

# Green Chemistry

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



[www.rsc.org/greenchem](http://www.rsc.org/greenchem)



## Application of mild autohydrolysis to facilitate the dissolution of wood chips in direct-dissolution solvents

Somdatta Deb,<sup>a</sup> Sara R. Labafzadeh,<sup>a</sup> Unna Liimatainen,<sup>a</sup> Arno Parviainen,<sup>a</sup> Lauri K. J. Hauru,<sup>b</sup> Shoaib Ahzar,<sup>c</sup> Martin Lawoko,<sup>c</sup> Tuomas Kulomaa,<sup>a</sup> Tia Kakko,<sup>a</sup> Juha Fiskari,<sup>a</sup> Marc Borrega,<sup>b</sup> Herbert Sixta,<sup>b</sup> Ilkka Kilpeläinen,<sup>a\*</sup> and Alistair W. T. King<sup>a\*</sup>

Received 00th January 20xx,  
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Wood is not fully soluble in current non-derivatising direct-dissolution solvents, contrary to the many reports in the literature quoting wood 'dissolution' in ionic liquids. Herein, we demonstrate that the application of autohydrolysis, as a green and economical wood pre-treatment method, allows for a massive increase in solubility compared to untreated wood. This is demonstrated by the application two derivitising methods (phosphitylation and acetylation), followed by NMR analysis, in the cellulose-dissolving ionic liquids 1-allyl-3-methylimidazolium chloride ([amim]Cl) and 1,5-diazabicyclo[4.3.0]non-5-enium acetate ([DBNH][OAc]). In addition, the non-derivitising tetrabutylphosphonium acetate ([P<sub>4444</sub>][OAc]):DMSO-d<sub>6</sub> electrolyte also allowed for dissolution of the autohydrolysed wood samples. By combination of different particle sizes and P-Factors (autohydrolysis intensity), it has been clearly demonstrated that the solubility of even wood chips can be drastically increased by application of autohydrolysis. The physicochemical factors affecting wood solubility after autohydrolysis are also discussed

### Introduction

Wood is often commented to be the most abundant renewable feedstock for future production of materials, chemicals and energy. Wood is known to be 'recalcitrant' towards chemical conversion but the well-documented cellulose dissolution capability of basic ionic liquids<sup>1-4</sup> and electrolytes<sup>5-7</sup> is commonly used to justify the ability to dissolve wood in similar solvents. Kilpeläinen *et al.*<sup>8</sup> showed that wood can be dissolved and chemically modified from chloride ionic liquids, such as, [amim]Cl but the degree of degradation caused during this dissolution and modification steps were not apparent. At least a highly acetylated and chloroform-soluble polymeric material was identified by NMR analysis. However, Kyllönen *et al.*<sup>9</sup> has subsequently shown that wood is simply not soluble in non-derivatising direct-dissolution solvents, such as ionic liquids, without some kind of mechanical or chemical pre-treatment. It is suggested, that this is in part due to lignin-carbohydrate complexes (LCCs).<sup>10-13</sup>

These are often regarded to be covalent linkages between mainly hemicelluloses and lignin and render the LC matrix resistant to dissolution. However, the presence of LCCs, as covalent linkages, in native (untreated) wood is disputed.

In any case, in order to maintain the molecular weight (MW) of the constituent polymers during homogenisation, methods for selective deconstruction of the wood matrix are required. This would allow for the application of these high MW polymers in a wide range of potential materials applications, i.e. preparation of synthetic regenerated fibres, films, thermoplastics, compositing and formation of polymeric blends, before or after further chemical modification.

Previously Hauru *et al.*<sup>14</sup> showed that a better solubilisation of wood and more efficient fractionation was possible after an initial autohydrolysis pre-treatment, followed by ionic liquid fractionation, compared to the untreated birch wood. This was demonstrated for particle sizes similar to sawdust-size. Autohydrolysis is the treatment of wood under hydrothermal conditions, by which some components of wood are extracted. Acetic acid released during initial hydrolysis, of acetate groups mainly on hemicelluloses, further catalyse hydrolysis and release of the wood components.<sup>15,16</sup> There are several synonyms or related processes, such as, hot water extraction, pressurised hot water extraction, steam pre-treatment, steam explosion, etc. All result in extraction of hemicelluloses and some lignin, to different degrees. Autohydrolysis is also implemented in industry, as a pre-treatment prior to kraft pulping, to produce dissolving-grade pulp (high purity and molecular weight cellulose designed for polymeric chemical or textile uses). The resulting pulp is typically called pre-

<sup>a</sup> Laboratory of Organic Chemistry, Department of Chemistry, University of Helsinki A.I. Virtasen aukio 1 (Chemicum), P.O. Box 55, 00014 University of Helsinki, Finland. E-mail: [alistair.king@helsinki.fi](mailto:alistair.king@helsinki.fi), [ilkka.kilpelainen@helsinki.fi](mailto:ilkka.kilpelainen@helsinki.fi); Tel: +358505279446

<sup>b</sup> Department of Forest Products Technology, School of Chemical Technology, Aalto University, Finland.

<sup>c</sup> Wallenberg Wood Science Center (WWSC), Department of Fiber and Polymer Technology, Royal Institute of Technology (KTH), Stockholm, Sweden.

† Footnotes relating to the title and/or authors should appear here.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

hydrolysis kraft (PHK) pulp. As a stand-alone process there are several advantages to the use of autohydrolysis, i) The process is relatively 'green' as water is used, ii) Hemicelluloses may be recovered and converted to a wide range of useful chemicals, such as, xylitol, prebiotics, antioxidants, pharmaceuticals and cosmetics ingredients, etc.<sup>17</sup> Most PHK mills however simply combust the extract for its calorific value. iii) Solid wood residue enriched in cellulose and lignin can be further utilised in alternative applications, such as in biocomposites.<sup>17</sup>

As, the aim of this study is to assess the effect of autohydrolysis on the solubility of wood in non-derivatising direct-dissolution solvents, 3 experimental elements were combined: 1) preparation of wood samples of different particle sizes to assess how wider particle size ranges affect solubility, 2) autohydrolysis of wood samples to varying degrees to assess how the intensity of autohydrolysis affects solubility and 3) correct choice of analytical procedures to quantify wood solubility. This last point was satisfied by using ionic liquids to dissolve/swell the wood samples, prior to NMR analysis. Two of these methods also involved chemical derivatisation and one did not, to demonstrate that dissolution was not an artefact of the chemical modification steps. Therefore, the goals of the present manuscript concern the investigation of autohydrolysis, as a sustainable wood pre-treatment method, to allow for much more thorough dissolution and chemical modification of wood chips, as the most suitable low-cost feedstock for bulk industrial application.

