Volume 1 | Number 1 | Jan 2013 | Pages 1–100

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## ARTICLE

# Fractionation of 'water-soluble lignocellulose' into C$_5$/C$_6$ sugars and sulfur-free lignins

Mats Käldström, Niklas Meine, Christophe Farès, Roberto Rinaldi* and Ferdi Schüth*

Recently, we demonstrated the mechanocatalytic depolymerization of lignocellulosic substrates as a powerful methodology that fully converts lignocellulosic substrates into 'water-soluble lignocellulose.' We now show that the saccharification of the aqueous solution of depolymerized beechwood, pinewood and sugarcane bagasse (at 140 °C for 1 h) produces a high yield of sugars (*e.g.* 88-92 % glucose, 3.5-8 % glucose dimers and 93-98 % xylose relative to the glucan and xylan fractions, respectively) and leads to precipitation of sulfur-free lignins. Noteworthy, the formation of furfurals is suppressed because the 'water-soluble lignocelluloses' undergoes hydrolysis at relatively low temperatures. At 140 °C, 5-hydroxymethylfurfural and furfural are formed in yields not exceeding 1.4 and 5.7 %, respectively. The separation of the carbohydrate fraction (as C$_5$ and C$_6$ sugars) from the lignin fraction is thus feasible by simple filtration.

## Introduction

Mechanical forces have been exploited for wood processing for the past few hundreds of years. A representative example is the mechanical pulping of wood, which has been in place for more than 100 years. As such, the paper industry produces about 35 million tons of pulp per year.[1] In mechanical pulping, the internal processes leading to the deconstruction of wood are predominantly physical in nature.[2] Surprisingly, although mechanical processing of biomass has been long established,[3, 4] the combination of acid catalysis and mechanical forces in a 'one-pot process' has not yet been extensively studied for performing processes that are chemical in nature, such as catalytic biomass conversion. Only recently have some reports on solvent-free approaches for the acid-catalyzed depolymerization of cellulose – driven by mechanical forces – emerged in literature.[5-11] In this context, the high depolymerization efficiency, that is, the full conversion of plant biomass into water-soluble products, was realized through the impregnation of cellulosic fibers with catalytic quantities of HCl or H$_2$SO$_4$ before milling of the dry substrate, as recently demonstrated by us.[8]

The impregnation of acid onto the cellulose surface mitigates contact problems found by Blair *et al.*[5] for the reaction in the presence of solid acids. Hence, cellulose undergoes deep depolymerization and is thus fully converted into 'water-soluble oligosaccharides' (WSO) within 2 h of milling.[8] We demonstrated the WSO as a unique replacement for glucose and xylose, enabling high-yield production of sugar alcohols[9] or furfural and 5-hydroxymethylfurfural (HMF).[12] Moreover, first
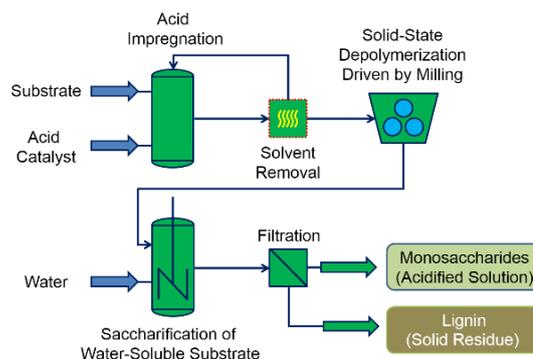


**Scheme 1.** Schematic representation of the method for fractionation of plant biomass into water-soluble monosaccharides and lignins.

analyses of the results on lab-scale indicate that the mechanocatalytic depolymerization of lignocellulosic biomass could well become both economically and energetically sustainable.[8]

Recently, we reported that the full conversion not only of cellulose, but also of native lignocellulosic substrates (*e.g.* pinewood, beechwood, switchgrass and sugarcane bagasse) into 'water-soluble wood' can also be achieved by the mechanocatalytic approach.[8] The separation of the water-soluble oligosaccharides from the water-soluble lignin fragments, however, has remained an unsolved challenge until now.

We have now found a simple method for the separation of sulfur-free lignins from $C_5$ and $C_6$ sugars. Scheme 1 depicts the four key steps of the fractionation approach for plant biomass conversion under low-severity conditions:

(1) Wet-impregnation of the substrate with acid (which includes solvent removal and its reuse) or dry-impregnation by exposing the substrate to gaseous HCl (thus eliminating the need for solvent removal and recycling);[8]

(2) Deep' depolymerization of acid impregnated biomass by mechanocatalysis;

(3) Saccharification of the 'water-soluble lignocellulose';

(4) Separation of the lignin from the solution of $C_5$ and $C_6$ sugars by filtration.

In this report, we describe the fundamental chemistry of the fractionation process in detail, and establish 'water-soluble lignocellulose' as a highly promising feedstock for the production of sugars and sulfur-free lignins, compared with "regular" water-insoluble lignocellulose. This paper is structured as follows. First, we address the separation of $C_5$ and $C_6$ sugars from lignin by saccharification in aqueous solution. Next, the chemical properties of the lignin precipitates are presented in detail. Finally, the factors responsible for the precipitation of lignin are proposed.

