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Catalytic Upstream Biorefining through Hydrogen Transfer Reactions: Understanding the Process from the Pulp Perspective

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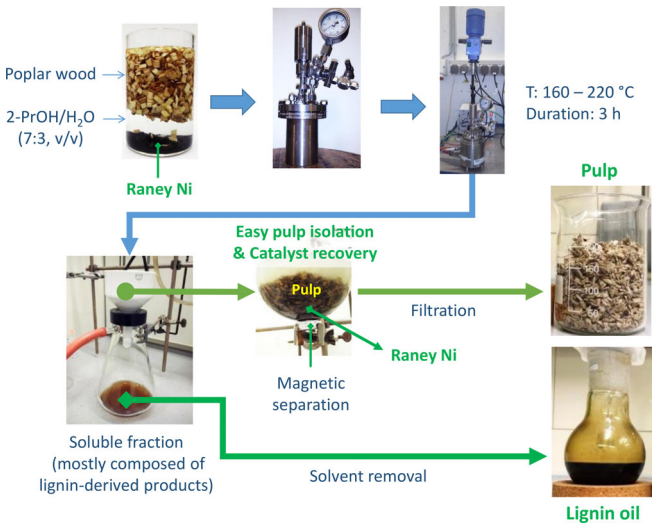
Catalytic upstream biorefining (CUB) encompasses processes for plant biomass deconstruction through the early-stage conversion of lignin by the action of a hydrogenation catalyst. CUB processes produce lignin as an extensively depolymerised product (i.e., a viscous lignin oil) and render highly delignified pulps. In this report, we examine CUB from the pulp perspective. Notably, Raney Ni plays an indirect role in the processes that occur within the lignocellulose matrix. As there are negligible points of contact between the poplar wood chips and Raney Ni, the catalyst action is limited to the species leached from the matrix into the liquor. Nevertheless, the substantial changes in the liquor composition (through the decomposition of carboxylic acids and H-transfer reductive processes on the lignin fragments) have significant implications for the pulp composition, degree of polymerisation and morphology. Compared with organosolv pulps, CUB pulps show higher xylan retention, higher delignification, and higher polymerisation degree. Moreover, the correlation between these properties and the performance of the enzymatic hydrolyses of CUB and organosolv pulps reveals that the high susceptibility of CUB pulps is mostly caused by their lower residual lignin contents.

Introduction

In the recent years, research into lignin valorisation has gained fresh momentum that has brought about advances in both plant bioengineering and the catalytic processing of lignin.[1,2] In the broader context of catalysis, the relationship between this discipline and lignin valorisation is no longer limited to the processing of technical (recalcitrant) lignin wastes, generated by the pulping and paper industry, but has expanded to offer innovative solutions for the pulping process itself and efficient methods for lignocellulose fractionation or deconstruction (here referred to as catalytic upstream biorefining or CUB).[1,3] In addition to delignified pulps, these processes render lignin streams of low molecular weight (M_w).[4–7] This new lignin stream enables subsequent catalytic upgrading (i.e., catalytic downstream biorefining) to achieve highly efficient energy and carbon balance, as shown by several studies.[4,8–10]

Recently, we introduced a CUB process performed in the presence of Raney Ni as the catalyst for H-transfer reactions (i.e., hydrogenation, hydrodeoxygenation, and hydrogenolysis) and 2-propanol as a component of the lignin-extracting solvent mixture and an H donor.[4] We discovered that the addition of a hydrogenation catalyst to the conventional organosolv process led to the early-stage catalytic conversion of lignin (ECCL) oligomers.[1,4,11] Remarkably, the lignin is not isolated as a red-brown polymeric solid like that from the organosolv process; instead, a brownish viscous oil is isolated (Scheme 1). Lignin oils comprise up to 50–60% monophenols (with $M_w < 250$ Da).[4] The other products (≈40–50%) found in



Scheme 1. Steps of CUB in the presence of Raney Ni and visual appearances of the isolated fractions.

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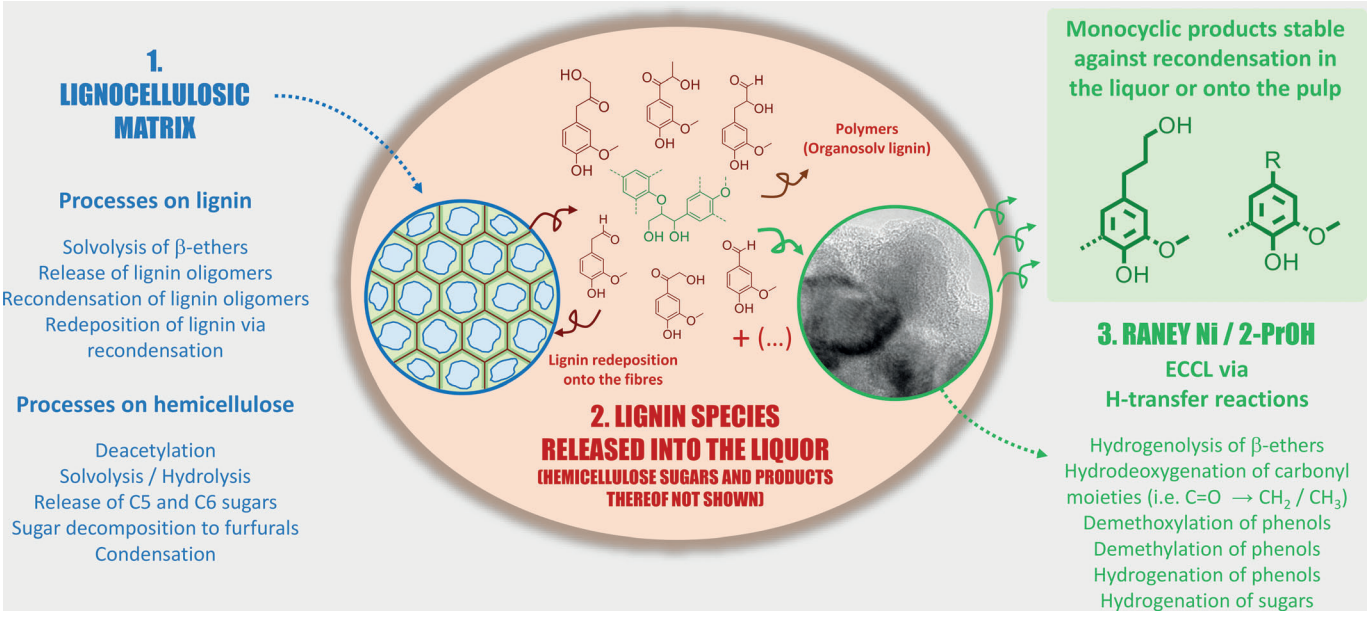
the lignin oil are dimers and trimers and, to a lesser extent, lignin oligomers and polyols (i.e., hydrogenolysis products from hemicellulose sugars).[4]