## Experimental

Various wood samples of different particle sizes were prepared by combinations of Wiley milling, sieving and planetary milling steps (Figure 1).

### Wood Samples: 'Sawdusts'.

Silver birch (*Betula pendula*) chips and autohydrolysed birch chips were Wiley milled to pass a 22-mesh (1 mm) sieve.

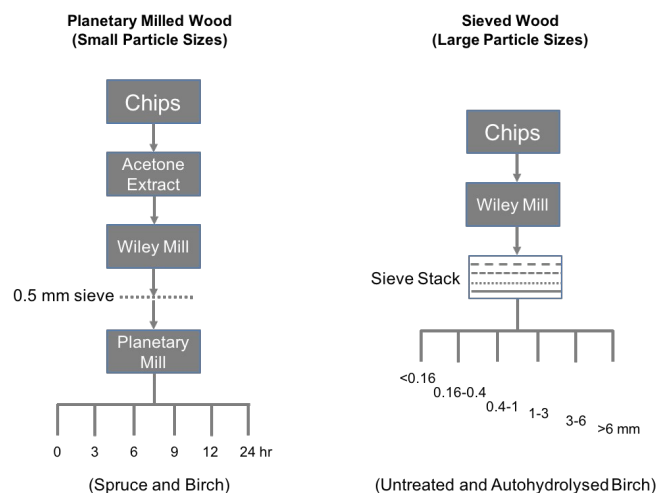


Figure 1: Methods for production of the particle-sizes used in this study.

### Wood Samples: Planetary Milled Wood.

Soxhlet-extracted (acetone, 24 hr) Norway spruce (*Picea abies*) and birch wood chips were Wiley milled to pass a 40-mesh (0.5 mm) screen. These samples were then planetary milled using a PM400 Retsch Planetary Ball Mill, to the required degree (Figure 1). The following conditions were used: 36 g of extractive-free wood samples were planetary ball milled at 3000 rpm from 0 - 24 hr.

### Wood Samples: Larger Particle Size-Ranges.

Starting unextracted wood chips and autohydrolysed wood chips were Wiley milled without sieving to collect the bulk effluent (Figure 1). This was then sized using a sieve stack (< 0.16 mm, 0.16-0.4 mm, 0.4-1.0 mm, 1-3 mm, 3-6 mm and > 6 mm, Figure 2).

### Wood Samples: Autohydrolysed Wood Chips.

Autohydrolysed birch wood was prepared by extracting wood chips with hot water at various intensities (by varying the extraction temperature and isothermal extraction time), according to a previous publication by Borrega *et al.*<sup>18</sup> The intensity of hot water extractions are typically described using the P-Factor, in units of hours.<sup>19</sup> This is a measure of cumulative conversion of hemicellulose (based on hydrolysis kinetics) under given temperature conditions and over time. It is derived by the time-dependent integration of the rate constant from a modified Arrhenius equation, using one activation energy for xylan hydrolysis (125.6 kJ/mol<sup>19</sup>). In the previous publication by Borrega *et al.*,<sup>18</sup> pressurised hot water was re-circulated through the chip bed (1 kg of chips, 3 kg of deionised water) in a batch reactor (10 L). After the treatment, the water extract was drained and the wood residue was recovered and stored in a freezer. These were then used in the present publication after the various mechanical treatments and sieving steps. The conditions used to prepare the samples analysed in this study are presented in Table 1 and are based on the previous publication by Borrega *et al.*<sup>18</sup>

### <sup>31</sup>P NMR Procedure.

Preparation of 1-allyl-3-methylimidazolium chloride ([amim]Cl) and the <sup>31</sup>P NMR analysis was performed according to a previous publication by Kyllönen *et al.* (ESI)<sup>9</sup> Briefly, wood samples were heated at various times and temperatures in dry [amim]Cl. This allows for significant swelling of the biomass and complete dissolution in some cases. Hydroxyl groups were then functionalized as phosphite esters (Figure 3) and the whole spectral region (151.5-133 ppm) was then integrated against the internal standard to give a value of 'available hydroxyls' (mmol/g). This is essentially a measure of the solubility of the wood samples as those hydroxyl groups that are still in the solid and gel state are not visible using this solution-state NMR technique. For the > 3 mm particle size range the particles may have required splitting to fit into an 8 ml sample vial for the derivatisation reaction.



Figure 2: Large particle sizes produced during Wiley milling and sieving.

Table 1. Parameters for autohydrolysis of the birch chips.<sup>18</sup>

Sample <sup>a</sup>	Reactor Set Temp. (°C)	Isothermal Reaction Time (min) <sup>a</sup>	Reactor Heat-Up Time (min)	P-Factor (hr)
180:0	180	0	18	51
200:0	200	0	21	167
180:10	180	10	27	262
180:30	180	30	47	766
200:10	200	10	29	1057
220:2	220	2	25	1322
200:30	200	30	49	3600
220:25	220	25	47	8166

<sup>a</sup>This is the reaction time at the set temperature, after the reactor heat up time.

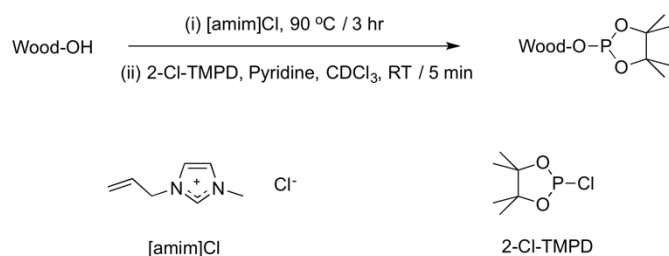


Figure 3: <sup>31</sup>P NMR analysis procedure for quantifying wood hydroxyls.

