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Research Article

Mild Organosolv Lignin Extraction with Alcohols: The Importance of Benzylic Alkoxylation

Douwe S. Zijlstra, Ciaran W. Lahive, Coen A. Analbers, Monique B. Figueirêdo, Zhiwen Wang, Christopher S. Lancefield, and Peter J. Deuss*

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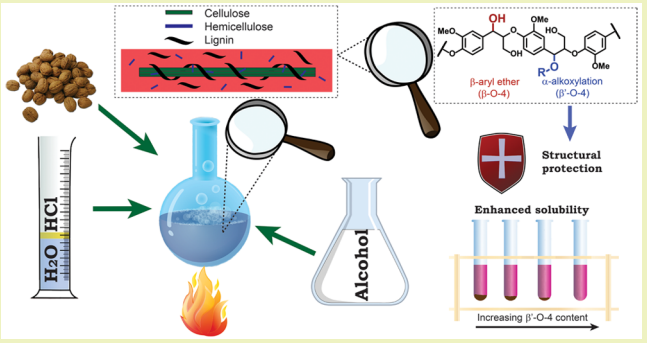
ABSTRACT: Lignin holds the key for maximizing value extraction from lignocellulosic biomass. This is currently hindered by the application of fractionation methods that significantly alter the lignin structure to give highly recalcitrant materials. For this reason, it can be highly beneficial to use less-severe fractionation conditions that allow for efficient extraction of lignin with retention of the β-aryl ether (β-O-4) content. Here, we present a detailed study on mild alcohol-based organosolv fractionation with the aim of understanding how to achieve a balance between efficiency of lignin extraction and the structure of the resulting lignin polymers, using walnut shells as model biomass. Monitoring different extraction conditions reveals how the structure of the extracted lignin changes depending on the extraction conditions in terms of molecular weight, alcohol incorporation, and H/G/S ratios. Moving from ethanol to *n*-pentanol, it was revealed that, in particular, alcohol incorporation at the benzylic α-position of β-aryl ether units not only plays a key role in protecting the β-O-4 linking motif but more importantly increases the solubility of larger lignin fragments under extraction conditions. This study shows that α-substitution already occurs prior to extraction and is essential for reaching improved extraction efficiencies. Furthermore, α-substitution with not only bulky secondary alcohols and tertiary alcohols but also chloride was revealed for the first time and the latter could be involved in facilitating α-alkoxylation. Overall, this study demonstrates how by tuning the fractionation setup and conditions, the resulting lignin characteristics can be influenced and potentially tailored to suit downstream demands.



KEYWORDS: *lignin, mild organosolv extraction, benzylic alkoxylation, β-O-4 retention, solvent effects*

INTRODUCTION

In order to realize the implementation of biomass as a renewable feedstock for the chemical industry, efficient resource utilization as well as maximum value extraction are essential.[1−3] This is particularly important for lignocellulose as around 30% of its main constituents, in the form of lignin, is typically burned as a low-value fuel.[4] Therefore, new high-value applications for lignin from lignocellulose are sought. As an aromatic biopolymer, the production of aromatic platform chemicals from lignin is the preferred route.[5−8] Currently, two main approaches are being pursued (Figure 1). The first approach is the valorization of waste streams of existing lignocellulose biorefineries such as kraft lignin produced in paper mills. Although depolymerization of these technical lignins is possible, it typically requires harsh conditions that result in a complex mixture of products.[9−11] This limitation is the result of the severe processing conditions applied during fractionation, which leads to a lignin with a complex, condensed C−C bonded chemical structure, thus hindering selective depolymerization into selected aromatic monomers. The second approach is to avoid condensation reactions and address lignin depolymerization at an early stage of the lignocellulose conversion process, often termed lignin-first or early-stage lignin biorefining.[12−14] This can be achieved by either performing the fractionation under mild conditions to obtain lignins with a more native-like C−O bonded structure by preserving the β-aryl ether units[15] or by integrating the depolymerization into the fractionation process.[16−18] An example of the latter is the application of reductive fractionation to extract and depolymerize lignin into specific alkyl-phenolics in high yield.[19−28] Reductive depolymerization is only one of many selective lignin depolymerization methods and yields a specific selection of phenolic products.[18,29−34] For the implementation of higher value lignin products in a biorefinery scheme, a more diverse chemical platform is

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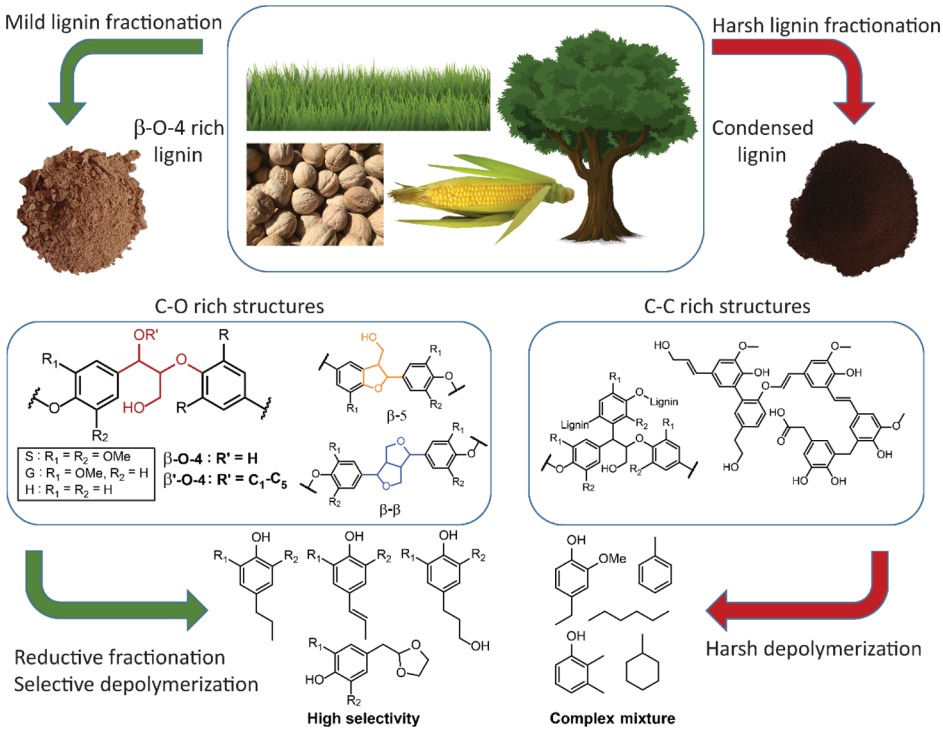

Figure 1. Influence of the fractionation conditions on the structural composition of the obtained lignin and possibilities for further depolymerization.

desired.[24] There are many other elegant depolymerization methods that cannot be implemented during fractionation, but rather require isolated lignins.[7] Most selective depolymerization methodologies developed focus on lignin β-aryl ether units, which are the main C−O bonded linking motif in native lignin. Their retention in the extracted lignin structure is thus essential.[35−38]