## Results and Discussion

### Separation of the fermentable sugars from lignin

For any biorefinery scheme, the separation of the different lignocellulose fractions is crucial. Considering the complexity of the "water-soluble wood" composition,[8] the question arises whether *it is possible to separate the sugar fraction from the lignin fragments.* In our first report on the mechanocatalytic depolymerization of biomass,[8] we showed that the soluble products of $H_2SO_4$-impregnated cellulose undergo full saccharification by merely heating its aqueous solution at 130 °C for 1 h without extra addition of acid. Glucose and xylose yields close to 100 % were obtained. No extra acid is needed for the saccharification because the initial acid content (from the impregnation step) is not destroyed by milling the acid-impregnated substrate as revealed by titration of the acid content before and after milling.

A rather different result is obtained by heating a 10 % aqueous solution of depolymerized beechwood, pinewood or sugarcane bagasse (pH 1). Fig. 1 shows the aqueous solution of beechwood before and after treatment at 140 °C for 1 h. Here, the saccharification of the 'water-soluble beechwood' leads to the precipitation of a solid residue. The solution color changes from dark reddish brown to pale yellowish (after removal of the precipitate). Table 1 summarizes the results obtained from the saccharification of the water-soluble substrates (beechwood, pinewood and sugarcane bagasse).

Table 1 summarizes the sugar and furfural yields obtained by the saccharification of the 'water-soluble lignocelluloses.' At 140 °C for 1 h, glucose yields exceeding 88 % relative to the

glucan fraction (refer to Supporting Information for the substrate composition, Table S1) in addition to xylose yields exceeding 93 % relative to the xylan fraction were achieved. Clearly, the formation of dehydration products and humins is suppressed because the saccharification of 'water-soluble lignocelluloses' takes place at relatively low temperatures, compared with conventional processes.[13, 14] At 140 °C, HMF and furfural are formed in yields not exceeding 1.4 and 5.7 %, respectively.



**Fig. 1.** Fractionation of lignocellulose into $C_5$ and $C_6$ sugars and sulfur-free lignin by saccharification of a solution of depolymerized beechwood at 140 °C for 1 h.

**Table 1**. Product yields obtained from the saccharification of 'water-soluble lignocelluloses' at 140 °C for 1 h.

| Substrate | Product yields (%) | | | | |
|---|---|---|---|---|---|
| | Glucose Dimers | Glucose | HMF | Xylose | Furfural |
| Beechwood | 3.5 | 88 | 1.4 | 93 | 5.7 |
| Pinewood | 6.6 | 88 | 1.4 | 98 | 1.7 |
| Sugarcane bagasse | 8.0 | 92 | 3.0 | 95 | 5.0 |

The high sugar yields obtained by the saccharification of the water-soluble substrates at 140 °C serve as a preliminary indication that the precipitate is mostly composed of lignin. To verify whether the precipitate is indeed lignin, the precipitates and the corresponding lignins extracted by the organosolv method were compared by FTIR. It is clear from Fig. 2 that the fingerprint region of the FTIR spectra of the precipitates are very similar to those collected from the corresponding organosolv lignins, thus confirming that the precipitates are made of lignin.
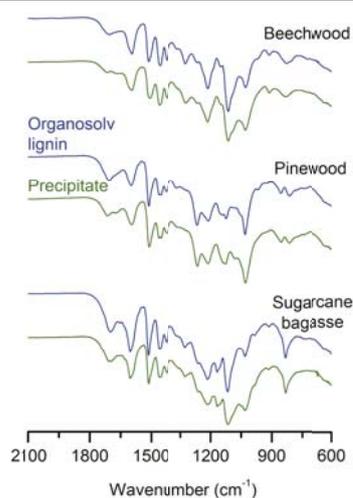
**Fig. 2.** FTIR-spectra of the organosolv lignins of beechwood, pinewood and sugarcane bagasse (in blue) compared with the corresponding lignin precipitates (in green) formed by the saccharification of the 'water-soluble lignocellulose' at 140 °C for 1 h.

### Properties of the lignin precipitates

The lignin precipitates are brownish (beechwood and pinewood) or pale brown powders (sugarcane bagasse), as shown in Fig. 3. Table 2 summarizes the elemental composition found for the lignin precipitates and the corresponding organosolv lignins.
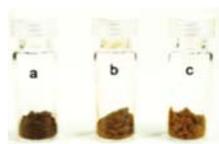


**Fig. 3.** Lignin precipitates obtained from 'water-soluble lignocelluloses': a) beechwood, b) sugarcane bagasse and c) pinewood after saccharification at 140 ºC for 1 h.

The elemental composition of the beechwood lignin precipitate is in close agreement with values reported for beechwood MWL (milled wood lignin) by Björkman and Person (C: 60.3 %, H: 6.3 % and O: 33.3 %) and with those determined by us for the corresponding organosolv lignin.[15] The elemental composition of the pinewood lignin precipitate shows, however, a lower C-content than that reported for pinewood MWL (C: 64.0 %, H: 6.1 %, 29.8 %) and that obtained for organosolv pinewood lignin. This result clearly shows that pinewood lignin precipitate still contains traces of carbohydrate impurities. Lastly, the elemental analysis for the sugarcane lignin precipitate revealed a lower C-content than that found for the corresponding organosolv lignin.