### Ionic Liquid Acetylation and NMR Analysis.

Acetylation was performed by dissolving the wood samples in 1,5-diazabicyclo[4.3.0]non-5-enium acetate ([DBNH][OAc]) followed by acetylation with isopropenyl acetate (Figure 4). [DBNH][OAc] was prepared according to a previous publication by Parviainen *et al.* (1:1 addition of DBN and acetic acid in the absence of solvent).<sup>3</sup> Freshly prepared [DBNH][OAc] (1.90 g) was then added to the wood samples (100 mg) in a screw-top glass vial and heated, with stirring at 130 °C, for 1 hr under argon. The mixture was allowed to cool to 80 °C and isopropenyl acetate (670 μl) was added. The reaction mixture was then stirred vigorously with a spatula and after few minutes the vial was left in an oil bath at 80 °C for 30 min. Water (50 ml) was added and the mixture was refluxed for 1 hr to remove traces of the ionic liquid. The product was filtered and dried under vacuum. All samples had changed from the original dark brown to a light brown colour. The product was extracted with chloroform (20 ml) with gentle heating and filtered through a G3 glass sinter. Chloroform was removed by rotary evaporation. The dried filtrate and the undissolved material were weighed and yields were determined.

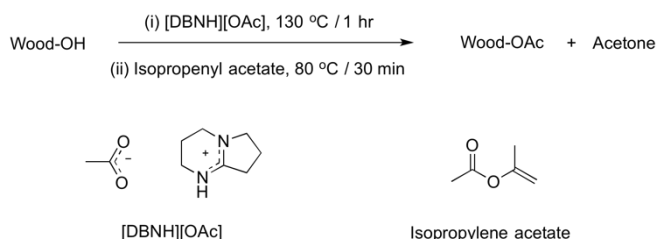


Figure 4: Mild acetylation procedure applied to the acetylation of autohydrolysed birch samples.

The ATR-IR and <sup>1</sup>H NMR spectra (chloroform-d<sub>1</sub>) were recorded for all samples. DEPT-edited HSQC were also recorded for some samples.

### Ionic Liquid Electrolyte Dissolution and NMR Analysis

<sup>1</sup>H NMR analysis of dissolved wood was possible, under non-derivatising conditions, through the use of tetrabutylphosphonium acetate ([P<sub>4444</sub>][OAc], Figure 5) as the DMSO-d<sub>6</sub> electrolyte. [P<sub>4444</sub>][OAc]:DMSO-d<sub>6</sub> stock solution (200 μl, 50:50 wt%) was added to the wood samples (33.8 mg) in an 8 ml sealable vial. Further DMSO-d<sub>6</sub> (400 μl) was added and the samples were heated, with stirring, for 3 hr at 100 °C. The solutions (600 μl) were transferred to a 5 mm NMR tube while hot, via needle and syringe. <sup>1</sup>H NMR spectra (at 65 °C) were recorded for all. [P<sub>4444</sub>][OAc] was prepared according to a previous publication by Holding *et al.* (ESI)<sup>4</sup>

## Results and Discussion

### Solubility of Unextracted Wood (Low Particle Size-Range)

Both spruce and birch were planetary milled from the sawdust and subjected to the <sup>31</sup>P NMR analysis conditions. The results show that only after extended milling times are the samples fully soluble (Figure 6), with ~ 12 mmol/g representing the total number of hydroxyl groups in wood. This is consistent with what was published previously for Norway spruce by Kyllönen *et al.*<sup>9</sup> The results show that for both hard and softwood species mechanical degradation is required to reach full solubility under mild dissolution conditions (90 °C, 3 hr) in [amim]Cl. The solubility was also investigated as a function of temperature and time (Figure 7). If the samples are heated at 100 °C or below over an extended period there is no increase in the available hydroxyls as a function of time.

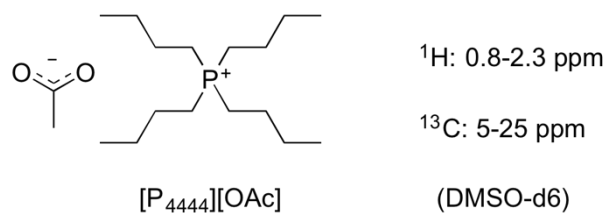
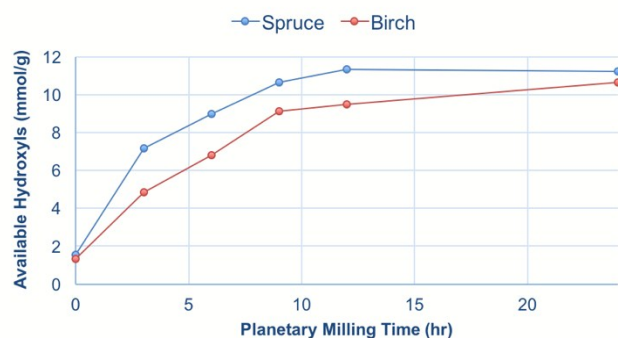
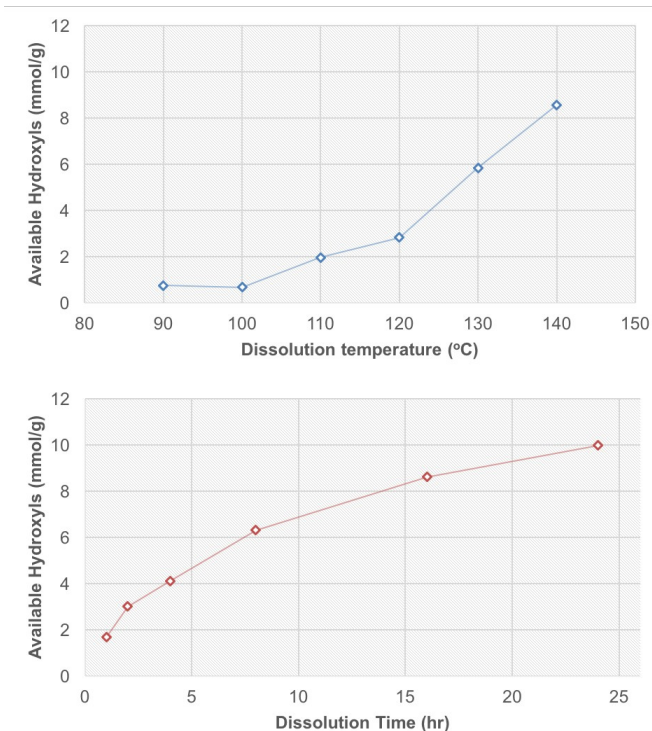


Figure 5: Structure of [P<sub>4444</sub>][OAc], used for NMR analysis of wood samples as the DMSO-d<sub>6</sub> electrolyte, under non-derivatising conditions.



**Figure 6:** Increase in solubility of spruce and birch after extended planetary milling, as determined by [amim]Cl dissolution and  $^{31}\text{P}$  NMR analysis.<sup>9</sup> Solubility is represented by the 'available hydroxyls' corresponding to the number of measurable phosphite esters.



