectra with the NIST11 and NIST11s MS libraries. These products were grouped according to the nature of ring structure and functional groups. All the quantitative analyses of liquid phase products were based on 1D GC-FID. Experimentally determined weight response factors of cyclohexane, cyclohexanone, ethyl benzene and ethyl guaiacol were used to cover all the possible lignin monomers related to *n*-dodecane as the internal standard.

3.4.2. Gel Permeation Chromatography (GPC)

GPC analysis was performed with Shimadzu Prominence-I LC-2030C 3D apparatus equipped with two columns (Mixed-C and Mixed-D, Polymer Laboratories) in series and

a UV–Vis detector at 254 nm. The columns were calibrated with polystyrene standards. Analyses were performed at 25 °C using tetrahydrofuran (THF) as eluent. Samples were prepared at a concentration of 2 mg/mL in non-stabilized THF and then filtered using a 0.45 µm filter membrane. An automated peak integration function was employed, using Shimadzu Labsolutions CS software, in order to calculate an approximation for the areas under the gel permeation chromatograms.

3.4.3. ^1H - ^{13}C HSQC NMR Analysis

All NMR spectra were recorded using a VARIAN INOVA 500 MHz spectrometer equipped with a 5 mm ID AutoX ID PFG probe. For analysis of the lignin residue, approximately 100 mg of lignin residue was dissolved in 0.7 mL dimethylsulfoxide- d_6 (DMSO- d_6). The ^1H - ^{13}C HSQC NMR spectra were obtained using the phase-sensitive gradient-edited HSQC program (gHSQCAD). The main parameters were as follows: 16 scans, acquired from 0 to 16 ppm in F2 (^1H) with 1200 data points (acquisition time 150 ms), 0 to 200 ppm in F1 (^{13}C) with 256 t1 increments (acquisition time 10 ms) and 2 s relaxation delay. Data were processed using the MestReNova software. The residual DMSO solvent peak was used as an internal reference ($\delta_{\text{C}} = 39.5$ ppm; $\delta_{\text{H}} = 2.50$ ppm). A semi-quantitative analysis of the HSQC spectra was performed by integration of the correlation peaks in the different regions of the spectra using MestReNova software according to a method described in the literature [45,52–55]. The relative quantity of side chains involved in the inter-unit and terminal substructures is expressed as a number per 100 aromatic units (S + G).

4. Conclusions

In this study, we optimized the solvolytic extraction of lignin for the two-step lignin-first upgrading of lignocellulosic biomass. The first step of the process involves the acid methanolysis of biomass to a crude lignin oil composition, at moderate temperatures (160–200 °C) and with a 2 h reaction time. We explored the influence of biomass and sulfuric acid loading on the product distribution and the quality of the extracted lignin fractions. High methanol/biomass ratios (15.9/1 and 9.5/1 *w/w*) exhibit complete delignification of birchwood at 180 °C. Higher biomass loadings (6.3/1 *w/w*) require the implication of elevated pressure to keep methanol in the liquid phase, overcoming mass transfer limitations and poor delignification degrees. This allowed us to explore even higher biomass loadings. The ratio of 2.3:1 *w/w* was the maximum that could be applied, and 86.4 wt% of the lignin could be extracted at 180 °C under 0.24 wt% of acid loading. Together with the lignin, hemicellulose is also solubilized in the lignin oil phase, mostly in the form of methyl xylosides. It was found that a high degree of delignification is always accompanied by a large extent with C5 sugar release. The combination of high methanol/biomass ratios and high acid loadings improves delignification and results in the complete disappearance of ether linkages in the lignin, also promoting lignin depolymerization. Lower ratios, acid loading and pretreatment time help to preserve the number of β -O-4' linkages. Lignin repolymerization cannot be prevented due to the formation of reactive intermediates such as benzylic cations and unsaturated monomers. Catalytic depolymerization of the resulting lignin oils in the presence of H_2 and Pd/C indicates that lignin can be further depolymerized to phenolic monomers. The presented results demonstrate the link between lignin quality and potential for the extracted lignin to be further depolymerized. Overall, the extraction and depolymerization demonstrates a trade-off between the lignin oil extraction yield and the retention of the native aryl-ether structures and thus the potential of the lignin oils to be used as substrate for the production of chemicals for high-value applications.

5. Patents

A patent application entitled “A method for obtaining a lignin oil composition using a compressed gas and acid assisted process” with PCT application number PCT/EP2020/077411 was filed on 30 September 2019 by the inventors P.D.K., X.H., M.D.B. and E.J.M.H.

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Conflicts of Interest: The authors declare no conflict of interest.

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