More recently, the concept of ECCL has also been explored in the presence of supported noble-metal catalysts under H_2 pressure[5,12] or through internal H-transfer reactions,[7] and similar results have been achieved regarding the isolation of a highly depolymerised lignin stream. Therefore, heterogeneous catalysis can play a decisive role in tailoring the properties of the lignin streams for subsequent downstream processing.[1,3] However, the use of Raney Ni in the ECCL remains very advantageous, as this catalyst (0.5 US$ mol^{-1})[13] is much less expensive than Ru (137 US$ mol^{-1}) or Pd (1865 US$ mol^{-1}).[14] Most importantly, owing to the magnetic properties of Raney Ni, the separation of the catalyst from the pulp can be achieved readily by simple magnetic separation, which allows for catalyst recycling.[4] Remarkably, in recycling experiments, we observed no deterioration of the catalytic performance with respect to the properties of the lignin oil.[4] As the catalyst is readily separable from the pulp, the product shows a visual appearance similar to that of the initial wood chips (Scheme 1); however, the product is a 'fluffy' material owing to its low lignin content (wood: 25–30% vs. pulp: 3–5%).[4,11]

Regardless of the type of hydrogenation catalyst utilised in CUB, there are always three distinct phases in which the chemical processes occur: (1) the lignocellulosic matrix, (2) the liquor and (3) at the hydrogenation catalyst, as represented in Scheme 2.

In the lignocellulosic matrix [Scheme 2(1)], upon the impregnation of the fibres with the solvent mixture (liquor), the solvolytic processes may already initiate at temperatures as low as 160 °C.[4] Lignin oligomers can be released readily from the matrix by cooking the lignocellulose at 160–220 °C in solvent mixtures (e.g., C_1–C_4 alcohols/water)[15] with a Hildebrand parameter (δ) of (22 ± 5) $MPa^{1/2}$.[16,17] Under these conditions, the deacetylation of hemicelluloses constitutes another key chemical process that occurs within the plant tissue. Owing to the release of acetic acid, the medium (liquor) becomes acidified (pH 4–5);[4,18–24] consequently, the rate of hemicellulose hydrolytic processes increases.[11] Unsurprisingly, the xylan retention decreases with the acidification of the liquor. Surprisingly, although the β-ether moieties of lignin are prone to acid-catalysed hydrolysis,[24–27] the lignin content released into the liquor is pH-independent (within the pH range from 4.5 to 9.5), as we recently found for CUB and organosolv processes performed on poplar wood.[11]

In the liquor [Scheme 2(2)], which also includes the liquid absorbed or entrapped in the matrix, the lignin fragments (e.g., Hibbert ketones and aldehyde intermediates) are prone to several types of reaction.[1,28] For instance, under the conditions of organosolv pulping, lignin fragments undergo recondensation, which transforms the initially reactive lignin biopolymer into a complex mixture of degraded polymers containing strong C–C bonds.[1] Nonetheless, in the presence of a hydrogenation catalyst [Scheme 2(3)], the carbonyl groups of the C_3 side chains of the lignin-derived phenols undergo reductive processes to form alcohol, methylene or methyl groups.[4,11,29,30] As a consequence, the early-stage catalytic conversion of lignin fragments leads to their "passivation", which protects them from recondensation processes not only in the liquor but also within the lignocellulosic matrix, as evidenced by the systematically lower content of residual lignin found in CUB pulps compared to those for organosolv lignins obtained under varying conditions.[11] Therefore, even though the contact between the lignocellulose and the hydrogenation catalysts is inadequate for their direct action onto the substrate,



Scheme 2. Schematic representation of the primary chemical processes involved in CUB for the early-stage catalytic conversion of lignin (ECCL). For clarity, hemicellulose sugars and their degradation products were omitted.

the alteration of the chemical species present in the liquor is anticipated to have implications for the pulp quality.

Motivated by the intricacy of the interconnected processes among the three phases (Scheme 2) and by the macroscopic properties of catalyst-free pulps obtained from the CUB process in the presence of Raney Ni, we began to study the CUB process from the pulp perspective. In this report, we compare the pulps obtained from CUB and the conventional organosolv process in a mixture of 2-propanol/water (7:3 v/v) as the pulping liquor and examine the impact of the passivation of the lignin fragments on the pulp composition and structure. Finally, we correlate the results obtained from the enzymatic hydrolysis of the pulps with their properties and draw some conclusions on the microscopic properties of the pulps.