Many methods have been developed to extract high β-O-4 lignin. One of these is mild organosolv extraction, which is contrasted by technical organosolv extraction by the application of lower temperatures (<150 °C). When alcohols are used, with ethanol (EtOH) in particular being very popular, this process typically yields clean lignins with excellent retention of the high β-aryl ether content, and contains very low carbohydrate, ash, and other nonlignin-derived impurities.[36,37,39] It has been shown that when alcohol solvents are used, they can be incorporated in the β-aryl ether units via α-alkoxylation, resulting in the β'-O-4 motif (Figures 1 and S6).[36] Alcohol incorporation is believed to be important as it traps reactive benzylic cations formed under acidic pretreatment conditions, especially when higher acid concentrations are applied, and thereby preventing undesired condensation reactions.[36] However, currently, there is limited understanding of the intricate relationship between the extraction solvent incorporation and the characteristics of the obtained lignin. The studies that do focus on the influence of the organosolv conditions on the lignin characteristics mainly consider the influence of time, catalyst, and temperature on the degree of delignification and enzymatic digestibility of the remaining cellulose.[40−49] The property of the obtained lignin that is consistently reported is the average molecular weight (MW), which decreases with increasing temperature. However, the processing conditions do have an influence not only on the MW but also on the chemical structure of the extracted fraction of the lignin. Important parameters for further application of lignin are, for example, the number of linking motifs, aliphatic and phenolic −OH content, and H/G/S ratio of the obtained lignin. A measure for the harshness of the extraction, which takes extraction time, temperature, and acidity into consideration, is the severity factor.[50] This factor is often used to quantify the effect of extraction conditions on the quality of the obtained cellulose fibers[51] as well as the delignification and purity of the obtained lignin.[52] However, most of the extraction methods to which this parameter has been applied are in the range of 150−230 °C, which corresponds to an extraction severity factor in the range of 0.5−3.0, assuming a 1 h extraction time and a mildly acidic extraction mixture at pH 2.5. Recent studies using alcohols and relatively mild conditions (80−160 °C) did show that there are some marked changes to the lignin associated with extraction time and the extraction solvent composition. However, these cannot be directly related to the severity factor.[36,39,53−55] For example, clear differences in lignin yield, S/G/H ratio, and MW can be seen in lignin fractions collected at different extraction times. These results are not always consistent as under some conditions an increase of more than twofold in the lignin MW was reported, whereas in other studies the MW was reported to decrease in conjunction with an increase in phenolic groups and thus possible degradation. In general though, milder fractionation conditions result in lignin fragments with a higher MW. Bauer et al.[39] studied the effect of mild ethanosolv extraction (80 °C, extraction severity factor of 0.8) on the lignin properties, including the distribution of linking motifs under reflux conditions. An increase in β'-O-4 units, together with a slight increase in the MW, was reported when using higher EtOH concentrations as well as longer reaction times. We recently reported that lignins suitable for selective depolymerization should be extracted in the range of

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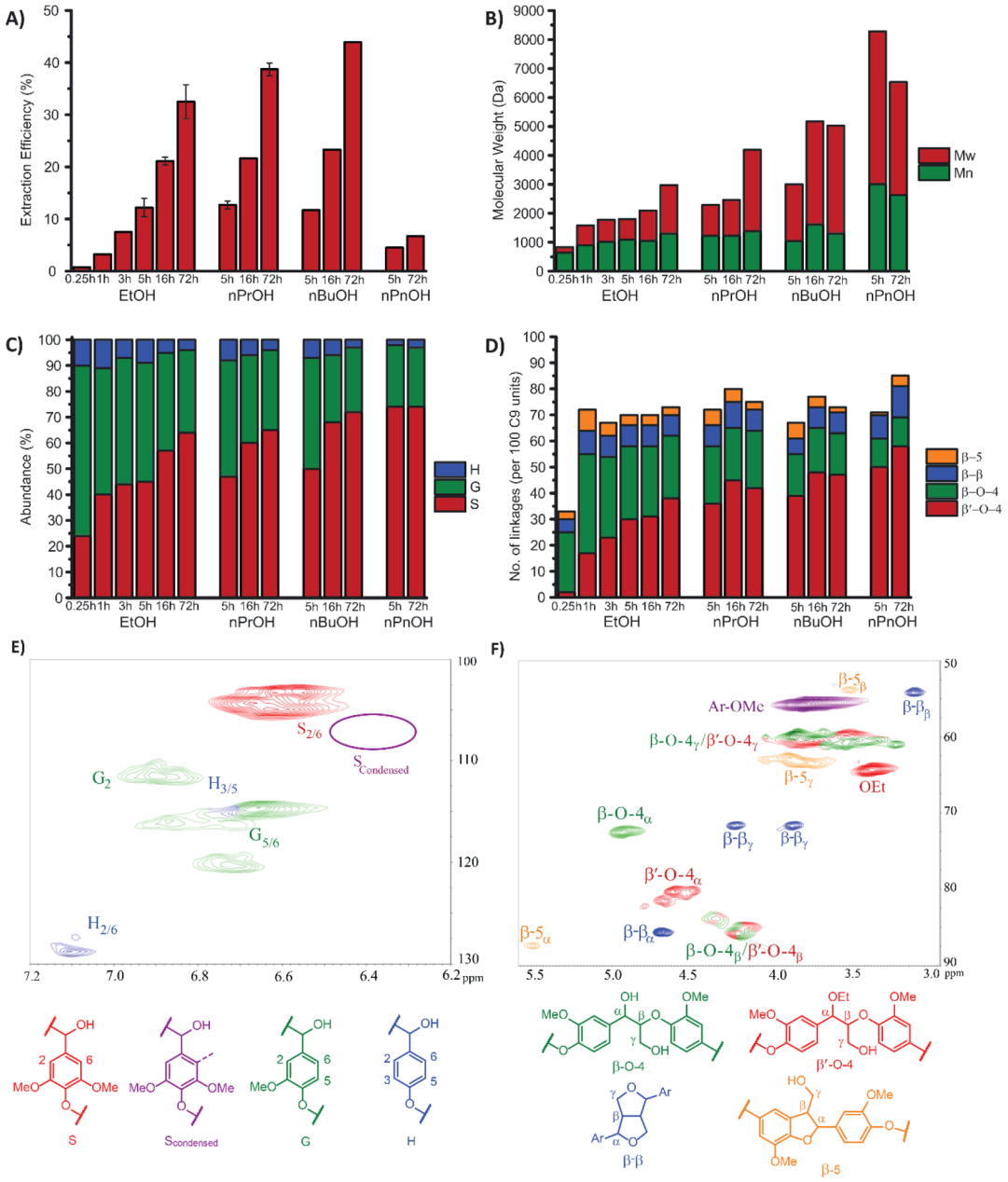

Figure 2. Overview of the properties of the lignins obtained by mild organosolv extractions (4:1 alcohol/H_2O, 0.24 M HCl, 80 °C) at different extraction times, showing (A) extraction efficiency (expressed as % of the original amount of lignin in the biomass used as determined by Klason, corrected for alcohol incorporation as shown in Supporting Information 2.2), (B) M_w distributions as determined by GPC (THF) and (C) H/G/S ratio, (D) the number of linking motifs of the obtained lignins determined by HSQC NMR (d_6-acetone), (E) 2D-HSQC spectra of the aromatic region of a 16 h ethanosolv extraction, and (F) 2D-HSQC spectra of the oxygenated aliphatic region of a 16 h ethanosolv extraction.

80−120 °C.[35−37,56] At these conditions, a higher catalyst loading and longer extraction times are required to obtain reasonable levels of delignification. As a result, the extraction severity factor is approximately 2.1 and is comparable to most reported lignin fractionations, but in contrast to the other reports, excellent retention of the lignin β-aryl units is observed. This is in part due to the formation of β'-O-4 linking motifs by solvent incorporation. Lancefield, Westwood, and co-authors demonstrated the benefits of using *n*-butanol (*n*BuOH),[36,55] resulting in an almost twofold higher lignin yield compared to EtOH at the same conditions. The MW of the obtained lignin was higher compared to EtOH as well as the β'-O-4 content. Furthermore, an increase in α-ethoxylation was observed by increasing the extraction temperature from 80 to 110 °C. These trends are also in agreement with our previous reported results.[37] α-Alkoxylation can also be used to open new reaction pathways by acting as a protecting group. This was demonstrated by introducing an azide at the γ-position, as well as γ-oxidation to a γ-carboxylic acid[55] and the formation of a γ-aldehyde that allows for subsequent reactions to γ-alkynes,[57] decarbonylation,[58] and monomers.[58,59] This demonstrates the potential of lignin with a high degree of α-alkoxylation combined with high β-aryl ether content.