**Table 2.** Elemental composition of the lignin precipitates, obtained by the saccharification of $H_2SO_4$ water-soluble beechwood, pinewood and sugarcane bagasse at 140 ºC for 1 h, and the corresponding lignins isolated by the organosolv process.

| Sample | Contents (*daf*, %) | | | | |
|---|---|---|---|---|---|
| | C | H | N | S | O[a] |
| $H_2SO_4$-impregnated beechwood | 40.4 | 5.8 | 0.1 | 2.5 | 50.3 |
| Beechwood lignin precipitate | 60.9 | 5.8 | 0.2 | 0.0 | 33.1 |
| Organosolv beechwood lignin | 61.8 | 5.9 | 0.0 | 0.0 | 32.3 |
| $H_2SO_4$-impregnated pinewood | 42.9 | 6.2 | 0.2 | 2.6 | 48.1 |
| Pinewood lignin precipitate | 63.4 | 6.4 | 0.3 | 0.0 | 29.9 |
| Organosolv pinewood lignin | 68.2 | 6.3 | 0.9 | 0.0 | 24.6 |
| $H_2SO_4$-impregnated sugarcane bagasse | 39.8 | 5.9 | 0.4 | 3.1 | 51.2 |
| Sugarcane bagasse lignin precipitate | 61.2 | 5.8 | 0.5 | 0.0 | 32.4 |
| Organosolv sugarcane bagasse lignin | 66.7 | 5.6 | 0.5 | 0.0 | 27.2 |

[a] Determined by difference.

Most importantly, the elemental analysis results show that sulfur contents dramatically decrease from ca. 3 % (which corresponds to the acid loading of 0.9 mmol $H_2SO_4$ per gram of substrate), *to levels below the detection limit of this method* (< 0.05 %). Therefore, even if the lignin were sulfonated by mechanocatalytic reactions (we have not found so far any experimental evidence for this), the very low sulfur-content in the lignin precipitate leads to the conclusion that such sulfonic acid groups would be hydrolyzed under the saccharification conditions. This proposition is plausible because sulfonic groups on aromatic rings substituted with electron donating groups (*e.g.* methoxyl and alkyl groups) are known to be prone to undergo hydrolysis at temperatures as low as 100 °C.[16]

The very low sulfur content in the lignin precipitates distinguishes these materials from technical lignins obtained by current pulping processes (*e.g.* Kraft and Sulfite processes) which may contain up to 9 % sulfur. This low sulfur content suggests advanced utilization of the lignin precipitates for the production of high value products (*e.g.* chemicals,[17, 18] fuel additives[18-20] and production of high-quality carbon fibers[21]).

To evaluate the solubility of lignin precipitate formed at 140 ºC, the solubility was initially tested at concentrations of 1 mg mL^{-1} in different solvents (ethanol/$H_2O$ 7:3 v/v, DMSO, THF and 1,4-dioxane). The lignin precipitates obtained from processed beechwood and sugarcane bagasse were completely soluble in a 1 mol L^{-1} solution of NaOH, while that from pinewood was only partially soluble in this solution. Unlike the organosolv lignins, which are soluble in ethanol/$H_2O$ 7:3 v/v, DMSO, THF and 1,4-dioxane, the lignin precipitates were only partially soluble in these solvents. Additionally, the lowest solubility was found for the pinewood lignin precipitate, which was almost not soluble in any of the organic solvents tested.

The lower solubility of the lignin precipitates, compared to the corresponding organosolv lignins, suggests the presence of lignin with high molecular weight. These large structures are very likely produced by condensation involving position 5 on the G units[22] or positions 3 and 5 of the H units, forming highly stable C—C bonds. Gellerstedt *et al.* proposed a reaction scheme, showing the condensation as a side reaction concurrent to the depolymerization of lignin upon the cleavage of α-O-4 (Scheme 2).[22] The condensation extent of lignin, by the route