**Figure 7:** Solubility of birch wood sawdust after heating in [amim]Cl at different temperatures over 3 hr heating time (top) and at 120 °C for extended heating times (bottom).

However, if the pre-dissolution temperature is increased from 90 °C to 140 °C, for a dissolution time of 3 hr the solubility increases. If the dissolution time at 120 °C is increased from 1-24 hr the solubility also increases. As this increase in solubility is not observed at < 100 °C this is likely due to degradation occurring at the elevated temperature.

This is quite consistent with early reports of depolymerisation of cellulose or lignin in chloride ionic liquids<sup>20,21</sup> and confirms the need for lower temperatures (100 °C or lower) to preserve polymer molecular weights. This is particularly relevant for analytical procedures using [amim]Cl

and other chloride anion analogues. Other analogues, in particular containing the acetate anion, are also shown to be reactive towards cellulose. This topic has been thoroughly discussed in previous reports.<sup>22,23</sup>

### Solubility of Autohydrolysed Birch (Sawdust)

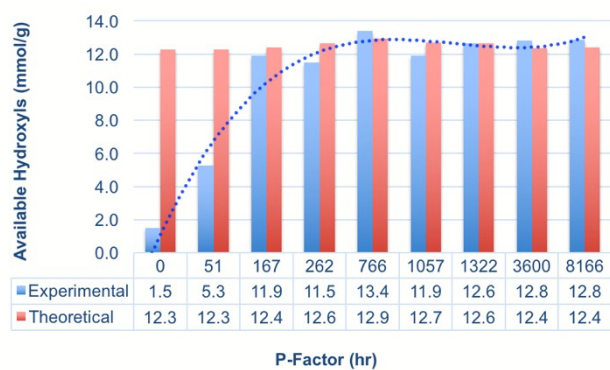
To measure the effect of autohydrolysis intensity on wood solubility, autohydrolysis was then applied to birch chips according to Borrega *et al.* (Table 1)<sup>18</sup> The chips were then Wiley milled to pass a 1 mm sieve (sawdust size) and were then subjected to  $^{31}\text{P}$  NMR analysis (Figure 8). For comparison, the theoretical OH content was also calculated based on the chemical composition of the different biomass samples,<sup>18</sup> before and after autohydrolysis (cellulose content, xylan content, lignin content and acetyl content). The values were determined using equations 1 and 2:

$$\text{OH}_{(\text{Th})} = \text{OH}_{(\text{Cell})} + \text{OH}_{(\text{Xyl})} + \text{OH}_{(\text{Lig})} - \text{OH}_{(\text{Ac})} \quad (1)$$

$$\text{OH}_{(\text{X})} = \frac{\text{XOH} * \%_{(\text{X})}}{100} \quad (2)$$

where  $\text{OH}_{(\text{X})}$  (mmol/g) are the different theoretical hydroxyl contributions to equation 1,  $\%_{(\text{X})}$  are the different mass percentages of those species in the wood samples and XOH (mmol/g) are the available hydroxyls for the different species:  $\text{OH}_{(\text{Th})}$  is the theoretical hydroxyl content of the whole biomass,  $\text{OH}_{(\text{Cell})}$  is the theoretical cellulose hydroxyl content,  $\text{OH}_{(\text{Xyl})}$  is the theoretical xylan hydroxyl content without acetyl functionalities and  $\text{OH}_{(\text{Ac})}$  is the theoretical hydroxyl content consumed by residual acetate groups. In the second equation, CelloH (18.5 mmol/g) is the available hydroxyls per unit mass of cellulose (3/162). XyloH is the available hydroxyls per unit mass of xylan (14.2 mmol/g) and was determined by King *et al.*<sup>24</sup> based on data published by Timell concerning the sugar composition of xylan.<sup>25</sup> LigOH is the available hydroxyls per unit mass of lignin (ESI) and are mostly predicted values based on  $^{31}\text{P}$  NMR analysis of milled wood lignin, after autohydrolysis of birch wood at increasing P-Factors.<sup>26</sup> Only lower P-Factors were analysed but LigOH values for higher P-Factors are estimated based on the fact that higher intensity treatments cause significant condensation of lignin. AcOH is the unavailable hydroxyls per unit mass of acetyl (23.3 mmol/g) incorporated into the biomass, as acetate esters.  $\%(\text{Cell})$  is the percentage by mass of cellulose in the samples,  $\%(\text{Xyl})$  is the percentage by mass of xylan in the samples,  $\%(\text{Lig})$  is the percentage by mass of lignin in the samples (based on klason and acid-soluble lignin),  $\%(\text{Ac})$  is the percentage by mass of acetyl species in the biomass samples. All values are given in the ESI.

The results in Figure 8 clearly show that application of a P-Factor close to 200 hr allows for practically complete solubilisation of the sawdust samples, as opposed to almost completely insoluble samples for the 0 hr P-Factor (untreated samples). P-Factors of around 800-1000 hr are typically those which are applied during pre-hydrolysis for PHK pulp

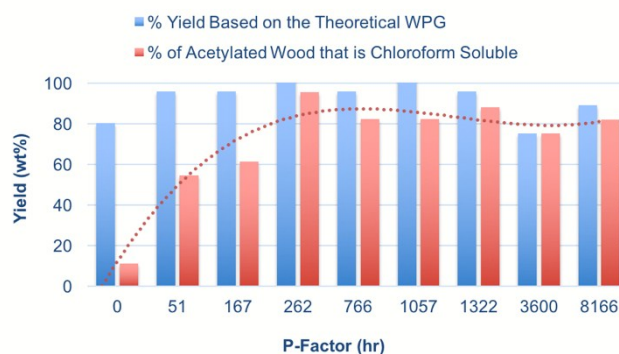


**Figure 8:** Solubility (available OH) of autohydrolysed sawdust samples, with increasing P-Factor, compared to the theoretical available OH values from sugar, lignin and acetyl analyses<sup>18</sup>.

production. Clearly mild autohydrolysis is a good economic substitute for costly planetary milling when one wishes to achieve a more complete chemical modification of wood.