Results and Discussion

Impact of the catalyst on the pulp composition

Compared in Table 1 are the percentages of isolated fractions (the values are given relative to the initial weight of poplar wood used in each experiment), pulp compositions, C balances and delignification degrees obtained by the organosolv process and CUB. Before an in-depth discussion of the results listed in Table 1, the meaning of each class of results deserves careful consideration. Regardless of the presence of Raney Ni, the cooking of wood releases lignin fragments and hemicellulose sugars into the liquor, to an extent that depends on the cooking temperature[24,25,31–36] and solvent properties.[16,17] Logically, the leaching extent of these species determines the percentage of isolated fractions (i.e., a soluble fraction comprising lignin and hemicellulose-derived products and an insoluble fraction, the pulp). Notably, the percentage of these isolated fractions does not correspond to a regular yield and, therefore, should not be interpreted as the (sole) parameter to measure the fractionation performance. To evaluate the efficiency of the fractionation process, the extent of pulp delignification is one of the most important parameters, together with the content of xylans remaining in the pulp. Notably, these parameters should also be evaluated with caution if processes performed in different solvents are compared. As the solubility of the low-molecular-weight species will differ dramatically from solvent to solvent, lignin and oligosaccharide species may stay sorbed in the lignocellulosic matrix, and this hinders the evaluation of the overall process performance. Also important is the fact that the percentage of isolated fractions does not necessarily have to equal 100%. Owing to deoxygenation processes (e.g., for the C_3 side chains of the lignin building units or the dehydration of hemicellulose sugars), a considerable loss of weight may occur in the resulting soluble fraction. This observation holds especially true for CUB, as the presence of a hydrogenation catalyst leads to considerable hydrodeoxygenation of the lignin stream (not only in the C_3 side chain but also through the demethoxylation on the phenolic ring). For this reason, to evaluate the mass flow throughout the fractionation process, the C balance seems to be a better indicator than the mass balance. After the consideration of these points, it is now possible to discuss the dataset presented in Table 1 in detail.

At first sight, the most intriguing question to emerge from the comparison between the percentages of isolated fractions from CUB and the organosolv process relates to the trends of the soluble-fraction percentages with the process temperature. Indeed, for the organosolv process, the soluble fraction increases steadily (from 10 to 36%) as the temperature increases (from 160 to 220 °C). Conversely, for CUB, the percentage of soluble fraction already shows a plateau at (24±2)% from a temperature of 180 °C.

From the examination of the pulp composition and delignification extent, the higher percentage of soluble products clearly does not translate into a greater delignification extent in the organosolv pulps. Surprisingly, for the organosolv process, delignification does not exceed (75±2)% in the pulps obtained from processing at temperatures of 180 °C or higher. These values agree with those reported for the ethanol organosolv pulping of poplar wood.[26,37] Conversely, for CUB, delignification increases steadily with the process temperature and reaches 87% in the pulp obtained from the treatment at 220 °C.

The recondensation of the lignin fragments dissolved in the liquor with those fragments still attached to the lignocellulose

Table 1. Comparison of pulp properties obtained from organosolv and CUB processes under identical process conditions.[a]

Entry[a]	Process type	T [°C]	Isolated fractions [% relative to initial substrate weight]		C balance [mol/mol]	Pulp composition [wt%][b,c]				Delignification extent [%]
			soluble	pulp		glucans	xylans	lignin	others[d]	
1	organosolv	160	10	86	1.0	48	15	20	17	33
2	CUB	160	15	81	1.0	57	14	14	15	53
3	organosolv	180	30	70	1.0	79	11	7	3	77
4	CUB	180	25	71	1.0	66	11	11	12	63
5	organosolv	200	33	53	1.0	79	6	8	7	73
6	CUB	200	22	55	0.8	80	9	6	5	80
7	organosolv	220	36	46	1.0	81	3	7	9	77
8	CUB	220	26	52	0.8	84	7	4	5	87

[a] General experimental conditions: poplar wood (16.5 g), 2-propanol/water (7:3 v/v, 140 mL), 3 h. The CUB experiments were performed with the addition of Raney Ni (wet, 10 g); [b] Obtained from analytical saccharification with sulfuric acid; [c] Unprocessed poplar wood composition: glucans 52 wt%, xylans 17 wt%, lignin 30 wt%, others 1 wt% (dry and ash-free values). [d] As part of the unidentified other products, acid-soluble lignin can constitute approximately 5 wt% of poplar.

matrix constitutes a well-known problem of conventional pulping processes.[1,38,39] Accordingly, for the conventional pulping process to provide highly delignified pulps, highly severe conditions are often required. Herein, the passivation of the lignin fragments dissolved in the liquor seems to alleviate the problem of lignin redeposition onto the cellulose fibres. As a result, greater extents of delignification are achieved by CUB compared with those for organosolv processes performed under identical conditions. The high delignification effected by CUB constitutes a distinct advantage over organosolv processes performed at 180–200 °C. At these temperatures, for further delignification in the organosolv process, an acid catalyst should be added (e.g., acetic acid or formic acid).[40, 41] This process option results in the hydrolysis of hemicellulose and, therefore, little xylan retention in the pulps. Noteworthy, in an application context broader than those of biofuels and chemicals production, low xylan retention does not necessarily constitute a trade-off. This is because there is a very lucrative market for high-purity, microcrystalline cellulosic fibres for which pulps with low xylan-content could show highly interesting, value-added uses.

Regarding the xylan retention, the organosolv process performed at 200 or 220 °C leads to the removal of more xylans than the experiments performed at 160 or 180 °C. This observation is in line with previous reports on other organosolv treatments of poplar wood.[33,40] Surprisingly, however, the xylan contents of the pulps obtained from CUB at 200 and 220 °C (9 and 7 %, respectively) are higher than those from the organosolv process (6 and 3 %, respectively). The higher retention of xylans achieved by CUB resulted in a lower percentage of soluble fraction compared with those for the corresponding organosolv experiments.

In a previous study,[11] we found that CUB systematically produces pulps with higher xylan content than those from organosolv treatment under the same process conditions. In that study, a comparison of the final pH values of the liquors from CUB with those from the organosolv process revealed ΔpH values of approximately 1–2. Moreover, in CUB, the increase in the pH value of the medium suppressed the hydrolysis of xylans.[11] Consequently, an increase in the xylan retention from 10 (at pH 4.5) to 60 % (at pH > 7.5) was achieved.[11] In the current study, although the pH value of the liquor from the organosolv process at 200 °C was 4.3, the liquor recovered from CUB had a final pH value of 5.5. Again, the current results provide evidence that Raney Ni degrades part of the acetic acid and formic acid released from the lignocellulosic matrix.

Finally, it is clear from Table 1 that the relative glucan content increases steadily with the process temperature from 48–57 % for pulps obtained at 160 °C (Entries 1 and 2) to 81–84 % for those produced at 220 °C (Entries 7 and 8) for both processes. Unsurprisingly, the increase in the relative glucan content is caused by the more extensive removal of lignin and hemicellulose. This observation indicates that higher process temperatures lead to increased delignification, and temperatures between 180 and 200 °C offer the best compromise between delignification and xylan retention in this case.