proposed in Scheme 2, is most likely to be greater in pinewood lignin than in beechwood lignin due to the high fraction of G units occurring in the structure of pinewood lignins (about 98 %, as determined elsewhere).[23]
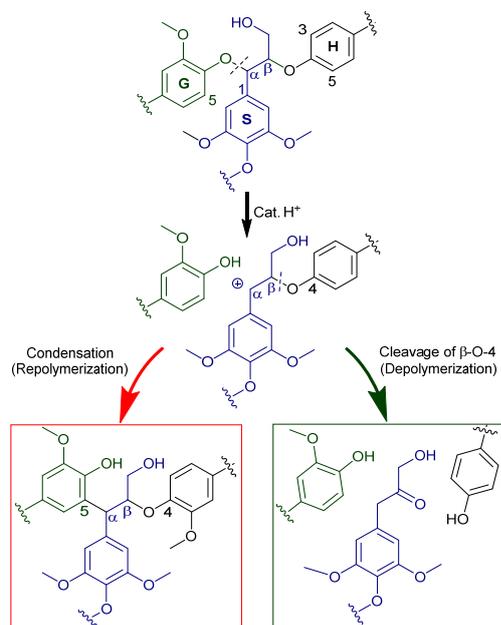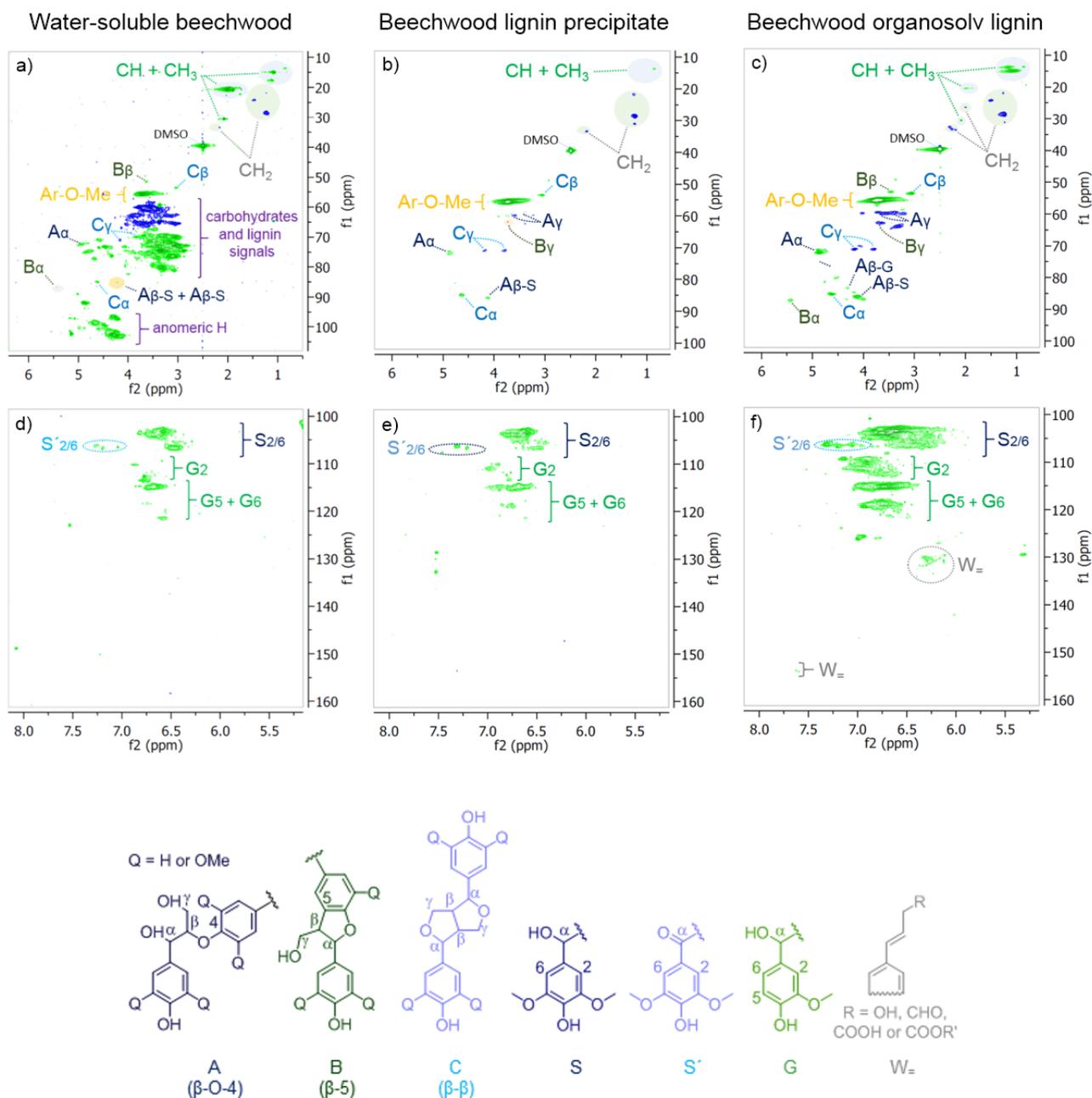
**Fig. 4.** HSQC NMR from the 'water-soluble beechwood', DMSO-soluble fraction of lignin precipitate, and organosolv beechwood lignin dissolved in DMSO-$d_6$. The spectra display (a-c) the region of the $C_3$ side-chains of the lignin units, and (d-f) aromatic region of lignin. The correlation signals in green correspond to CH and $CH_3$ groups, while those in blue to $CH_2$ groups.

In order to assess the nature of the chemical entities present in the 'water-soluble beechwood', organosolv beechwood lignin, and in the DMSO-soluble fraction of the beechwood lignin precipitate (which corresponds to about 70 % of the sample), HSQC NMR spectra of the samples dissolved in DMSO-$d_6$ were collected. Fig. 4 shows the HSQC spectra in the region of the $C_3$ side-chain of the phenyl propane units (Fig. 4a-c) and in the aromatic region (Fig. 4d-f).

From Fig. 4a-c, it is possible to identify the presence of lignin subunit structures A, B and C and thus the corresponding

interunit linkage types – β-O-4 (by the presence of the correlation signals, $A_\alpha$ and $A_\beta$), β-5 ($B_\alpha$ and $B_\beta$) and β-β ($C_\alpha$, $C_\beta$ and $C_\gamma$). Fig. 4a shows the HSQC spectrum of 'water-soluble beechwood', which is also fully soluble in DMSO-$d_6$. Due to the prominent and overlapping carbohydrate contours, it is very challenging to resolve the correlation signals for the $^1$H-$^{13}$C pairs corresponding to $A_\gamma$ ($\delta_C/\delta_H$, 59.7/3.6 ppm and 59.4/3.7 ppm) and $B_\gamma$ ($\delta_C/\delta_H$, 62.8/3.7 ppm). Worth mentioning, the correlation signal for $B_\alpha$ shows a very low absolute intensity, and is thus hardly seen in the magnification applied to Fig. 4a.

Next, Fig. 4b displays the HSQC spectrum of the DMSO-soluble fraction of the beechwood lignin precipitate. No cross signal attributable to the carbohydrate fraction was found in the spectrum. Interestingly, the phenylcoumaran-type structures (B, β-5) were not detected in the HSQC spectra (Fig. 4b). Finally, Fig. 4c shows the HSQC spectrum of organosolv beechwood lignin. In this spectrum, the cross signals characteristic of the substructures containing β-O-4, β-5 and β-β linkages are found.