To aid the development of lignin-first-type methods as well as methods for obtaining lignins suitable for selective depolymerization and modification, it is important to gain a

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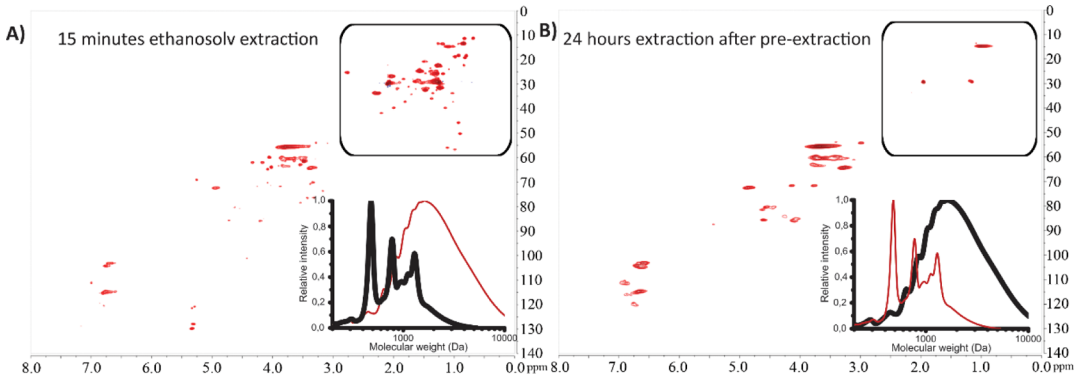

Figure 3. 2D-HSQC spectra (d_6-acetone) of lignin obtained by batch extractions in an acidic 4:1 EtOH/H_2O mixture, 0.24 M HCl, and 80 °C showing (A) lignin extracted in the first 15 min showing many signals in the aliphatic (0−3 ppm) region and being low in MW and (B) lignin obtained by a 24 h ethanosolv batch extraction with a short pre-extraction, showing a clean aliphatic region and only signals in the aromatic (6.0−7.5 ppm) and the oxygenated aliphatic (3.0−5.5 ppm) region and being high in MW.

better insight into the changes of the extracted lignin in terms of alcohol incorporation, MW, and H/G/S ratio during mild organosolv processing, while maintaining good extraction efficiencies. Additionally, an improved understanding of the influence of the reaction parameters on specific lignin properties will aid the development of smart extraction procedures that reduce the need for subsequent modification steps. Here, we study mild organosolv extractions in detail using walnut as a model lignocellulose substrate, as it is a waste product which is high in lignin content and consists of a mixture of H/G/S units. We show the influence of different alcohols and extraction setups on the extraction efficiency, H/G/S ratio, MW of the obtained lignin, and focuson the role of the reactivity at the α position in the β-O-4 linking motif.

EXPERIMENTAL SECTION

Feedstock Preparation. The walnut shells were pretreated following a previously reported procedure.[37] The walnut shells were cut into small fragments by hammer cutters (two cutters with 5 and 2 mm sieves, respectively). Extractives were removed by a 2 h toluene extraction at reflux conditions. The toluene was removed by filtration and the walnut fragments were dried in a vacuum oven at 70 °C for 16 h. 2D HSQC (heteronuclear single quantum coherence) NMR spectroscopy (Figure S67) shows that neglectable amounts of lignin fragments are removed during toluene extraction. Figure S2 shows that almost all monophenolics and the majority of the fatty acids present in the walnut shells are removed by toluene extraction. In the final preparation step, the walnut fragments were ground in a rotary ball mill equipped with a 80 mL zirconium bowl with 7 20 mm Ø zirconium grinding balls. The bowl was half-filled with biomass. Dry-grinding was performed with eight cycles of 5 min at 450 rpm followed by a 10 min pause.

Typical Batch Mild Organosolv Extraction. 20 g Finely ground walnut shells (40.3% Klason lignin, structural composition S2.5 providing H/G/S ratio and linking motifs composition as determined by whole cell NMR, cellulolytic enzyme lignin (CEL), and milled wood lignin (MWL) were placed in a 250 mL round-bottom flask equipped with a magnetic stirring bar. The desired alcoholic solvent (160 mL), 40 mL of H_2O, and 4 mL of a 37% (12 M) aqueous HCl solution were added and the setup was equipped with a reflux condenser. The mixture was heated and stirred at 80 °C (oil bath temperature). After the desired extraction time, the mixture was cooled to room temperature, the extract was collected by filtration and concentrated by rotary evaporation. The obtained solid was redissolved in a minimal amount of acetone and the lignin was subsequently precipitated by addition to 300 mL of water. Saturated aqueous Na_2SO_4 was added for *n*BuOH and *n*PnOH extracts to enhance flocculation. The product was collected by filtration, washed with water, and air-dried overnight to obtain organosolv lignin as a powder, which was analyzed by NMR (d_6 acetone with a few drops of D_2O) and gel permeation chromatography (GPC) [tetrahydrofuran (THF)] according to previously described procedures (see S1 for more information including HSQC peak assignment for d_6 acetone, an overlay with DMSO-d_6 is provided in Figure S64).[37] The mass balance of a 5 h ethanosolv extraction is provided in the Supporting Information, section 2.6.

RESULTS AND DISCUSSION

The starting conditions for this study were based on previous work where extractions at 80 and 120 °C were compared for different lignocellulose sources.[37] High-quality lignins in terms of linking motifs (57 β-O-4 linking motifs per 100 aromatic units) can be obtained by a 5 h EtOH/H_2O extraction with 0.24 M HCl at 80 °C. The extraction efficiency based on the starting lignin content (40.3% determined by Klason, corrected for the weight addition for alcohol incorporation, see S1) of the isolated lignin powder was determined to be 15.2%. This is likely an underestimation because of recovery losses as Klason analysis of the residue of this specific experiment showed 23% delignification. Nevertheless, isolated lignin yields and quality proved reproducible based on a set of five extraction experiments. Overall, the applied extraction conditions give a severity factor of around 1, which is typical for relatively mild lignin extractions. Nevertheless, the conditions are slightly different to most literature[54] because of the lower temperature, which is balanced out by the higher acid concentration. These conditions were chosen as in our experience temperature is the determining factor for lignin quality and the acidity can be counteracted by the use of alcohols as extraction solvents. The absence of the $S_{condensed}$ signal in the 2D HSQC spectra of the obtained lignins showed the effective prevention of condensation reactions.[60] HCl performs significantly better than both H_2SO_4 and HBr in terms of extraction efficiency and β-O-4 retention (Table S3), in accordance with previous reports using lignin and β-O-4 model compounds.[29,61,62] Here, HBr likely causes faster and less-selective β-O-4 breakdown, whereas H_2SO_4 leads to increased amounts of aldehydes upon β-O-4 degradation. We also investigated the EtOH/H_2O ratio, as the work of Bauer showed that a 19:1 EtOH/H_2O ratio gave similar yields when lignin was extracted from *Miscanthus giganteus*.[39] However, for our walnut shell powder the extraction efficiency was markedly lower at a higher alcohol concentration (6.0% for 19:1 vs 12.5% for 4:1). Additionally,

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the total β-O-4 content was significantly lower, with 42 linking motifs for a 19:1 ratio compared to the 57 linking motifs in the 4:1 EtOH/H_2O system.