#### Dissolution vs 'Reactive Dissolution'

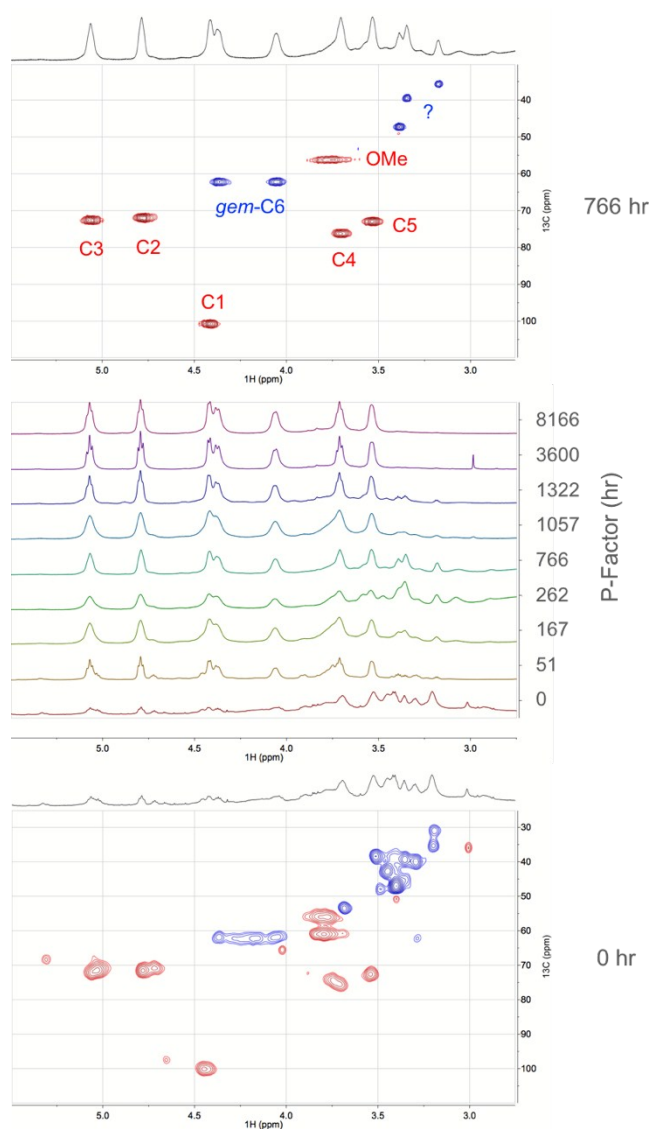
The reagent used in the <sup>31</sup>P NMR phosphitylation procedure is rather reactive and some degree of 'reactive dissolution' may be expected. Reactive dissolution has been observed previously for cellulose modification where cellulose can be dissolved, in the absence of direct-dissolution solvents, particularly using acid chlorides as acylation reagents.<sup>27,28</sup> However, in this context we wish also to avoid other possible degradation effects produced by the high reactivity or the phosphitylation reagent. Therefore, two additional mild procedures were tested: 1) acetylation in [DBNH][OAc] with isopropenyl acetate, and 2) direct-dissolution into [P<sub>4444</sub>][OAc]:DMSO-d<sub>6</sub>. NMR analysis was performed on the acetylated materials after extraction with chloroform and also on the biomass dissolved in the [P<sub>4444</sub>][OAc]:DMSO-d<sub>6</sub> electrolyte solutions. [DBNH][OAc] has been used previously for the acetylation of hemicelluloses, with acetic anhydride as reagent.<sup>29</sup> An even milder procedure has however been developed using the lower reactivity acetate ester, isopropenyl acetate (Figure 9).<sup>30</sup> Acetylation using these reagents is expected to avoid any issues with reactive dissolution. The sawdust samples (5 wt%) were first dissolved over 1 hr at 130 °C to pre-swell the biomass. Some degradation is expected under these conditions but is not expected to be significant due to the shorter heating times and degradation seems to occur to a much lesser extent in [DBNH][OAc], compared to chloride ionic liquids. However, no accurate study has been done on this to date. Isopropenyl acetate was then introduced and the reaction quenched. The filtered and dried products were then extracted with chloroform to give an additional measure of the solubility of the samples. The yields after acetylation and chloroform extraction are given in Figure 9. Here we can clearly see that there is a dramatic increase in the chloroform solubility of the acetylated autohydrolysed samples, compared to the untreated samples. The weight percent gains (WPGs) compared to the theoretical WPGs



**Figure 9:** Yields after [DBNH][OAc] dissolution, acetylation (Figure 5) and solubilisation of autohydrolysed sawdust samples into chloroform.

are all above 75 %, even for the untreated sample. This nicely corresponds with the ATR-IR analysis (ESI) showing high degrees of acetylation for all samples. At intermediate P-Factors the WPGs are almost 100 % of the theoretical WPGs indicating more thorough acetylation. At higher P-Factors (>3000 hr) the yields slightly decrease again indicating that further degradation of the samples may reduce yields, after aqueous precipitation. It is common that low molecular weight samples may not fully precipitate upon addition of non-solvent. However, as the samples were only acetylated on a 100 mg scale, there may also be error due to unavoidable losses during filtration and transfer of samples. Above a P-Factor of around 260 hr the chloroform-soluble portion of the recovered acetylated material reaches 93 %, compared to 14 % for the untreated wood sample. As with the <sup>31</sup>P NMR analysis this indicates a very significant increase in solubility of wood after an autohydrolysis P-Factor of around 200 hr. All samples after extraction with chloroform and drying in a rotary evaporator are film-forming (ESI). For direct comparison of the [amim]Cl-<sup>31</sup>P NMR results with the [DBNH][OAc]-acetylation results, the <sup>31</sup>P NMR results can be plotted against the acetylation results (ESI). While some error exists, clearly there is a strong correlation between the two analytical procedures, indicating that reactive dissolution during the phosphitylation procedure does not have a significant effect when dissolving the minimally treated (autohydrolysed) samples. However, the yields from acetylation are not close to 100 %, as in the case of the <sup>31</sup>P NMR procedure indicating that there is some effect. Importantly the increase in solubility on going from untreated up to a P-Factor of around 200 hr is significant using both procedures. NMR analysis was performed on the acetylated materials (Figure 10). This included <sup>1</sup>H NMR on all acetylated samples and DEPT-edited HSQC on the untreated sample (0 hr P-Factor) and the sample autohydrolysed to a P-Factor of 766 hr. From Figure 10 we can see that as P-Factor increases the cellulose in the samples (<sup>1</sup>H: C3 – 5.07 ppm, C2 – 4.80 ppm, C1 – 4.42 ppm, *gem*-C6 – 4.38 & 4.06 ppm, C4 – 3.71 ppm, C5 – 3.54 ppm) clearly becomes soluble. A relatively significant peak which may correspond to the lignin methoxy (<sup>1</sup>H/<sup>13</sup>C: 3.76/56.25 ppm) is present. Some unidentified peaks upfield of the cellulose backbone (<sup>1</sup>H: 3.5 ppm) are also present. At this

point in the 766 hr P-Factor sample these are thought to be lignin resonances which are possibly methylene species ( $\text{CH}_2$ ), alpha to both ketone and oxygen. This is however speculative without much more detailed analyses. For the untreated sample (0 hr P-Factor) there are more unknown resonances that may be related to extractives, saccharides and lignin. While there are peaks that roughly correspond to acetylated cellulose, these are rather weak, relative to the unknown resonances, indicating minimal solubilisation of cellulose during the acetylation and chloroform extraction. There are also several unidentified saccharide resonances, consistent with solubilisation of mainly the lower molecular weight fractions and not the cellulose.