Fig. 4d-f reveal information concerning the aromatic units of lignin. Notably, the spectrum for 'water-soluble beechwood' sample (Fig. 4d) and its lignin precipitate (Fig. 4e) are very similar. These spectra do not display the cross signals for Ar-CH=CH-R structures found for the organosolv beechwood lignin (W=, Fig. 4f). Accordingly, these unsaturated structures may have undergone polymerization already in the mechanocatalytic depolymerization step or were not even formed by mechanocatalytic reactions, in contrast to the organosolv process.

Table 3 summarizes the distribution of lignin subunit linkages β-O-4 (as $A_\alpha$), β-5 (as $B_\alpha$) and β-β (as $C_\alpha$) in addition to the relative composition of S and G units present in both lignins.

**Table 3.** Distribution of lignin subunit linkages β-O-4 (as $A\alpha$), β-5 (as $B\alpha$) and β β (as $C\alpha$), and relative composition of sinapyl (S) and guaiacyl (G) units in lignin as estimated by HSQC NMR from the 'water-soluble beechwood', DMSO-soluble fraction of lignin precipitate, and organosolv beechwood lignin dissolved in DMSO-d$_6$.

| Sample | Linkage distribution | | | Content (%)[a] | |
|---|---|---|---|---|---|
| | β-O-4 | β-5 | β-β | S | G |
| H$_2$SO$_4$-processed beechwood[a] | 2.0 | 0.3 | 1.0 | 61 | 39 |
| Beechwood lignin precipitate | 1.9 | 0.0 [b] | 1.0 | 60 | 40 |
| Organosolv beechwood lignin | 2.5 | 0.5 | 1.0 | 66 | 34 |

[a] the correlation signal for H$_{2/6}$ entities was not detected in the beechwood samples; [b] not detected.

The beechwood lignin precipitate shows a distribution of lignin subunits linkages similar to that found for the parent material, 'water-soluble beechwood.' In regard to the relative composition of sinapyl (S) and guaiacyl (G) units, the DMSO-soluble fraction of the beechwood precipitate shows a higher content of G units (40 %) compared with organosolv lignin (34 %) but still similar to that found for the parent material. These observations indicate that the lignin precipitate is similar to the lignin contained in 'water-soluble beechwood'. Apparently, the mechanocatalytic depolymerization of beechwood causes more significant alterations (*e.g.* condensation and S-unit conversion) in the native structure of lignin than the subsequent aqueous-phase saccharification itself.

### Insights into processes leading to the precipitation of lignin

The precipitation of lignin from water-soluble lignocellulose involves complex processes occurring in the carbohydrate and lignin fractions dissolved in water. In order to shed light into these processes, we investigated the effect of temperature on the formation of the lignin precipitate. The evolution of the chemical composition of the lignin-based precipitate upon the saccharification of water-soluble beechwood, pinewood and sugarcane bagasse was also examined.

Fig. 5 shows the evolution of the yield of cellobiose, glucose and xylose relative to the corresponding glucan and xylan fractions in beechwood, pinewood and sugarcane bagasse.
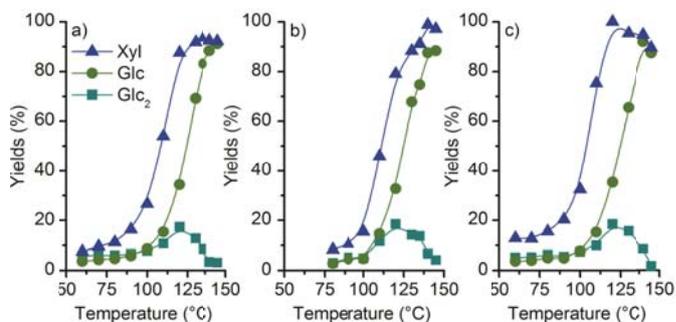


**Fig. 5.** Yields of glucose dimers (Glc$_2$), glucose (Glc) and xylose (Xyl) after saccharification of water-soluble a) beechwood, b) pinewood and c) sugarcane bagasse at different temperatures for 1 h.

The release of xylose starts at temperatures about 20 to 30 °C lower than that found for the release of glucose. This result confirms the previous data collected in the saccharification of solid wood in diluted H$_2$SO$_4$, showing that xylans are more prone to hydrolysis than glucans. Furthermore, in all the experiments, the formation of cellobiose reached a peak at 120 °C.

In spite of the solubility of the depolymerized lignocelluloses in water, the temperature, at which the maximum yield of xylose is obtained, depends on the substrate type and is ordered as follows: sugarcane bagasse (120 °C, 99 %) < beechwood (135 °C, 94 %) < pinewood (140 ºC, 99 %). Surprisingly, this trend corresponds to the results obtained from the dilute acid hydrolysis of solid lignocellulose. The results show the xylans of the water-soluble sugarcane bagasse as more easily hydrolysable than those of beech- and pinewood samples. In turn, the maximum yield of glucose is reached at about 140 °C as indicated in the following: pinewood (88 %, 145 ºC) ≈ beechwood (92 %, 145 °C) < sugarcane bagasse (95 %, 140 °C).