Next, the influence of extraction time was studied for up to 72 h, with concurrent increases of the severity factor up to 2.1. A gradual increase in extraction efficiency was observed in the first 5 h, with an increment of approximately 2.5% per hour (Figure 2A). At longer extraction times, the yield of lignin also increased, although at a much lower increment (21.3% after 16 h and 31.3% after 72 h). GPC analysis of the obtained lignin showed an increase in MW (Figure 2B, 15 min: M_w = 831 Da, 72 h M_w = 2978 Da). Another clear change was observed in the S/G ratio, which switched from mainly G (0.4 after 15 min) to mainly S (2.0 after 72 h) upon increased extraction time (Figure 2C, determined from the regions as shown in Figure 2E). The latter is more reminiscent of the S/G ratio in the native lignin as determined by whole-cell NMR and CEL isolation (1.6−1.8, Table S2.5). On the other hand, the H signal is more unreliable and is known to be overestimated in HSQC NMR.[63] The increase in S/G ratio is also observed in other studies using mild organosolv extraction conditions.[37,39,55] and also in a study on reductive fractionation by Román-Leshkov et al.[64] The number of linking motifs is low after 15 min (33 per 100 C9 units,) but shows a substantial increase when the extraction time is increased to 1 h (72 per 100 C9 units) and remained stable at longer extraction times (Figure 2D, determined from the regions as shown in Figure 2F). This showed that even with a higher severity factor, lignins with a high amount of β-O-4 linking motifs can be obtained under the selected conditions in contrast to other studies with similar severity factors.[40,44,53,65] As the lignin quality is low after 15 min (Figure 3A), this short extraction can be efficiently applied as a pre-extraction, resulting in a clean lignin with a higher structural quality for the second extract (Figure 3B). A closer examination of the distribution of the linking motifs from the 1 h extraction onward showed an increase in the β'-O-4 units, the result of α-ethoxylation occurring postextraction, which is accompanied by a decrease in the "native" β-O-4 units showing an overall stable total amount of β-O-4 linking motifs. The temperature and the solvent thus clearly play a more significant role compared to the acid strength than the previously used severity factor value accounts for. The number of β-5 linking motifs decreases with increasing time. This is attributed to the decrease of G-units over time. Surprisingly, the $\beta-\beta$ content is nearly constant for all extraction times, even though the percentage of S-units increases significantly at longer extraction times. One explanation for this observation is that $\beta-\beta$ units are suspected to be nucleating sites for lignification, and thus found toward the center of lignin chains, resulting in a proportional decrease in their abundance as the MW increases.[64] A comparison of the number and composition of linking motifs between the 72 h ethanosolv lignin and CEL shows excellent retention of the β-O-4 (62 vs 63 per 100 C9 units), $\beta-\beta$ (8 vs 12 per 100 C9 units), and β-5 (3 vs 6 per 100 C9 units) structural motifs, confirming the extraction of lignin with a structure that resembles to a large extent that of the bulk lignin in the feedstock.

Next, we investigated the effect of switching to other linear primary alcohols (homologous series of EtOH to pentanol (*n*PnOH)), focusing on longer extraction times (Figure 2A). Extractions were also performed with methanol and *n*-hexanol (MeOH and *n*HxOH, Tables S7 and S8), which showed similar trends as those described below, but did not allow for direct comparison because of the use of a different extraction setup to accommodate temperatures above the boiling point (for MeOH) and troublesome lignin precipitation after *n*HxOH extraction. With the exception of *n*PnOH, it can be seen that the extraction efficiencies after 5 h are very similar for the different alcohols, but after a prolonged extraction time (72 h) an increased efficiency is observed from EtOH (32.5%) to *n*-propanol (*n*PrOH, 38.7%) to *n*-butanol (*n*BuOH, 43.9%). The latter is remarkably high considering these mild conditions and the benefit of using *n*BuOH is in accordance with earlier reports.[56,66,67] Just like EtOH, an increase in M_w, amount of S units, as well as changes in the amounts of the different linking motifs could be observed for *n*PrOH and *n*BuOH for increasing extraction times (Figure 2B−D). *n*PnOH-extracted lignin is a clear exception to the general trends showing no increase in β'-O-4 linking motifs in time, a much higher MW, and a relatively high amount of S units already at short extraction times. Notably, in none of the above experiments was significant condensation of the lignin observed as judged by the absence of any signal for $S_{condensed}$ in the HSQC NMR spectra, which matches the high overall number of β-O-4 linking motifs.

The increasing yield for longer linear alcohols can be related to the Hildebrand solubility parameters (eq S11 in the Supporting Information section 1) of the different mixtures.[67] This parameter provides a numerical estimate of the degree of interaction between materials. Materials with similar solubility parameters are typically able to interact with each other, resulting in miscibility or solvation. The Hildebrand solubility parameters of alcohol/H_2O mixtures (4:1 EtOH/H_2O 30.8 $MPa^{1/2}$, 4:1 *n*PrOH/H_2O 29.2 $MPa,^{1/2}$ and 4:1 *n*BuOH/H_2O 28.1 $MPa^{1/2}$)[68] are in the range of the reported values of organosolv lignins (25−26 $MPa^{1/2}$ up to 30 $MPa^{1/2}$),[69,70] but these were extracted at harsher conditions and thus are chemically significantly different compared to the lignins obtained here. The much lower extraction efficiency after 72 h with *n*PnOH (6.7%) can be explained by the poor miscibility of *n*PnOH with water, resulting in a pure *n*PnOH layer, which has a markedly lower Hildebrand solubility parameter (21.7 $MPa^{1/2}$) and is therefore less efficient as an extracting solvent. Recent work by Sameni et al. showed that acetylation of −OH groups resulted in lower values for the Hildebrand solubility parameters than for the initial lignin samples.[71] The same is expected for α-alkoxylation as this effectively replaces an −OH group in lignin. Thus, a higher degree of α-alkoxylation leads to a lower Hildebrand solubility parameter. This fits with the higher degree of α-alkoxylation for lignins isolated by extractions with long-chain alcohols as these have a lower Hildebrand solubility parameter. This trend can also be seen in work by Wu et al. in which efficient α-ethoxylation gives high yields for extractions with an EtOH/H_2O mixture, whereas inefficient α-butoxylation results in a low extraction efficiency in a *n*BuOH/H_2O mixture.[49] Moreover, unlike other lignin samples, all the extracted lignins in this work were remarkably soluble in the d_6 acetone/D_2O mixture used for NMR analysis and in particular those with a higher alcohol incorporation showed instant dissolution. Previous experimental work already showed that lignins with higher α-alkoxylation percentages have better solubility in THF.[39] We tested the solubility of our ethanosolv and pentanosolv lignin in EtOH/H_2O and *n*PnOH/H_2O mixtures (Figure S3). The lignins were fully soluble in their own extraction mixtures (even at RT), but

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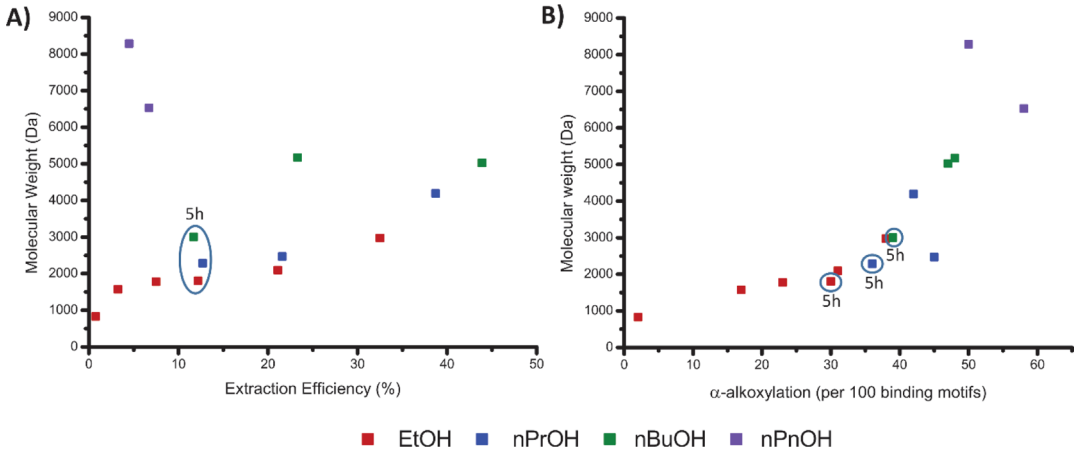

Figure 4. Plots of the properties of the obtained lignin, showing correlations between (A) extraction efficiency and MW and (B) α-alkoxylation and MW. Encircled are the 5 h datapoints for EtOH, *n*PrOH, and *n*BuOH.

in the other solvent the solubility was poor (for ethanosolv in *n*PnOH/H_2O) to incomplete (for pentanosolv in EtOH/H_2O). For ethanosolv, poor solubility was expected based on the lower degree of α-alkoxylation, which results in a higher Hildebrand solubility parameter for ethanosolv lignin, which is too high to be adequately soluble in *n*-pentanol.