Degree of polymerisation

The quantity of pulp obtained by the fractionation process is an important descriptor of process performance. However, to assess the process fully, the assessment of the pulp quality not only by its composition but also by the degree of polymerisation (DP) is essential. The DP indicates the number of monomeric units (described as anhydroglucose units, AGU) that constitute the polymer chain. It is well-known that the DPs of celluloses can be affected by pretreatments that hydrolyse glycosidic bonds (e.g., acid pretreatments).[42,43] Both the organosolv and the CUB processes presented here were performed without added acids. However, the acidic environment developed by the release of acids during lignin solvolysis and affected by Raney Ni may to some extent exert different effects on cellulose to those already found for hemicellulose.

To assess the effect of CUB upon the DP of the pulp, organosolv and CUB pulps were derivatised to the corresponding cellulose tricarbanilate and then analysed by gel permeation chromatography (GPC).[43] The distributions of the apparent DPs for the CUB and organosolv pulps are compared in Figure 1. Both processes resulted in pulps that exhibited two maxima in the apparent DP distribution curves (Figure 1 a). The first maximum (≈30 AGU) is most likely associated with the residual hemicellulose fraction in the pulp, as indicated by the correlation between the area of the deconvoluted peak and the respective xylan content of the pulp (Figure 1 b). Importantly, having assigned the secondary peak to hemicelluloses (which comprise C_5 and C_6 sugars), one should bear in mind that the value of 30 AGU gives a rough estimate of the M_W of the hemicellulose retained in the pulp, because C_5 sugars undergo carbanilation to form dicarbanilate derivatives instead. Accordingly, the normalisation factor of 519 g mol^{-1} (as for tricarbanilated AGU) results in an underestimation of the actual DP values of the hemicelluloses in the samples.

In the apparent DP distribution curves (Figure 1 a), the main peak corresponds to the cellulosic fraction. Interestingly, at a temperature as low as 160 °C, the effect of the processing of poplar wood in the presence of Raney Ni upon the apparent DP of the pulp is already evident. Indeed, the organosolv pulps show a shift in the apparent DP distribution curves to values lower than those of the CUB counterparts. For the pulps obtained from the process performed at 160 °C, the absolute maximum is located at approximately 6000 AGU for the organosolv pulp, whereas the value is approximately 8000 AGU for the CUB pulp. Both values compare to those found for native celluloses (3000–10 000 AGU).[43–45] With the increase in the process temperature, the reduction in the apparent DP of the cellulosic fraction is much more significant for the pulps obtained from the organosolv process than for those from the CUB process. For instance, the organosolv pulp obtained at 220 °C shows the main peak at approximately 600 AGU, whereas that from CUB is observed at approximately 2000 AGU.

Despite their low acid strengths, carboxylic acids can improve the rate of cellulose de-polymerisation. This effect becomes more significant at high temperatures (e.g., 200–240 °C)

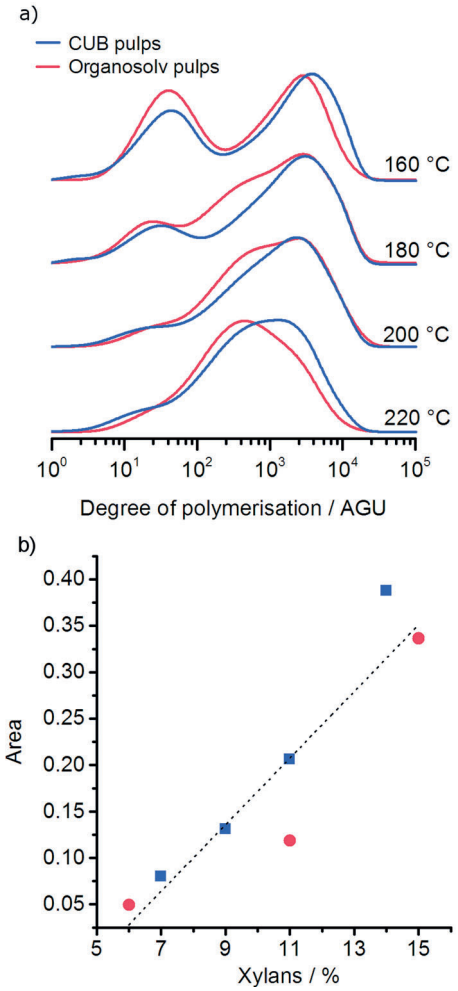


Figure 1. a) Distribution curves of apparent DPs for organosolv pulps (red lines) and CUB pulps (blue lines) produced at varying temperatures (DP values given as anhydroglucose units, AGU); b) correlation between xylan content and area of the GPC peak corresponding to low M_w compounds (red: organosolv pulps; blue: CUB pulps).

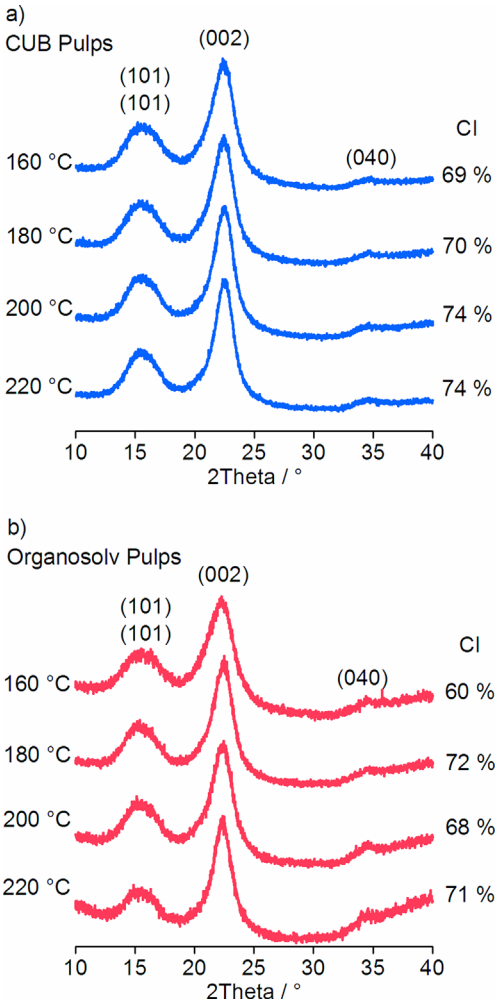


Figure 2. XRD patterns of a) CUB pulps and b) organosolv pulps at varying temperatures. The CI values are indicated.

and can even be useful for the production of fermentable sugars from cellulose.[46,47] This piece of information, together with the fact that Raney Ni decomposes part of the carboxylic acids released from the lignocellulosic matrix, clarifies the indirect effect of Raney Ni upon the preservation of high DP in CUB pulps.