Because the water-soluble substrates are subjected to lower temperatures than those applied for the saccharification of lignocellulose (*e.g.* by the two-stage, dilute H$_2$SO$_4$ acid process: 180 and 210 °C),[14] the dehydration of xylose to furfural proceeds to a lower extent. Under conditions leading to the highest yield of xylose, the yields of furfural do not exceed 1.5 %. Nonetheless, as the glucan fraction requires more severe conditions for full conversion into glucose, xylose undergoes further dehydration if not isolated from the reaction medium. In comparison, the furfural yields formed at 145 ºC follow the order: pinewood (2.4 %) < beechwood (7.9 %) ≈ sugarcane bagasse (8.4 %). Since glucose is more recalcitrant than xylose towards dehydration, the formation of HMF is lower than 1.5 % even at temperatures as high as 145 °C.

Fig. 6a displays the evolution of the lignin-based precipitate with the saccharification temperature. The amount of precipitate depends strongly on the saccharification temperature and its profile against the saccharification temperature significantly varies for the different substrates. For water-soluble beechwood, an increase in the saccharification temperature (from 60 to 90 °C) leads to a marked increase of the amount of precipitate (from 4 to 27 % relative to the initial substrate weight). For the water-soluble pinewood, no precipitate was formed until a saccharification temperature of 90 °C was reached. At this temperature, the formation of a precipitate corresponding to 33 % of wood material took place. Finally, for the water-soluble sugarcane bagasse, a large amount of precipitate was formed already at temperatures as low as 60 °C.
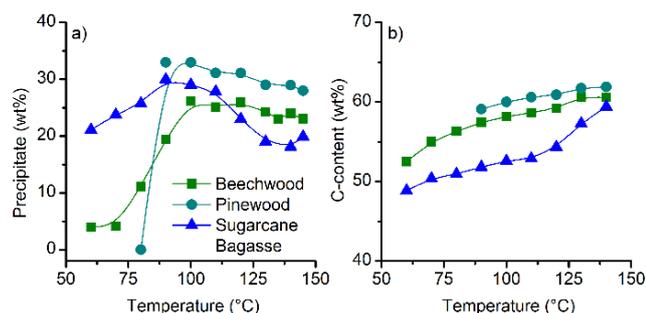


**Fig. 6.** a) Weight yield of precipitate formed by saccharification of 'water-soluble lignocelluloses' at different temperatures. b) C-content of the precipitates versus saccharification temperature.

In all cases, the amount of precipitate reached a peak at temperatures between 90 and 100 °C. In this temperature range, a substantial fraction of xylans already undergoes hydrolysis, releasing *ca.* 15-20 % xylose content into the solution. In turn, the glucan fraction remains almost unconverted, as revealed by the glucose yields not exceeding 5 %.

To assess the evolution of the composition of the lignin precipitates with the saccharification performed at varying temperatures, the C-content of the precipitate was determined by elemental analysis. Fig. 6b plots the carbon content of the precipitates as a function of saccharification temperature. The C-content of the precipitates increases with the saccharification temperature. It is evident that, by increasing the severity of the saccharification, the remaining linkages between lignin and a residual fraction of carbohydrates are hydrolyzed. At about 140 °C, the substrates have C-contents close to that found for the corresponding lignins isolated by the organosolv methodology (Table 2).

These observations suggest a plausible hypothesis about the nature of the aggregates present in the 'water-soluble lignocellulose'. Considering that the lignin fragments are covalently linked to highly hydrophilic hemicellulose fragments, full solubilization of the processed lignocellulosic substrates in water should be achieved when both lignin and the carbohydrate fraction are sufficiently depolymerized. Apparently, the partial depolymerization of the xylan

oligosaccharides should already be sufficient to destabilize the lignin fragments in solution, leading to lignin precipitation.

This proposition agrees very well with the fact that both hemicellulose and lignin are in the same environment in the plant cell wall, surrounding quite similarly the cellulosic core. Mass transport of these polymers is probably an inefficient process in the initial structure of the lignocellulosic matrix. Hence, lignin fragments formed by the cleavage of the α-O-4 and β-O-4 may react with the vicinal sugars from hemicellulose, due to their close proximity to each other. In contrast, the formation of a covalent linkage of lignin fragments to glucan fragments should rarely occur, as the cellulosic fibers constitute the core of the lignocellulose composite. Hence, the cellulosic fibers should come closer to the reactive lignin sites only after the disruption of the lignocellulosic matrix by the mechanical forces. At this point, however, a large proportion of the reactive sites of lignin should have already combined with themselves or hemicellulose fragments, thus making the formation of linkages between lignin and cellulose-derived fragments more difficult.

## Conclusion

We demonstrated 'water-soluble lignocelluloses' as suitable feedstock for the production of monosaccharides and sulfur-free lignins. The current methodology is suitable for different types of biomass, that is, softwood (*e.g.* pinewood), hardwood (*e.g.* softwood) and a perennial grass (*e.g.* sugarcane bagasse). This observation is of importance in order to meet the needs for the production of biofuels and platform chemicals from different lignocellulose residues. Remarkably, lignin is easily recovered as a sulfur-free material that closely resembles organosolv lignin. This feature could well open new horizons in lignin valorization, since the extraction of high quality lignins by the organosolv process is rather expensive.[24] The current results also indicate that the precipitation of lignin by the saccharification of the carbohydrate fraction involves complex processes (*e.g.* condensation and hydrolysis of ester linkages between lignin and the hydrophilic fragments of hemicellulose, to mention just a few). In addition, the destruction of micellar aggregates of lignin and oligosaccharides by the saccharification of hemicellulose may also be associated with the precipitation of lignin. Work is in progress to better understand the role of these specific processes in the physical chemistry of 'water-soluble wood.'