Apart from the clear increase in α-alkoxylation for both increasing extraction time and alcohol chain length, a few additional trends are observed. For a given extraction time, a clear increase in MW is observed with an increasing alcohol chain, which seems to also correlate well with the increase in yield with the exception of *n*PnOH (Figure 4A). The results thus indicate that shorter lignin fragments are extracted in the early stages and have good solubility, with larger fragments being extracted with longer extraction times. Even though the extraction efficiencies for EtOH, *n*PrOH, and *n*BuOH are comparable after 5 h, a clear difference can be seen in the relative MWs of the obtained lignins, with an increasing tendency for longer-chain alcohols to extract larger lignin fragments, which is in accordance with literature.[36,56] With increasing MW, the extent of α-alkoxylation also increases, which is very clear by noting the degree of α-alkoxylation at 5 h of extraction time: EtOH, 52%; *n*PrOH, 62%; and *n*BuOH, 71%. With 82%, *n*PnOH fits into this trend as well, regardless of the markedly lower extraction efficiency. By plotting the amount of α-alkoxylation versus the M_w (Figure 4B), a clear correlation between the M_w and α-alkoxylation could be observed for all linear alcohols, indicating that α-alkoxylation plays a role in the extraction of the larger lignin fragments. This is especially striking when looking at the GPC graphs for all the linear alcohols after a 5 h extraction (Figures 5 and S10−S16), where there is a clear difference between EtOH, *n*PrOH, and *n*BuOH compared to *n*PnOH. With an increasing alcohol chain length, a larger fraction of large lignin fragments is extracted, but for EtOH, *n*PrOH, and *n*BuOH the bulk of the lignin fragments are relatively small. With *n*PnOH, hardly any small lignin fragments are extracted, showing a significant higher MW compared to the smaller-chain alcohols. The trends for α-alkoxylation, extraction efficiencies, and MW are in correspondence with our recent work with the flow-through organosolv extractions performed at harsher conditions (120 °C, 0.18 M H_2SO_4), provided that the β-O-4 linking motifs are preserved.[56]

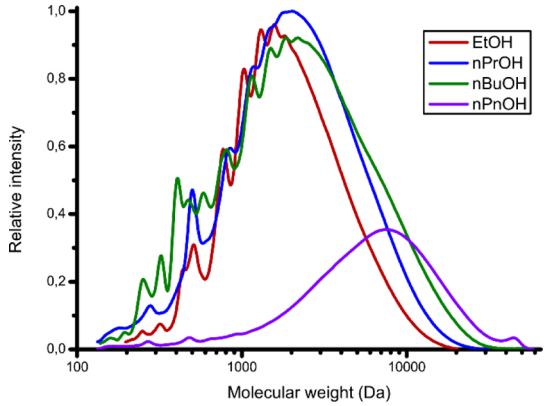

Figure 5. MW distribution of the lignins obtained after 5 h of extraction time for the applied linear alcohols as determined by GPC (THF), normalized for the yield.

From all these observations, we believe that alkoxy-incorporation and thus hydroxyl reduction is important for improving the solubility of large lignin fragments in these extraction solvents at mild conditions. This is underpinned by the lack of smaller fragments in pentanosolv lignin (Figure 5), which shows that fragments significantly rich in β-O-4 linking motifs combined with α-alkoxylation are required for extraction in an apolar solvent like *n*PnOH, which is further elaborated by the immiscibility with water causing a lower Hildebrand parameter. Because of the longer aliphatic chains, the effect of the α-alkoxylation on lowering the solubility parameter is expected to be higher. Combined with the reported negative influence of the aliphatic −OH groups on the solubility of lignin,[71] this explains that extractions with solvents like *n*BuOH and *n*PnOH gives lignins with higher MW as a higher percentage of α-alkoxylation is required for solubilization of larger fragments. It appears that the S content also relates to the MW and α-alkoxylation (Figures S8 and S9). Previous reports mention that H-groups are often end-groups[63] and hydrogenolysis occurs at a substantially higher rate for lignin end-units compared to internal units (peeling mechanism).[72] This can explain the abundance of G and H units in the first fractions, if both G and H units are abundant as (near) end-units. Furthermore, S-units favor the formation of β-O-4 groups compared to G and H units,[64] and are thus more affected by the improved solubility upon α-alkoxylation.

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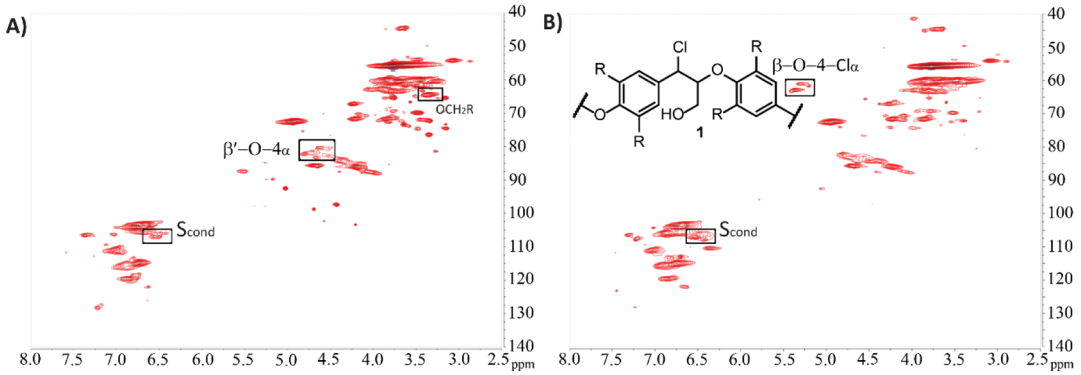

Figure 6. 2D HSQC spectra (d_6-acetone) of (A) lignin obtained after ethanosolv extraction (4:1 EtOH/H_2O, 0.24 M HCl, 80 °C, 1 h) and subsequent pure 1,4-dioxane extraction (0.24 M HCl, 80 °C, 5 h) showing α-alkoxylation and (B) lignin obtained by pure 1,4-dioxane extraction (0.24 M HCl, 80 °C, 5 h) showing chlorine incorporation in the β-O-4 motif. Condensation is observed in both products.

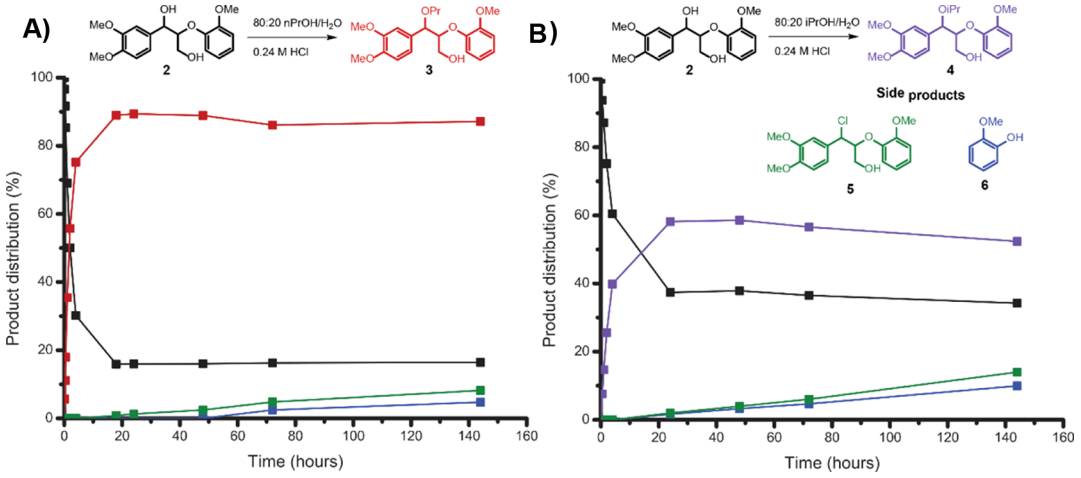

Figure 7. Reaction profile for (A) *n*PrOH and (B) *i*PrOH α-alkoxylation experiments on a model compound (**2**) showing *n*PrOH (**3**) and *i*PrOH (**4**) incorporation into the lignin structure under the extraction conditions determined by HPLC.