Pulp crystallinity

To assess the impact of the CUB upon the pulp crystallinity, XRD analyses were performed on the pulps obtained from the organosolv and CUB processes, and the crystallinity index (CI) values were determined. The XRD patterns obtained for these samples are shown in Figure 2. The reflections at $2\theta = 15$, 22.5, and 34.5° are characteristic of cellulose I[48] and were detected for the organosolv and CUB pulps. On the basis of the CI values, the CUB pulps show approximately 3–9% higher amounts of crystalline cellulose than the organosolv pulps in absolute terms, with the exception of the CUB pulp obtained at 180 °C.

Pulp morphology

To visualise and compare the morphological changes caused by the biomass fractionation processes, field-emission scanning electron microscopy (FESEM) images were obtained for each sample. The chip surface of poplar wood (Figure 3) is compact (even at high magnification, Figure 3b) and typical of a knife-milled sample (i.e., a sample with residues of particulate material are deposited on the external surfaces of the chips; indicated by the arrows in yellow). In contrast, the organosolv and CUB pulps both show detached fibres of varied thickness, which can be completely independent or partially connected to the main wood body (Figure 4).

A clear distinction between the low- (160–180 °C) and high-temperature (200–220 °C) treatments can be outlined in the morphology of the samples. On the one hand, the pulps obtained at 160 °C, as well as that recovered from CUB at 180 °C, exhibit individual thin fibres as well as agglomerates of thinner

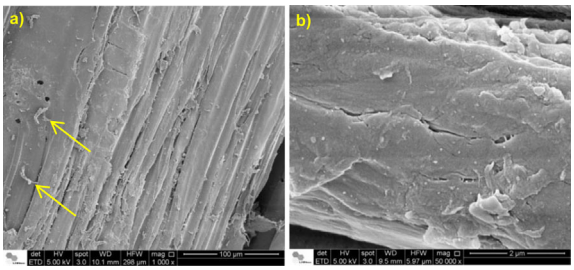


Figure 3. FESEM images of poplar wood surface: a) 1000× magnification (scale bar: 100 μm); b) 50000× magnification (scale bar: 2 μm). The arrows indicate particulate material deposited on the external surfaces of the poplar wood chips.

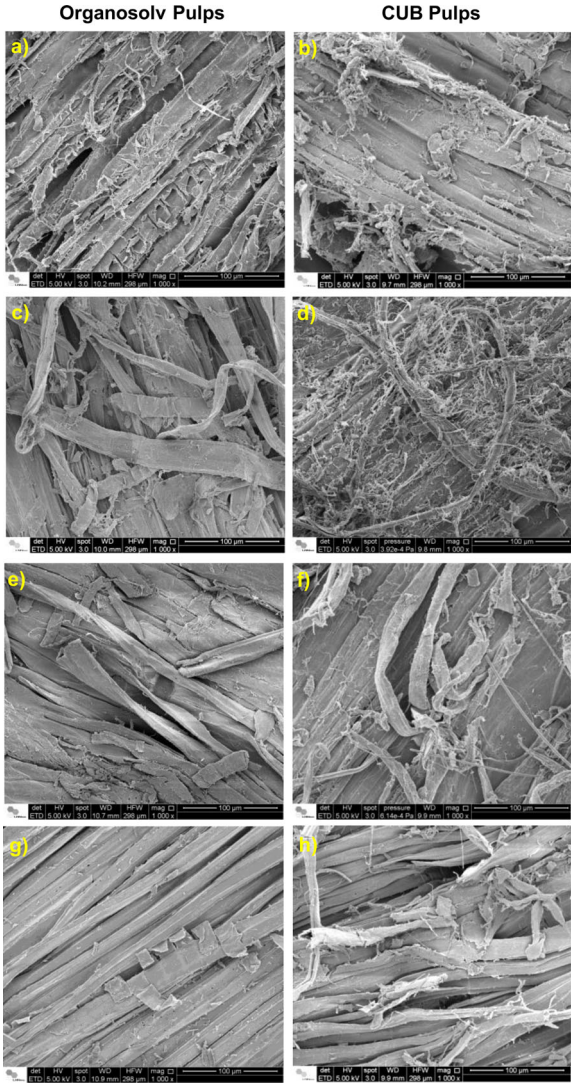


Figure 4. FESEM images of organosolv pulps (left) and CUB pulps (right) obtained at a) and b) 160, c) and d) 180, e) and f) 200, and g) and h) 220 °C. Scale bar 100 μm.

fibres (Figure 4, images a, b, and d). On the other hand, higher-temperature treatments result primarily in thick fibres with smooth surfaces (Figure 4, images c and e–h).