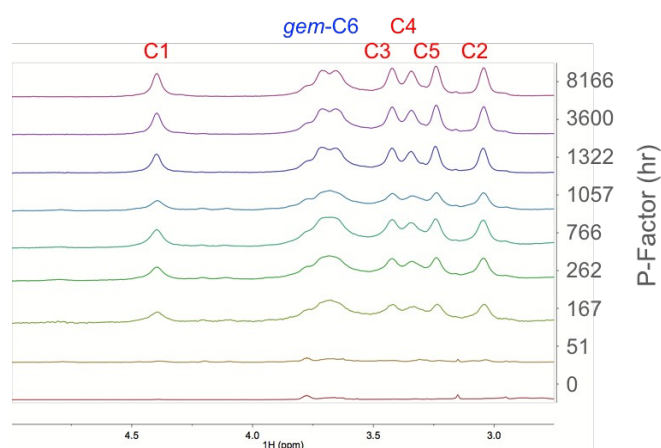
**Figure 10:**  $^1\text{H}$  NMR saccharide backbone region for the  $[\text{DBNH}][\text{OAc}]$ -Acetylated  $\text{CDCl}_3$ -soluble fractions for the different P-Factors of autohydrolysed birch wood (middle). The 2D spectra are DEPT-edited ( $\text{CH}$  &  $\text{CH}_3$  in red,  $\text{CH}_2$  in blue) HSQC spectra (above and below). Assignments in the 766 hr P-Factor HSQC are for the cellulose backbone peaks.

### Dissolution under 'Non-derivatising' Conditions

As a further demonstration of how reactive dissolution does not effect solubility at lower P-Factors, the samples were dissolved into the ionic liquid electrolyte  $[\text{P}_{4444}][\text{OAc}]:\text{DMSO-d}_6$ . Similar electrolytes have been used for the analysis of high molecular weight pulp samples, containing mainly cellulose.<sup>31,4</sup> All components of wood are soluble in these electrolytes, as demonstrated previously by Holding *et al.*<sup>4</sup> Therefore, if it is truly possible to solubilize autohydrolysed wood, this media should allow for this. 5 wt % samples were dissolved into the electrolyte solutions and  $^1\text{H}$  NMR analysis was performed (Figure 11). Clearly for the extended P-Factors the cellulose in the samples is dissolving, indicating a much more complete solubilisation of the samples. For the 0 and 51 hr P-Factors little cellulose is visible indicating poor solubility of the whole biomass sample. This is entirely consistent with the  $^{31}\text{P}$  and acetylation results.

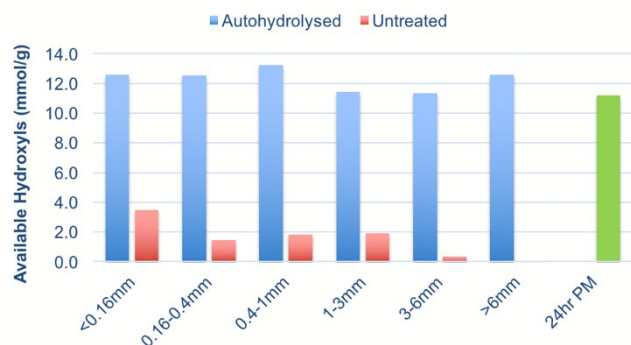
### Solubility of Autohydrolysed Birch (Chips)

From a technoeconomic standpoint, sawdust is not a suitable feedstock to use for bulk application. While it is readily available as a low-cost by-product from saw mills, it is only available on a limited scale industrially. Industrial chips are much more common and much lower cost compared to the additional milling steps required to reach lower particle sizes, such as sawdust. As such, we wished to investigate larger particle-size ranges to see if they could also be solubilised after autohydrolysis. To achieve this the starting untreated and autohydrolysed wood chips (1057 hr P-Factor) were Wiley milled and sieved to give different particle size ranges (Figure 2). These were then subjected to the  $[\text{amim}]\text{Cl}$  dissolution, phosphite ester derivatisation and  $^{31}\text{P}$  NMR analysis procedure



**Figure 11:**  $^1\text{H}$  NMR saccharide backbone region for the different P-Factors of autohydrolysed birch wood dissolved in  $[\text{P}_{4444}][\text{OAc}]:\text{DMSO-d}_6$ . The concentrations are the same for all spectra and normalised against the  $[\text{P}_{4444}][\text{OAc}]$  signals, i.e. solubility is represented by cellulose backbone signal intensity. The assignments are for the different cellulose backbone  $^1\text{H}$  signals, according to previous articles by Holding *et al.*<sup>31,4</sup>

(Figure 12). From the results presented in Figure 12 it is clear that decreasing particle size slightly improves solubility of untreated chips. However, after autohydrolysis to a P-Factor of 1057 hr all particle size ranges are equally soluble. Some error exists but the results clearly show that autohydrolysis can be used to drastically enhance the solubility of wood chips over the untreated chips, with the untreated chips being practically insoluble.



**Figure 12:** Solubility (available OH) of increasing particle size ranges, up to chip-size, after autohydrolysis of the birch wood chips at a P-Factor of 1057 hr. 24 hr PM refers to the experimental value for the untreated birch sample which was fully soluble, after 24 hr of planetary milling.