As it was shown that α-alkoxylation is an important parameter for determining both the amount and type of lignin extracted, the α-alkoxylation mechanism was studied by performing a set of control experiments. Sequential batch and flow-through extractions were performed with the benchmark ethanosolv conditions (see S1 and S2.5 for details). With these extractions, the influence of reactions occurring postextraction on the macromolecular properties of the lignin is limited because of the shorter exposure time of dissolved lignin to the reactive extraction medium. For both extractions, a markedly lower amount of α-alkoxylation was observed compared to the ethanosolv batch extractions, clearly showing that α-alkoxylation also occurs post-extraction. This is further evidenced by the differences between sequential batch and flow-through extractions. With flow-through extractions, the residence time of the solvent is constant and the amount of α-alkoxylation in the isolated lignin remains constant regardless of the extraction time. With the sequential batch extractions, the collection intervals were gradually increased, which was accompanied with an increase of α-alkoxylation in the lignin extracted with a longer extraction time.

Next, we performed experiments to investigate the degree of α-alkoxylation prior to the dissolution of the lignin fragments. For this purpose, a 1 h extraction was performed with the standard ethanosolv extraction conditions, yielding a lignin consisting of 55 β-O-4 units, of which 17 had the β'-O-4 motif. The recovered residue was extensively washed and subsequently extracted with 1,4-dioxane (100% 1,4-dioxane, 0.24 M HCl at 80 °C) for 5 h. Interestingly, the degree of α-alkoxylation was comparable with the lignin obtained after the 1 h ethanosolv extraction (60 β-O-4 linking motifs with 18 β'-O-4 units, Figure 6A). A similar result was obtained from a 1 h *n*PnOH pre-extraction followed by a 1,4-dioxane extraction, yielding a lignin with 16 β'-O-4 units. This value is remarkably similar to the experiment with ethanol, especially considering the observed differences between ethanosolv and pentanosolv extractions. Additionally, the residue from the *n*PnOH pre-extraction was investigated by NMR in d_5-pyridine/DMSO-d_6, revealing a signal for the α-alkoxylated β'-O-4 in addition to the regular β-O-4 signal (Figure S62). These results demonstrate that α-alkoxylation is not limited to the extracted lignin, but that it also occurs to lignin prior to extraction. This fits well with our conclusion that α-alkoxylation aids solubilization of larger lignin fragments.

The high lignin extraction efficiencies of 26.5 and 24.8% obtained during the 5 h of mild extraction with 1,4-dioxane following EtOH and *n*PnOH pre-extraction show that this sequential extraction is a method to obtain α-alkoxylated lignin in improved yield, especially in the case of *n*PnOH. The higher extraction efficiencies are due to the superior extraction ability of 1,4-dioxane as shown in a control experiment in which only the 5 h 1,4-dioxane extraction was performed, resulting in a

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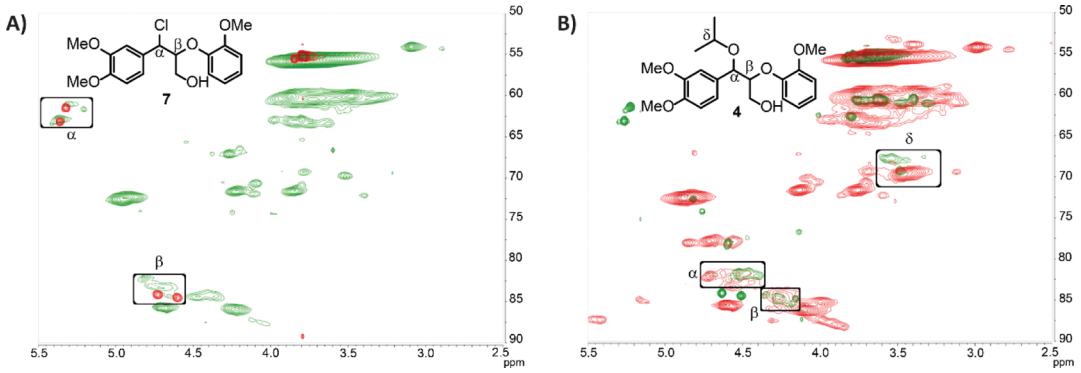

Figure 8. 2D HSQC overlay spectra (d_6-acetone) of (A) β-O-4-Cl model compound (**7**, red) and lignin obtained after 1,4-dioxane extraction (green) and (B) lignin after *i*PrOH extraction (red) and the model compound after partial *i*PrOH incorporation (**4**, green).

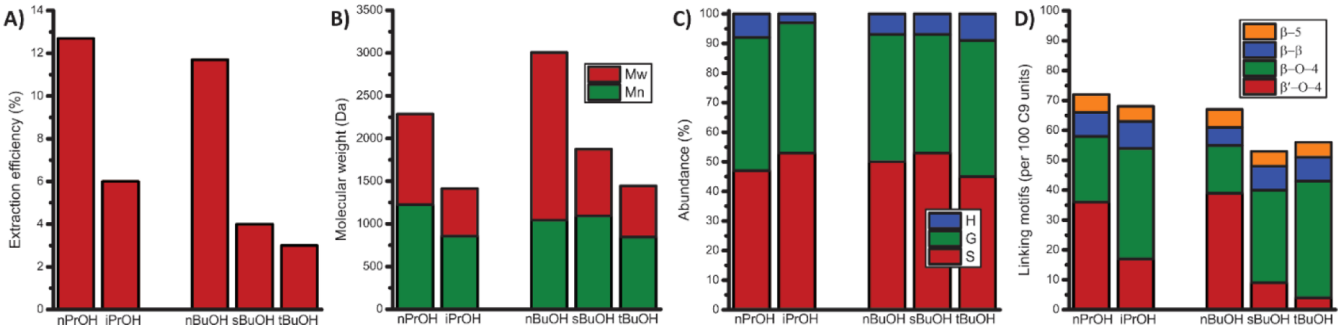

Figure 9. Comparison of the properties of the obtained lignins by extraction with branched alcohols 4:1 alcohol/H_2O mixture, 0.24 M HCl, 80 °C, 5 h of extraction time compared with their linear counterpart, showing (A) extraction efficiency, corrected for alcohol incorporation, (B) M_w distributions as determined by GPC (THF), and (C) H/G/S ratio and (D) number of linking motifs of the obtained lignins determined by HSQC NMR (d_6-acetone).

higher extraction efficiency (59.4%). However, the structural quality was much lower as the degree of S condensation was approximately 20% and only 30 β-O-4 linking motifs were measured (Figure 6B), highlighting the positive effects of alcohols as extraction solvents. As less condensation product could be observed in the 2D HSQC spectra of the 1,4-dioxane lignin obtained with alcohol pretreatment, it seems that this pretreatment has a beneficial effect on the structural integrity of the lignin, possibly because of the partial α-alkoxylation. Closer analysis of the 1,4-dioxane extracted lignin without EtOH pre-extraction showed a new signal at δ_C/δ_H 62.7/5.35 ppm, which was assigned as the α-chlorinated β-O-4 (**1**, assignment discussed in more detail below) corresponding to 10 β-O-4 linking motifs per 100 aromatic units. This is the first time such a motif was structurally assigned and present in organosolv lignin. Its occurrence could also play a role in preventing condensation, explaining the improved performance of HCl as acid (compared to HBr and H_2SO_4) in the mild organosolv extractions discussed above.

Because of the comparable α-alkoxylation into the unextracted lignin by EtOH and PnOH, alcohol incorporation experiments were conducted on a β-O-4 model compound (**2**). The α-alkoxylation experiments were performed with linear alcohols up to *n*PnOH and additionally the secondary alcohols *i*PrOH and *s*BuOH were included. All linear alcohols showed a similar reaction profile with 80–90% α-alkoxylation within 24 h and at longer reaction times the formation of α-chlorinated product (**5**) and guaiacol (**6**), indicating β-O-4 degradation. The results with model compounds clearly show that the deviations observed with the organosolv extractions are not caused by different reactivities of the alcohols, but should be caused by the aforementioned difference in solubility in which a higher degree of α-alkoxylation plays a significant role. The reaction profile of bulky alcohols showed a markedly different reaction profile with lower levels of α-alkoxylation and faster formation of side products at greater quantities (Figures 7 and S19–S25). This clearly shows the added stability that α-alkoxylation provides to the β-O-4 motif.