In the images recorded at higher magnification (Figure 5), especially those from pulps produced at 160 °C (Figure 5a and

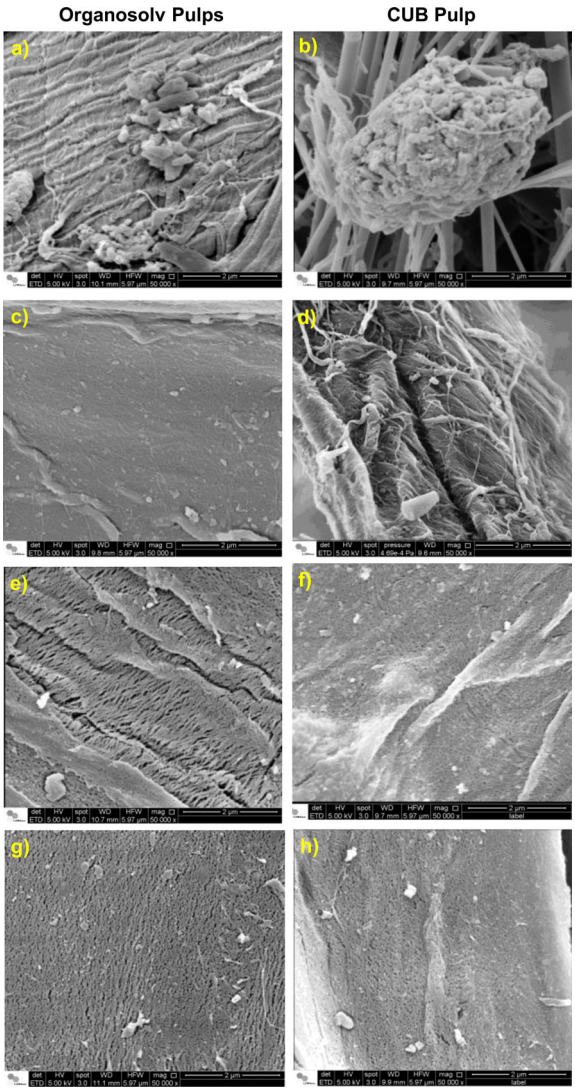


Figure 5. FESEM images of organosolv pulps (left) and CUB pulps (right) obtained at a) and b) 160, c) and d) 180, e) and f) 200, and g) and h) 220 °C. Scale bar 2 μm.

b), it is possible to visualise some agglomerates of material deposited on the chip surface. These are commonly assigned to lignin globules, which condense and migrate on the wood surface during fractionation pretreatments.[33,49] The high-magnification images also reveal different features between the organosolv pulps (Figure 5e and g) and the CUB pulps obtained from treatment at high temperatures (Figure 5f and h).

The organosolv pulps present fibrous and porous surfaces. In contrast, the CUB pulps show more-compact surfaces. Apparently, the low retention of xylans in the organosolv pulps seems to be responsible for the micro-defibrillation seen in their high-magnification FESEM images. Most likely, the removal of the xylan fractions located in the interstices of the cellulose fibrils leads to a better definition of the fibril reliefs. Overall, the effects of Raney Ni upon the pulp properties are indirect, but derived from the action of the catalyst on the soluble components released into the liquor (i.e., the decomposition of carboxylic acids and the hydrodeoxygenation of lignin fragments).

Enzymatic hydrolysis

In the context of our work, we chose to perform enzymatic hydrolysis of the organosolv and CUB pulps not only to demonstrate the potential of the pulps recovered from CUB as a source of fermentable sugars but also to gain further information on the accessibility of these materials to cellulases and provide further insights to be correlated with the material properties.[44,48] Accordingly, the organosolv and CUB pulps were subjected to enzymatic hydrolysis with a commercial cellulase preparation (Celluclast® from *Trichoderma reesei*).

The yields of glucose obtained by enzymatic saccharification, calculated relative to the total quantity of glucans determined through analytical saccharification with sulfuric acid, are shown in Figure 6.[50] As expected, untreated poplar wood is resistant to enzymatic hydrolysis and leads to a glucose yield as low as

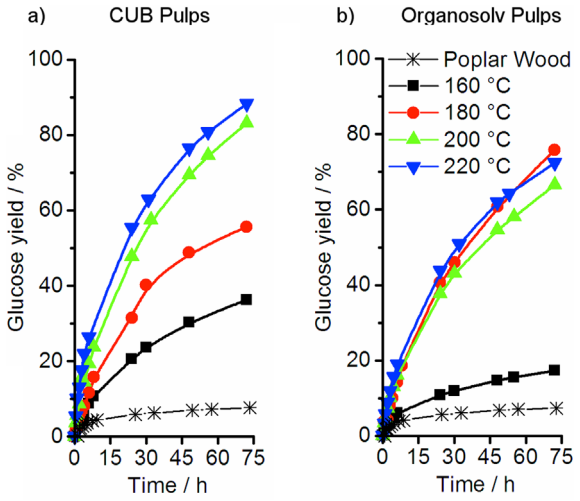


Figure 6. Glucose yields from the enzymatic hydrolysis of poplar wood, a) CUB pulps and b) organosolv pulps obtained at varying temperatures. Reaction conditions: substrate (equivalent to 1 g of glucans), Celluclast® (350 U g^{-1} substrate), pH 4.8 (acetate buffer), 45 °C.

10% after 72 h. It is clear from Figure 6 that the organosolv and CUB processes result in pulps more susceptible to enzymatic hydrolysis. The organosolv pulps (Figure 6b), except that obtained at 160 °C, exhibit high yields of glucose (65–75% after 72 h). There is no strong dependence of the yield of glucose on the temperature applied in the organosolv fractionation process. This observation agrees with those of other studies on the enzymatic hydrolysis of organosolv-treated poplar wood.[36,51] Conversely, the enzymatic conversion of CUB pulps depends strongly on the temperature applied during the process (Figure 6a). After 72 h of enzymatic saccharification, very high levels of glucose were found (from 36 to 88% as the pretreatment temperature was increased from 160 to 220 °C).

The action of cellulases on the cellulose fibres depends on various factors. However, the lignin content, which disturbs the adsorption of cellulases onto the cellulosic fibres, in addition to the intrinsic crystallinity of cellulose, which creates a structural protection against the action of the adsorbed cellulases, are recognised as the most important factors. Under the conditions of this study, the susceptibility of the organosolv and CUB pulps is expected to be influenced mainly by the quantity of residual lignin.[33,52–54] This is because of the quite similar crystallinity indexes of the pulps obtained at 180–220 °C, irrespective of the fractionation process. Nonetheless, the real effect of the crystallinity on the enzyme action has been under debate.[48] Although the global crystallinity of the sample (measured by XRD) increases owing to lignin and hemicellulose removal, the intrinsic cellulose crystallinity (which is relevant for the enzyme activity) may not change with the pretreatments.