### Mechanism Causing Increased Solubility

In Kyllönen *et al.*<sup>9</sup> it was argued that the insolubility of wood was due to the recalcitrant 'lignin-carbohydrate matrix (LCM)'. LCCs are of course implicated in this but it was not specifically argued that these were actual branching covalent linkages, between polysaccharide and lignin. Extensive covalent LCCs would bind the wood matrix components to rapidly approach a molecular weight equivalent of Avogadro's number, for that molar quantity of material (much too high MW to be soluble). As mentioned in the previous article the scepticism about the occurrence of covalent LCCs in native (unprocessed) woods is mainly due to the inability to isolate LCC fractions for high resolution analyses, without the need for chemically or mechanically harsh treatments. This is the Schrödinger's cat of wood chemistry. As a consequence, we would like to present several options for discussion concerning the solubility of autohydrolysed wood:

- 1) Increased Porosity – It has been known for some time that during autohydrolysis of wood, hemicelluloses and lignin are extracted. An article by Smiljanski and Stanković<sup>32</sup> have shown that there is an increase in sub-microscopic porosity of beech wood (*Fagus moesica*) as glucuronoxylans are extracted during pre-hydrolysis. This increase in porosity would clearly allow for much more rapid penetration of the wood matrix with reagents.
- 2) Increased Fibrillation – After autohydrolysis the wood chips become much softer and easier to break apart, parallel with the fibre orientation. In addition, the chips fibrillate quite rapidly in ionic liquid ([amim]Cl and [DBNH][OAc]) as opposed

to unextracted chips, which essentially do not fibrillate at all. This effect clearly also rapidly increases surface area, similar to increased porosity. This is mainly a result of removal of acid-soluble lignin and pectins from the middle lamella, which acts as a cement keeping the adjacent fibres together. It is not clear if this, combined with removal of hemicellulose (binder), is enough to allow for complete dissolution of the remaining wood matrix.

3) LCC Breakage – Autohydrolysis, by definition, is an acid-catalysed process for degradation and extraction of hemicelluloses. Therefore, acid-labile linkages are expected to be degraded during this process. The proposed LCC structures (benzyl ethers, benzyl esters and phenyl glycosides)<sup>10-13</sup> will have variable stabilities under pH conditions. If they are actually present in native wood then particular types are expected to be cleaved, potentially diminishing the overall molecular weight of the LCM low enough to be rendered into solution.

4) Lignin Condensation – At higher P-Factors lignin starts to condense.<sup>26</sup> This may have the opposite effect of LCC breakage, i.e. leading to reduced solubility of the matrix, if the condensed fragments become large enough in size. Therefore, keeping to low P-Factors will likely be beneficial for many reasons. Aside from the structural changes in wood, ionic liquids themselves are often the culprits when it comes to enhanced 'solubility' of wood in seemingly non-derivatising ionic liquids. There is now quite much evidence demonstrating that certain ionic liquids react with wood functionalities or catalyse reactions. This reactivity often goes hand-in-hand with the cellulose dissolution capability as both depend on anion basicity.<sup>22</sup> A good example of this is the conjugation of 1-ethyl-3-methylimidazolium acetate ([emim][OAc]) with reducing ends of cellulose.<sup>33,34</sup> This has recently been developed in an article by Clough *et al.*<sup>35</sup> where it has been shown that [emim][OAc] allows for conversion of the carbon in the glucopyranose ring into an imidazolium C1-formaldehyde adduct. 1-butyl-3-methylimidazolium chloride ([bmim]Cl) was shown to be an alternative structure that did not form this adduct, presumably due to reduced basicity of the anion.<sup>22</sup> Similar reports can also be found for reaction with lignin.

### Potential Applications and Sustainability

Autohydrolysis in itself is a pre-treatment method performed in water on a 2<sup>nd</sup> generation bio-based feedstock. It is both green and sustainable as it is already implemented for the production of PHK. It yields an extractives fraction which can be combusted for its calorific value (steam or electricity generation) or used for chemicals production. Under mild autohydrolysis conditions this fraction typically contains acetic acid and hemicelluloses, both valuable chemicals. In terms of chemistry, the hemicellulose (xylan) fraction may be chemically modified to polymeric chemicals or it is also already industrially converted to xylose (for xylitol production) and furfural. Polymeric hemicelluloses also already have wide application in the food industry. Regarding the sustainability of the subsequent direct-dissolution step on the residual chips,



the most obvious applications are in further chemical modification of the wood matrix or regeneration to films and fibres, e.g. a lyocell using ionic liquid as direct-dissolution solvent.<sup>33,34</sup> Providing low toxicity ionic liquids and reagents are used, the main stumbling block to sustainability is the removal of water from ionic liquids during the recycling stage, as typically water is used to precipitate the biomass. Therefore, the value of the product must be high enough to offset this cost or advances made that either minimise the use or energy consumption of water use.

## Conclusions

In this paper, we demonstrate that mild autohydrolysis can be applied to allow for almost complete solubilisation of wood chips in ionic liquid. Without the autohydrolysis pre-treatment both wood chips and particle sizes much lower than sawdust (<0.16 mm) are mostly insoluble, until you get down to the very small particle sizes that extended planetary milling affords. As autohydrolysis is an existing green and economical pre-treatment method, typically applied prior to kraft pulping, this could now open the door to much more thorough chemical modification of wood chips, as a low cost feedstock. This could most obviously lead to new applications in thermoplastics, films, polymer blends, composite materials etc. It could also lead to novel dissolution/regeneration applications, such as, a wood-based lyocell process where high tensile strength wood fibres are regenerated. The factors affecting solubility of autohydrolysed chips over unextracted chips are also discussed, with no significant conclusions other than to further clarify the different potential physiochemical factors involved. Understanding all of these processes is required to make predictions about wood recalcitrance. However, clearly there will be interesting opportunities in the future to start to dissect the different contributions to the recalcitrance of wood. New economical pre-treatments, e.g. mild acid/base, ammoniolysis, electron beam and redox processes, etc., may also prove to be more effective or economical than autohydrolysis and offer different application areas.