2D HSQC analysis of the model compounds after reaction showed matching signals of indicative cross-peaks (Figure 8A, green, δ_C/δ_H 82/4.5 63/5.28, 61/5.22 ppm) with those observed in lignin from 1,4-dioxane extraction (Figure 6B). To confirm this, a model compound with chlorine incorporation at the α-position was synthesized (**7**), of which the 2D HSQC spectra overlapped very closely to those of the lignin obtained by 1,4-dioxane extraction (Figure 8A, red). In a corresponding experiment using HBr instead of HCl, similar signals were observed, although to a lesser extent. These are tentatively assigned to an α-brominated β-O-4 compound (Figure S13). With H_2SO_4 as acid, such signals were not observed (Figure S14).

The experiments with model compounds showed that α-alkoxylation also occurs with bulky alcohols, but to a somewhat lower degree (60%). We were curious how this would translate to extractions performed on lignin. Extractions with sterically hindered alcohols have been reported for lignin extraction[73,74] and based on these reports limited α-alkoxylation was expected. Compared to *n*PrOH, a significant drop in extraction efficiency was noted for *i*PrOH (6.0 vs 12.7%, Figure 9A) accompanied by a significant decrease in the M_w (1415 vs 2287

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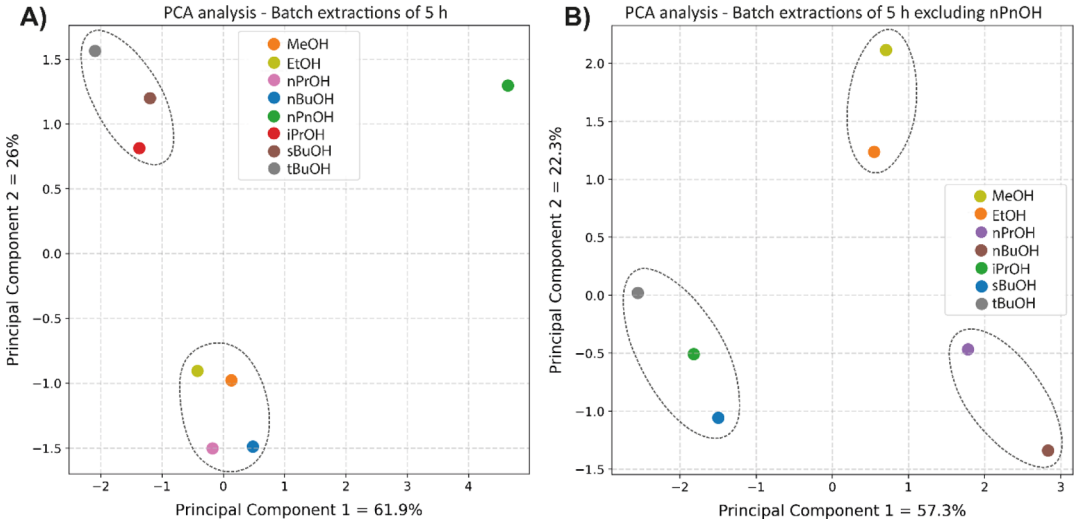

Figure 10. PCA of (A) all 5 h batch extractions (87.9% quality) and (B) 5 h batch extractions excluding *n*PnOH (79.6% quality).

Da, Figure 9B) and no notable change in the H/G/S ratio (Figure 9C). Nevertheless, to our surprise two clear new signals were detected by 2D HSQC analysis that mirrored the α-signal of the α-alkoxylated product with linear alcohols. Additionally, signals corresponding to isopropyl units were observed. Therefore, the new signal was attributed to α-alkoxylation with *i*PrOH. This was confirmed by overlap of the model compound with *i*PrOH incorporation as the indicated signals overlap well with the spectra of the model compound (Figure 8B, δ_C/δ_H 68/3.55, 84/4.63 ppm and δ_C/δ_H 84/4.51 ppm).

The number of β'-O-4 linking motifs was quite significant under our conditions, but markedly lower for *i*PrOH compared to *n*PrOH (17 and 36 β'-O-4 linking motifs respectively, Figure 9D). Extractions with *s*BuOH and *t*BuOH resulted also in lower extraction efficiency (4.0 and 3.0% respectively), lower M_w (1095 and 847 Da respectively), and lower degrees of α-alkoxylation (9 and 4 β'-O-4 linking motifs, respectively). The notable decrease in extraction efficiency is unexpected based solely on the Hildebrand solubility parameters of the solvent, as the values for *n*BuOH (23.1 $MPa^{1/2}$) and *i*PrOH (23.5 $MPa^{1/2}$)[68] are close to each other, but the extraction efficiencies differ from 11.7% for *n*BuOH to 6.0% for *i*PrOH. These results match the conclusion that α-alkoxylation aids solubilization of mainly larger lignin fragments, explaining why *i*PrOH gives lower yield and lower MW lignin. This is consistent with calculations on the negative influence of aliphatic −OH groups on the solubility.[71] To ensure that the lower yields with bulky alcohols were not the result of condensation reactions leading to insoluble residues, scanning electron microscopy (SEM) images were taken of the residue after *s*BuOH extraction. Recent literature showed the formation of spherical droplets upon lignin condensation because of pretreatment.[75] In our case, no spherical droplets could be observed, giving an identical picture compared to a 5 h mild ethanosolv extraction (Figures S4 and S5). Additionally, a second extraction on the *s*BuOH residue with a 1,4-dioxane/propanol extraction (5 h, 80 °C, 0.24 M HCl) gave good extraction efficiency (27%) and low levels of condensation (6% $S_{condensed}$), indicating that the low extraction efficiency with *s*BuOH is not due to lignin condensation.

Finally, principal component analysis (PCA) was performed to obtain an overview of the similarities among the extracted organosolv lignins, as well as to better visualize the main findings of this work. PCA is a statistical procedure that uses an orthogonal transformation to convert a set of possibly correlated variables into a set of linearly uncorrelated variables called principal components. The first principal component accounts for as much of variability of the data as possible and each succeeding component has the highest variance possible under the constraint that it is orthogonal to the preceding components. Datapoints that are close to each other represent extracted lignin with similar properties. A PCA performed with all the characterization results from the lignins obtained after 5 h of extractions revealed two clear clusters (Figures 10A and S32), consisting of linear and branched alcohols. In line with the results discussed in the previous sections, *n*PnOH is a clear outlier. The PCA was able to explain 87.9% of the data variance with the first two principal components (i.e., PC1 and PC2), which correspond to the *x* axis and *y* axis of the score plots, respectively. PC1 is heavily linked to α-alkoxylation, MW, and S/(G + H) and inversely to the β-O-4 and β-5 linking motifs, whereas the main deviator of the PC2 axis is the extraction efficiency (which indeed differs substantially between linear and branched alcohols, vide supra). As *n*PnOH deviates considerably from the other linear alcohols, a second PCA was performed excluding *n*PnOH (Figures 10B and S33). Again, clustering of the branched alcohols was observed. This time, however, the linear alcohols are further separated in two groups, that is, MeOH + EtOH and *n*PrOH + *n*BuOH. PC1 mostly separates linear and branched alcohols, being heavily linked to extraction efficiency, α-alkoxylation, and MW, while inversely linked to the β-O-4 linking motif. The linear alcohols are mainly separated by PC2, which is inversely linked to the β-5 linking motif and to a lesser extent to extraction efficiency. These results clearly show the influence of the extraction solvent on the properties of the obtained lignin.