To check whether there is a relationship between the residual lignin content of the pulps and the temporal evolution of the glucose yield, the data from Figure 6 was replotted against the lignin content of the pulps (Figure 7).

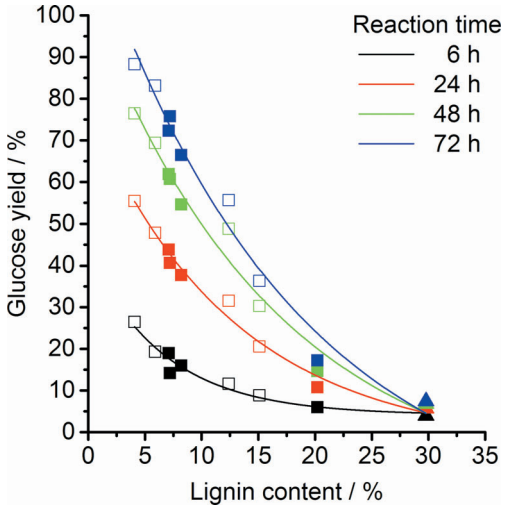


Figure 7. Correlation between the glucose yield from enzymatic hydrolysis (after the indicated hydrolysis period) and lignin content in poplar wood (solid triangles), organosolv pulps (solid squares) and pulps recovered from the CUB process (open squares) at varying temperatures.

The plot in Figure 7 shows that the glucose yield decays exponentially with the residual lignin content for all reaction times. Clearly, the pulps that underwent lesser delignification (Table 1, Entries 1, 2 and 4) are more resistant to cellulase activity. In contrast, the pulps that contain a lower quantity of residual lignin (Table 1, Entries 6–8) are more prone to enzymatic conversion.

The FESEM images (Figures 4 and 5) show that the plant tissue already undergoes substantial changes that lead to the formation of thin fibres at temperatures of 160 and 180 °C. Although one may intuitively expect that these morphological changes could improve the accessibility of the enzyme to cellulose, the relationship found in Figure 7 reveals that the morphology of the substrate seems to be less critical to the enzymatic conversion than its lignin content. It is important to bear in mind that the accessibility of cellulose is defined at a nanoscale level. Therefore, the unique texture of the substrates will probably have a secondary effect (if any) upon the enzymatic

conversion if the crystallinity and, therefore, the fibre (nano)environments accessible to cellulases are not modified.

Owing to the presence of xylanase enzymes in the Celluclast preparation, hemicellulose can also be saccharified during the enzymatic hydrolysis, and this results in the release of xylose into the solution (Figure 8). Notably, up to 87 and 67% xylose yields from organosolv and CUB pulps, respectively, could be obtained by the treatment with the Celluclast preparation. Apparently, the xylan fractions in these pulps should show distinct molecular features. Finally, no direct correlation between the xylose yield and residual lignin content could be found.

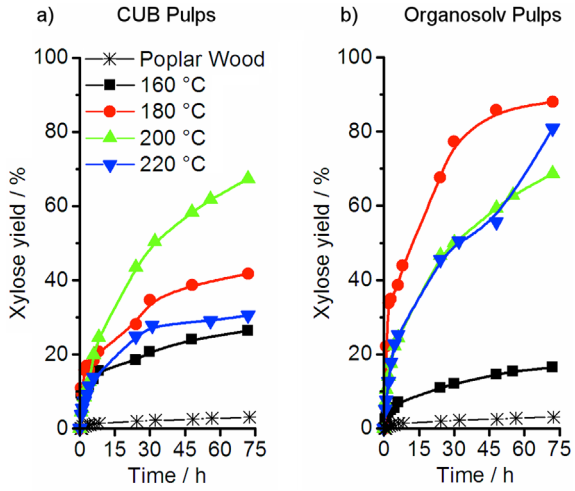


Figure 8. Xylose yields from the enzymatic hydrolysis of poplar wood and pulps recovered from a) the organosolv process and b) CUB in 2-propanol/water (7:3 v/v) at varying temperatures.

Conclusions

From an in-depth comparative analysis of the organosolv and catalytic upstream bioprocessing (CUB) processes, we arrived at the following conclusions:

1. Pulps of superior quality (regarding xylan retention and delignification) are obtained from CUB in comparison with those from the organosolv process.

2. Although the contact between pulp and Raney Ni is negligible, the presence of Raney Ni causes substantial changes in the liquor composition (i.e., the decomposition of carboxylic acids and H-transfer reductive processes on the lignin fragments).

3. The reductive processes on the lignin fragments seem to be conducive to the high delignification extents found in the CUB pulps. Most likely, the passivation of the lignin stream decreases the likelihood of lignin deposition onto the fibres through recondensation processes.

4. In the CUB liquor, the decrease in the acid content by a factor of 10 suppresses the partial de-polymerisation of cellulose as well as the hydrolysis of hemicelluloses. As a result, pulps with higher apparent degrees of polymerisation (DP) and higher xylan retentions are obtained by CUB compared with those for pulps from the organosolv process.

5. The morphology of the pulp is also affected by the chemical changes in the liquor composition caused by the presence of Raney Ni. The organosolv pulps present fibrous and porous surfaces. In contrast, the CUB pulps shows more-compact surfaces.

6. The morphological changes at this scale do not seem to have a significant role in the performance of the enzymatic hydrolysis.

7. CUB pulps show high susceptibility to enzymatic hydrolysis because of their lower residual lignin content compared with those of the organosolv pulps.

In summary, the superior properties of CUB pulps strongly suggest that these materials could hold a potential as a feedstock to produce paper and synthetic fibres (high-value products) along with biofuels and platform chemicals (low-to-intermediate-value products).

Experimental Section

Materials

Sulfuric acid (95–97%) and a commercial cellulase preparation (Celluclast® from *T. reesei*) were used as purchased from Sigma–Aldrich. Poplar wood (2 mm pellets, J. Rettenmaier & Söhne) was used as received. The pulps were produced by a catalytic biorefining process, as described elsewhere.[4] For all of the analysis (except the microscopic analysis) and for the enzymatic hydrolysis, the pulps were ground in a blender and sieved ($\leq$500 μm) before use.