## Experimental

**General.** Sulfuric acid (95-97 %, J. T. Baker), hydrogen chloride (99.8 %, Air Liquide) and diethyl ether (99 %, Aldrich) were used as received. Pellets from sugarcane bagasse, beech (*Fagus Sylvatica*) and scots pine (*Pinus Sylvestris*) were separately comminuted with a blender. The substrate sawdust was sieved. Powders with a particle size smaller than 250 μm were collected and used for the mechanocatalytic experiments.

**Wet impregnation of the substrates with an H₂SO₄ solution in diethyl ether.** The lignocellulosic substrate (10 g) was suspended in a 0.065 mol L$^{-1}$ H₂SO₄ solution in diethyl ether (150 mL). The suspension was shaken for 1 h (IKA shaker, KS 130 control, 350 rpm). The organic solvent was removed under reduced pressure at 40 °C. A fine powder with loose particles was obtained. This procedure led to an acid-loading of 0.8 ± 0.1 mmol H₂SO₄ per gram of substrate. The powder was immediately processed in a ball mill or stored in a closed vial and kept in a freezer (-10 °C) to prevent substrate decomposition that would normally occur to form grayish to black powder after several days of storage at room temperature.[9]

**Determination of acid loading.** Typically, 1 g of the acid impregnated substrate was suspended in 40 mL water. Subsequently, titration with a 0.0100 mol L$^{-1}$ NaOH solution was performed on a Metrohm Titrino Plus 848 automated titrator.

**Mechanocatalytic depolymerization.** The mechanocatalytic depolymerization of lignocellulose was performed in a stainless steel vial (12 mL; 5 stainless steel balls of 4 g each) using a planetary ball mill (Fritsch, Pulverisette P7). The acid-impregnated substrate (1 g) was processed at 800 rpm for 2 h (beechwood) or 3 h (pinewood and sugarcane bagasse). Under working conditions, the temperature inside the mill did not exceeded 42 °C after milling for 0.5 h. In experiments of longer duration, the mill was switched off every 0.5 h for 10 min to avoid overheating and thermal decomposition of the sample. Note that the experiment duration refers exclusively to the total milling time applied to the sample. The product was then collected and kept in an air-tight vial at - 10 °C prior to analysis or saccharification experiments.

**Extraction of lignin by the organosolv process.** Lignocellulose (16-17 g) was suspended in a 140 mL solution of ethanol:water (1:1, v/v) in a 250 mL autoclave equipped with a mechanical stirrer. The suspension was processed at 180 °C for 3 h. In sequence, the mixture was left to cool down to room temperature. A reddish-brown solution was obtained after filtering off the lignocellulose fibers (pulp). Ethanol was partially evaporated at 60 °C using a rotoevaporator. This procedure leads to lignin precipitation. The solid was collected by filtration and, in sequence, resuspended in hot water in order to remove hemicellulose sugars. Next, the suspension was filtered and the solid washed several times with hot water. Finally, the organosolv lignin was dried in oven at 40 °C for 24 h.

**Determination of the water-soluble products.** The processed substrate (0.500 g) was suspended in water (25 mL) and shaken for 5 min. The suspension was centrifuged for 10 min. The residue was washed one more time with water (25 mL), centrifuged and finally dried overnight at 90 °C. The weight of the solid residue was recorded. The solubility was then determined using the difference in weight, as described elsewhere.[8]

**Saccharification of 'water-soluble lignocellulose.'** The processed substrates were dissolved in water forming a 10 % solution with a pH value of 1. In a closed glass vial, the lignocellulose solution (9 mL) was heated at the indicated temperatures for 1 h. Upon heating, a solid residue was formed, which was isolated from the sugar solution by centrifugation. The precipitate was washed with 20 mL water six times. The aqueous solutions were combined and set aside for HPLC analysis. In turn, the solid residue was dried in an oven at 60 ºC for 24 h. The solid residue was weighed and stored at -10 °C.

**Sugar and furfural quantification.** HPLC analysis was performed on a Shimadzu LC-20 equipped with a column switcher combining two organic acid resin columns (100 and 300 mm in length and 8 mm inner diameter). An aqueous solution of trifluoroacetic acid (2 mmol L$^{-1}$) was used as the eluent (1 mL min$^{-1}$). Glucose, glucose-dimers and xylose were analyzed using an RI detector; furfural and HMF, with a UV-Vis detector operating at 280 nm. The yields of glucose, cellobiose and 5-hydroxymethylfurfural are given relative to the glucan content of the unprocessed substrate; the yields of xylose and furfural are given relative to the xylan content.[8]

**Solution NMR experiments.** All spectra were acquired at 25 °C with a Bruker AV spectrometer (400 or 500 MHz $^{1}$H frequency) equipped with a BBFO probe head with $z$-gradient. Spectral widths of 20 ppm were used for the 1D $^{1}$H spectrum. The relaxation delay for 1D $^{1}$H spectrum was 5.0 s following a 30-degree excitation pulse. For 1D inverse-gated $^{13}$C spectrum, the relaxation delay was set to 1.0 s following a 30-degree excitation pulse. $^{1}$H-decoupling with the Waltz-16 sequence was applied during acquisition. The number of collected points was 64 k for $^{1}$H and for $^{13}$C. The 1D $^{1}$H spectra were processed using an exponential weighting function (lb 0.2 Hz) prior to Fourier transform. The 2D HSQC NMR (Bruker standard pulse sequence "*hsqcetgpsi*" with delay optimized for $^{1}J_{CH}$ of 145 Hz) were set up with spectral widths of 20 ppm and 180 ppm for $^{1}$H- and $^{13}$C-dimensions, respectively. The number of collected complex points was 2,048 for $^{1}$H-dimension with a recycle delay of 3.13 s (3.0 s relaxation delay and 0.13 s acquisition time). The number of transients for the HSQC spectra was between 12 and 24, and 512 time increments were recorded in $^{13}$C-dimension resulting for in an overall experiment time of 6 to 12 h. For HSQC experiments, a squared cosine-bell apodization function was applied in both dimensions, followed by zero-filling to 1,024 points in the $^{13}$C-dimension prior to Fourier transform. The 1D $^{1}$H NMR and 2D HSQC NMR spectra were processed using MestReNova 8.1.1 software.