PCA was also applied to the complete set of batch experiments that were performed (Figures 11 and S34). Clustering was observed for the 5, 16, and 72 h extractions of the linear alcohols. The short-time extractions are clustered together with the branched alcohols, and *n*PnOH appears as an

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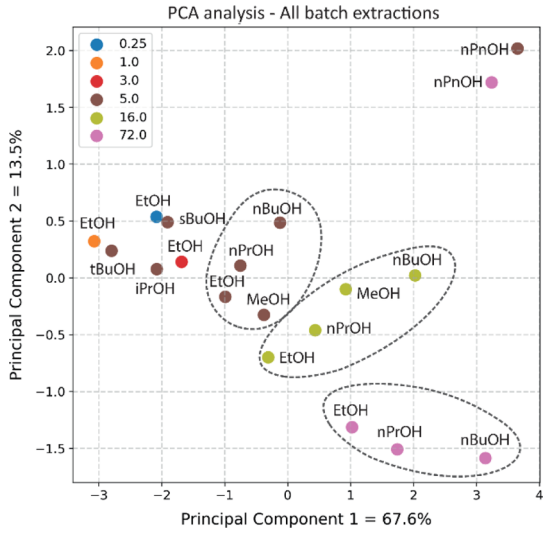

Figure 11. PCA of all batch extractions (81.1% quality).

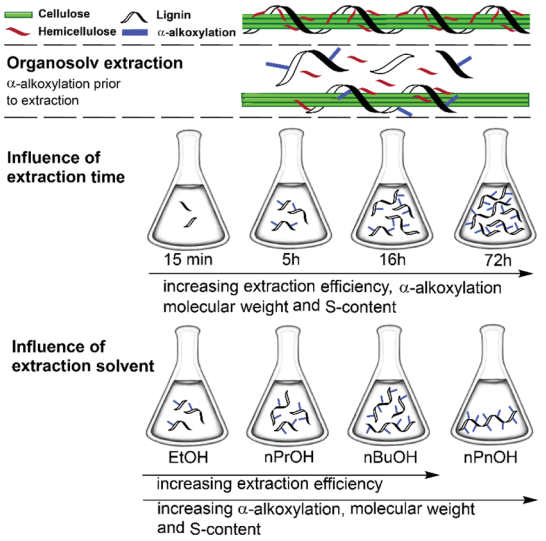

Figure 12. Overview of the most important trends observed in this paper: α-alkoxylation occurs prior to extraction and the influence of extraction time and extraction solvent on the properties of the obtained lignin.

outlier for both the 5 and 72 h extraction. PC1 is heavily linked to the β-O-4 linking motif, α-alkoxylation, MW, and S/(G + H). For PC2, the main deviator was again the extraction efficiency and to a lesser extent MW and the β-O-4 content. These results show the strong influence of extraction time on the lignin properties. To quantify the observed correlations, multivariable linear regressions were performed with all obtained data from the lignin extractions to investigate if extraction efficiency and MW could be expressed as a statistically significant combination of different lignin parameters (Figure S35). A linear fit for extraction efficiency was obtained with positive functions for the extraction time, S-content, and the β-O-4 content (for both the individual β-O-4 and β'-O-4 values and the total β-O-4 content). These results are in accordance with the trends discussed throughout the paper. The same parameters also showed positive functions for the MW, showing the interdependence of the extraction efficiency and the MW of the obtained lignin. Overall, the results clearly show the effects depicted in Figure 12, providing also quantitative correlations that can be useful for future explorations of mild organosolv lignin extraction with alcohols as solvent.

CONCLUSIONS

This research demonstrates that mild organosolv extractions with alcoholic solvents results in lignin with excellent retention of the high β-aryl ether content, but with partial α-alkoxylation of the obtained lignin by the solvent. Additionally, it is shown how the solvent and the reaction time affect the yield and chemical structure of the obtained lignins. Two-step extractions were successfully performed in which the properties of the lignin were tuned by adjusting the extraction conditions. A short ethanosolv pre-extraction followed by an ethanosolv extraction for a longer time results in a lignin with a higher structural purity in the second fraction as short-chain lignin fragments and other organic impurities are extracted in the first 15 min. A marked increase in α-alkoxylation into the β-O-4 motif with increasing alkyl chain length of the alcohol was observed, which was already observable after 5 h of extraction time, showing an increasing α-alkoxylation from EtOH (52%) to *n*PnOH (82%). However, two-stage extractions for both a 1 h ethanosolv and pentanosolv extraction, followed by a 1,4-dioxane extraction showed similar values for α-alkoxylation for the lignin isolated by the second step, showing that α-alkoxylation occurs to the same extent on the solid lignocellulosic biomass. This was also observed with incorporation experiments on model compounds, which showed that incorporation rates are not significantly different between linear alcohols. This difference is caused by the decreasing Hildebrand solubility parameter of the solvent mixture going from EtOH/H_2O to *n*PrOH/H_2O and *n*BuOH/H_2O mixtures to a pure *n*PnOH phase. The latter requires significantly higher degrees of α-alkoxylation for the lignin fragments to become soluble in the extraction mixture. Longer extraction times resulted in a further increase of alcohol incorporation, which was also accompanied by a higher average MW. Comparing the different alcohols, the average MW increased gradually from EtOH to *n*BuOH and showed a marked increase for *n*PnOH. The increase in the average MW of the obtained lignins also seems linked to an increase in the S-content. This effect holds up to *n*PnOH, which showed a markedly lower extraction efficiency, which is explained by the poor miscibility with water and thus requiring extensive α-alkoxylation before solubilization in the hydrophobic *n*PnOH layer. The use of sterically encumbered alcohols resulted in a lower extraction efficiency, which can be ascribed to lower, although surprisingly still observable, α-alkoxylation. With prolonged reaction times, the formation of a new linking motif was observed and identified as originating from α-chloro incorporation into the β-O-4 linking motif, which could also play a role in preventing condensation. Overall, the results show how the reactivity at the α-position of the lignin β-O-4 motif is important for efficient lignin extraction under mild organosolv conditions using alcohols. Although no full α-alkoxylation is achieved at set conditions, methodologies have been reported in which either full α-alkoxylation[58] or de-etherification to α-OH groups[36] can be achieved in a subsequent step. The former has shown to allow for new modification and depolymerization strategies and thus new applications for lignin.[58,76,77] Overall, these results and conclusions can be utilized to optimize extraction of such

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lignins for selective depolymerization procedures but importantly also for optimization of conditions for lignin-first methodologies such as reductive fractionation, as the choice of solvent also significantly influences the yield and phenolic composition in the subsequent steps.[78] Furthermore, implementing these mild organosolv extractions as the first step in a two-step extraction scheme, followed by a second step which applies harsher extraction conditions, could be industrially relevant. With this strategy, a high-value lignin stream can be obtained in the first stream, whereas high delignification is achieved in the second step.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.9b07222.

Experimental procedures for lignin extraction, lignin content determination, weight correction, synthesis of model compound, and model compound studies; analytical procedures for NMR, 2D HSQC, GC−MS, GPC, HPLC, UPLC, and statistical analysis (PCA and linear regression); analysis results; all organosolv extraction results and biomass analysis (GC−MS, SEM); all HSQC NMR data for the lignin used (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Peter J. Deuss − *Department of Chemical Engineering (ENTEG), University of Groningen, Groningen 9747 AG, The Netherlands;* orcid.org/0000-0002-2254-2500; Email: p.j.deuss@rug.nl

Authors

Douwe S. Zijlstra − *Department of Chemical Engineering (ENTEG), University of Groningen, Groningen 9747 AG, The Netherlands*

Ciaran W. Lahive − *Department of Chemical Engineering (ENTEG), University of Groningen, Groningen 9747 AG, The Netherlands*

Coen A. Analbers − *Department of Chemical Engineering (ENTEG), University of Groningen, Groningen 9747 AG, The Netherlands*

Monique B. Figueirêdo − *Department of Chemical Engineering (ENTEG), University of Groningen, Groningen 9747 AG, The Netherlands*

Zhiwen Wang − *Department of Chemical Engineering (ENTEG), University of Groningen, Groningen 9747 AG, The Netherlands*

Christopher S. Lancefield − *School of Chemistry and Biomedical Science Research Complex, University of St. Andrews and EaStCHEM, Fife KY16 9ST, U.K.*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssuschemeng.9b07222

Author Contributions

The paper was written through contributions of all the authors. All the authors have given approval to the final version of the paper.

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Notes

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