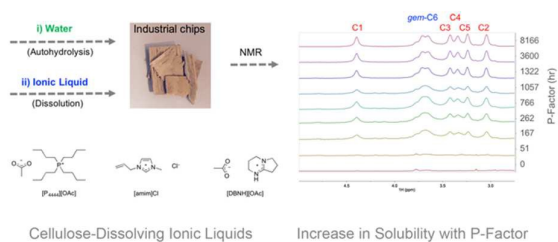
## Acknowledgements

We would like to acknowledge FIBIC (The Finnish Bioeconomy Cluster) Oy, now known as CLIC Innovation, for funding under the FuBio (Future Biorefinery) program. We would also like to thank the WWSC (Wallenberg Wood Science Center) for additional financial support

## References

- R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *J. Am. Chem. Soc.*, 2002, **124**, 4974-4975.
- Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, *Green Chem.*, 2008, **10**, 44-46.
- A. Parviainen, A. W. T. King, I. Mutikainen, M. Hummel, C. Selg, L. K. J. Hauru, H. Sixta and I. Kilpeläinen, *ChemSusChem*, 2013, **6**, 2161-2169.
- W. T. King, J. Asikkala, I. Mutikainen, P. Järvi and I. Kilpeläinen, *Angew. Chem. Int. Ed.*, 2011, **50**, 6301-6305.
- S. Spange, A. Reuter, E. Vilsmeier, T. Heinze, D. Keutel and W. Linert, *Polym. Chem.*, 1998, **36**, 1945.
- R. Rinaldi, *Chem. Commun.* 2011, **47**, 511-513.
- A. J. Holding, M. Heikkilä, I. Kilpeläinen and A. W. T. King, *ChemSusChem*, 2014, **7**, 1422-1434.
- I. Kilpeläinen, H. Xie, A. W. T. King, M. Granström, S. Heikkinen, and D. S. Argyropoulos, *J. Agric. Food Chem.*, 2007, **55**, 9142-9148.
- L. Kyllönen, A. Parviainen, S. Deb, M. Lawoko, M. Gorlov, I. Kilpeläinen and A. W. T. King, *Green Chem*, 2013, **15**, 2374-2378.
- D. Fengel and G. Wegener, Lignin-polysaccharide complexes. In *Wood chemistry, ultrastructure and reactions*, ed. W. De Gruyter, Berlin, 1984, 167-174.
- M. Lawoko, G. Henriksson and G. Gellerstedt, *Biomacromolecules*, 2005, **6**, 3467.
- M. Balakshin, E. Capanema, H. Gracz, H-M. Chang and H. Jameel, *Planta*, 2011, **233**, 1097-1110.
- M. Y. Balakshin, E. Capanema and H-M. Chang, *Holzforchung*, 2007, **61**, 1-7.
- L. K. J. Hauru, Y. Ma, M. Hummel, M. Alekhina, A. W. T. King, I. Kilpeläinen, P. A. Penttilä, R. Serimaa and H. Sixta, *RSC Adv.*, 2013, **3**, 16365-16373.
- G. Garrote, H. Domiguez and J. C. Parajo, *Holz. Roh. Werkst.*, 1999, **57**, 191-202.
- G. Garrote, H. Domiguez and J. C. Parajo, *J. Chem. Technol. Biot.*, 1999, **74**, 1101-1109.
- P. Gullon, A. Romani, C. Vila, G. Garrote and J. C. Parajo, *Biofuel. Bioprod. Bior.* 2012, **6**, 219-232.
- M. Borrega, L. K. Tolonen, F. Bardot, L. Testova and H. Sixta, *Bioresource Technol.* 2013, **135**, 665-671.
- H. Sixta, A. Potthast and A. W. Krottschek, *Chemical Pulping Processes: Handbook of Pulp – Vol 1.*, Ed. H. Sixta, Wiley-VCH, Weinheim, 2006. 343-345.
- G. Bentivoglio, T. Röder, M. Fasching, M. Buchberger, H. Schottenberger and H. Sixta, *Lenzinger Berichte*, 2006, **86**, 154-161.
- G. Laus, G. Bentivoglio, H. Schottenberger, V. Kahlenberg, H. Kopacka, T. Röder and H. Sixta, *Lenzinger Berichte*, 2005, **84**, 71-85.
- A. W. T. King, A. Parviainen, P. Karhunen, J. Matikainen, L. K. J. Hauru, H. Sixta and I. Kilpeläinen, *RSC Adv.*, 2012, **2**, 8020-8026.
- M. T. Clough, K. Geyer, P. A. Hunt, S. Son, U. Vagt and T. Welton, *Green Chem.* 2015, **17**, 231-243.
- A. W. T. King, L. Zoia, I. Filpponen, A. Olszewska, H. Xie, I. Kilpeläinen and D. S. Argyropoulos, *J. Agric. Food Chem.*, 2009, **57**, 8236-8243.
- T. E. Timell, *Wood. Sci. Technol.*, 1967, **1**, 45-70.
- T. Rauhala, A. W. T. King, G. Zuckerstatter, S. Suuronen and H. Sixta, *Nordic Pulp Paper Res. J.*, 2011, **26**, 386-391.
- S. Labafzadeh, J. Kavakka, K. Sievänen, J. Asikkala and I. Kilpeläinen, *Cellulose*, 2012, **19**, 1295-1304.
- T. Kulomaa, J. Matikainen, P. Karhunen, M. Heikkilä, J. Fiskari and I. Kilpeläinen, *RSC Adv*, 2015, **5**, 80702-80708.
- A. M. Stepan, A. W. T. King, T. Kakko, G. Toriz, I. Kilpeläinen and P. Gatenholm, *Cellulose*, 2013, **20**, 2813-2824.
- T. Kakko, S. Asaaki, A. W. T. King, M. Hummel, H. Sixta and I. Kilpeläinen, *ISWFPC 2015 (Extended Abstract Proceedings – P8)*, 22-25.
- A. J. Holding, V. Mäkelä, L. Tolonen, H. Sixta, I. Kilpeläinen and A. W. T. King, *ChemSusChem*, 2016, cssc.201501511R1.
- S. Smiljanski, S. Stanković, *Cellulose Chem. Technol.*, 1974, **8**, 283-294.
- T. Liebert and T. Heinze, *Bioresources*, 2008, **3**, 576-601.
- G. Ebner, S. Schiehser, A. Potthast and T. Rosenau, *Tetrahedron Lett.*, 2008, **49**, 7322-7324.
- M. T. Clough, K. Geyer, P. A. Hunt, S. Son, U. Vagt, T. Welton, *Green Chem.*, 2015, **17**, 231.
- H. Sixta, A. Michud, L. Hauru, S. Asaadi, Y. Ma, A. W. T. King, I. Kilpeläinen and M. Hummel, *Nordic Pulp Paper Res. J.*, 2015, **30**, 43-57.
- Y. Ma, S. Asaadi, L.-S. Johansson, P. Ahvenainen, M. Reza, M. Alekhina, L. Rautkari, A. Michud, L. Hauru, M. Hummel and H. Sixta, *ChemSusChem*, 2015, **8**, 4030-4039.

TOC Image:



TOC Text:

Autohydrolysis, a green and economical pre-treatment process, allows for dissolution and chemical modification of wood chips