Pulp characterisation

Humidity and ash content: All of the analysis and enzymatic hydrolysis results were calculated on a dry and ash-free basis. The humidity of the substrates and pulps was determined from the weight loss at 105 °C for 10 min. Typically, the pulps (2–3 g) were analysed in a thermal balance (Ohaus MB25). For each sample, this analysis was repeated at least three times.

For the determination of the ash content, the pulp or starting material ($\approx$100 mg) was placed in a quartz crucible. The sample was then burned in a ventilated muffle oven under a controlled temperature program (25 to 450 °C at 7 °C min^{-1}, 450 to 750 °C at 2.5 °C min^{-1} and 750 °C for 2 h). The crucibles were quenched to room temperature in a desiccator. After the crucibles had cooled to room temperature, the ash was determined by weight difference. For each sample, this analysis was repeated at least four times.

Pulp composition: The contents of glucans, xylans, and lignin were determined by analytical saccharification with sulfuric acid. Typically, a ground and sieved sample (500 μm, 50.0 mg) was suspended in a 72% sulfuric acid solution (0.5 mL) under stirring at 38 °C for 5 min. Distilled water (10 mL) was added to the suspension. The saccharification was then conducted under stirring at 130 °C for 1.5 h. The suspension was left to cool to room temperature and then filtered. The filtrate was analysed by HPLC. The determination of the sugar content was performed with a PerkinElmer Series 200 HPLC instrument equipped with a Nucleogel Ion 300 OA column (Macherey–Nagel). The analyses were performed at 80 °C with a 5 mM H_2SO_4 solution as the eluent (0.5 mL min^{-1}). For the determination of the lignin content, the same saccharification procedure was used; however, the determination was initiated with 500 mg

of sample, and the volume of a 72% sulfuric acid solution and water were scaled up to 5 and 100 mL, respectively. After the saccharification at 130 °C for 1.5 h, the reaction mixture was filtered through a previously weighed 1 μm Millipore filter. The solid was washed with distilled water until neutral pH and then dried in a forced-ventilation oven at 60 °C for 2 d. The weight of this dried solid was considered as the residual lignin in the carbohydrate fraction. The determination of the glucan, xylan, and lignin contents was performed at least in three replicates for each sample.

Crystallinity: Powder XRD patterns were recorded with a PANalytical X'Pert PRO diffractometer with CuK_α radiation within a 2θ range from 10 to 40°.

Degree of polymerisation: The pulp was derivatised to obtain cellulose tricarbanilate (CTC) for GPC analysis. Dry pulp (25 mg) was suspended in anhydrous DMSO (5 mL) and phenyl isocyanate (0.5 mL) in a sealed glass vial. The suspension was stirred at 80 °C for 24 h. The derivatisation of holocellulose leads to its dissolution in DMSO. Next, the solution was cooled to room temperature, and methanol (1 mL) was added to "neutralise" the phenyl isocyanate. The product was then washed three times with methanol/water (3:7 v/v, 30 mL), centrifuged and dried at 60 °C overnight. For the GPC analysis, CTC (20 mg) was dissolved in THF (2 mL), and the solution was filtered. The analysis was performed with a PerkinElmer Series 200 GPC instrument equipped with a TSKgel Super HZM-M column (Tosoh Bioscience). The analyses were performed at 50 °C with THF as the eluent (0.2 mL min^{-1}). The degrees of polymerisation were calculated by normalising the apparent molecular weight values to 519 g mol^{-1} (tricarbanilated AGU).

Field Emission Scanning Electron Microscopy

Poplar wood and its organosolv and CUB pulps were imaged by FESEM. Before analysis, dried samples were coated with gold (40 mA, 60 s) in an SCD 050 sputter coater (Oerlikon-Balzers). The sample imaging was performed with a high-resolution scanning electron microscope equipped with a field emission gun (FEI, Quanta 650, USA). Both the coater and the microscope were available at the National Laboratory of Nanotechnology (LNNano) in Campinas-SP, Brazil. The images were obtained under vacuum with a 5 kV accelerating voltage and a secondary electron detector. A large set of images were obtained from different areas of the samples (at least 20 images per sample) to guarantee the reproducibility of the observed features.

Enzymatic hydrolysis

The enzymatic hydrolysis was performed in a jacketed reactor (150 mL) containing a 1 wt% (dry basis) suspension of the substrate dispersed in 0.1 M acetate buffer (100 mL, pH 4.5). The mixture was stirred at 45 °C. The reaction was initiated by the addition of Celluclast® to the suspension (0.5 mL, 350 U). At defined intervals, aliquots (≈1 mL) of the reaction mixture were taken for the determination of released sugars. The samples were heated immediately at 100 °C for 10 min to inactivate the enzymatic preparation. Next, the aliquots were centrifuged and filtered. The filtered samples were then analysed by HPLC (PerkinElmer Series 200) with a Nucleogel Ion 300 OA column (Macherey–Nagel) with H_2SO_4 5 mM as the mobile phase at a flow rate of 0.5 mL min^{-1} and 80 °C.

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Keywords: biomass · carbohydrates · enzyme catalysis · heterogeneous catalysis · nickel

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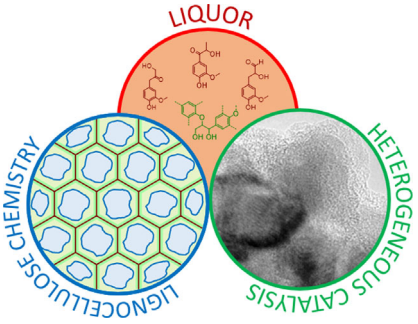
Union is strength: In catalytic upstream biorefining (CUB), the action of Raney Ni is limited to the species leached from the plant tissue into the liquor. Nevertheless, the substantial changes in the liquor composition (through the decomposition of carboxylic acids and H-transfer reductive processes on the lignin fragments) have major implications for the pulp composition, degree of polymerization, and morphology.



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Catalytic Upstream Biorefining through Hydrogen Transfer Reactions: Understanding the Process from the Pulp Perspective

VIP