Noteworthy, HSQC spectrum data must be interpreted with caution, since the $^{1}J_{CH}$ dependence of polarization transfer in HSQC experiments is not suppressed in regular HSQC pulse sequences.[25] As a result, the absolute intensity of cross peaks are not fully quantitative in the entire spectral range.[25-27]

Regular HSQC NMR experiments still offer extremely valuable (direct) semiquantitative information for characterization and comparison of lignins as well as whole plant cell compositions.[23, 28, 29] Semiquantitative determination of volume integral ratios is possible for $^1H$—$^{13}C$ pairs in a similar chemical environment (*e.g.* $C_\alpha$—$H_\alpha$ signals for the side-chain of lignin units or the $C_2$—$H_2$ and $C_6$—$H_6$ signals for lignin aromatic units), due to the fact that the $^1J_{CH}$ values for the specific entities are reasonably similar.[23, 28] Accordingly, for the different regions of the HSQC spectra, semiquantitative analysis was performed separately by integration of $^1H$—$^{13}C$ pairs of interest.[23, 28]

**FTIR analysis.** The FTIR spectra were collected on a Bruker Vertex 70 spectrometer using a Zn-Se ATR probe. For each spectrum, 128 scans were recorded at 4 cm$^{-1}$ resolution.

**Elemental analysis.** The CHNS/O elemental analysis was performed on triplicates for each sample (2 mg) on a Vario Micro cube elemental analyzer.

## Acknowledgments

## Notes and references

$^a$ Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, 45470 – Mülheim an der Ruhr, Germany. *Emails*: rinaldi@kofo.mpg.de; schueth@kofo.mpg.de

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## Graphical Abstract
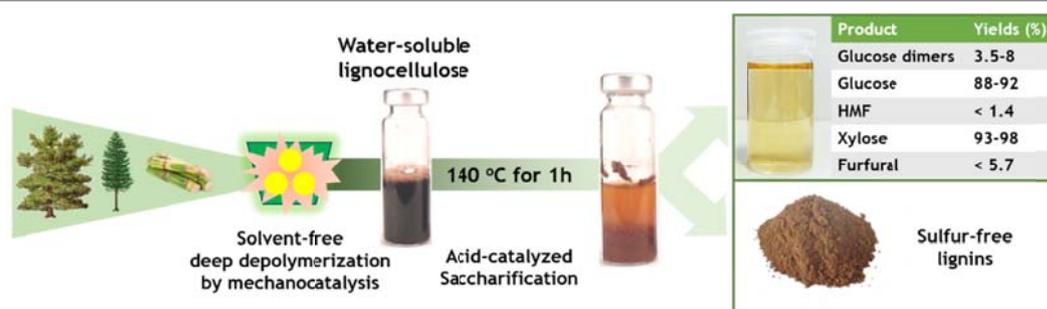


Lignocellulose undergoes deep depolymerization by a mechanocatalysis, quantitatively forming 'water-soluble lignocellulose.' The saccharification of the aqueous 'wood solution' at 140 °C for 1 h forms $C_5$ and $C_6$ sugars at high yields in addition to sulfur-free lignins.

## Broader Context

There is a substantial need for processes able to convert the whole plant biomass into $C_5$ and $C_6$ sugars and provide technical sulfur-free lignins suitable for subsequent catalytic valorization to platform chemicals and biofuels. In this context, the mechanocatalytic depolymerization of lignocellulosic substrates is a powerful methodology that fully converts lignocellulosic substrates into 'water-soluble lignocellulose.' Herein, we demonstrate that the saccharification of the aqueous solution of depolymerized beechwood, pinewood or sugarcane bagasse (at 140 °C for 1 h) produces a high yield of $C_5$ and $C_6$ sugars and leads to precipitation of sulfur-free lignins that are similar to those obtained by the organosolv process.

# Electronic Supplementary Information

# Fractionation of 'water-soluble lignocellulose' into monosaccharides and sulfur-free lignins under low-severity conditions

Mats Käldström, Niklas Meine, Christophe Farès, Roberto Rinaldi* and Ferdi Schüth*

Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, D-45470 - Germany

Table S1. Composition of the lignocellulosic substrates prior to impregnation with $H_2SO_4$.

| Substrate | Composition (wt. %) | | | | | |
|---|---|---|---|---|---|---|
| | Glucans | Xylans | Lignin | Humidity | Ash | Others |
| Beechwood (hardwood) | 41 | 21 | 24 | 5 | 0.4 | 8.4 |
| Pinewood (softwood) | 43 | 18 | 27 | 5 | 0.2 | 6.8 |
| Sugarcane bagasse | 41 | 19 | 20 | 7 | 2 | 11 |